The Development of a Bulk Bin, Deep Layer, Down Welling, Mussel Purification System

Seafish Report No.456

July 1994

The Sea Fish Industry Authority

Seafish Technology

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Summary

This report describes the development of a bulk bin, mussel purification system which has been developed by Seafish. Work on this project has been underway for a number of years. It started with small scale trials conducted at the Seafish Fish Laboratory in Hull and has lead to the development of the first commercial plant at Myti Mussels in North Wales.

This report outlines the physical parameters required to depurate mussels in deep layers. A practicable, easy to operate, deep layer mussel purification system has been developed by simple modification of standard pallet bins which are connected to a sump tank and a water circulation system. The water flows downwards through the mussels at a high rate. Each pallet bin holds approximately 300kg of mussels. This system is aimed at large-scale operators and is geared to the use of mechanical handling systems.

Contents

1. Introduction

In the past few years Seafish has developed four modular standard design purification tanks. (Ref. 1-7). Two of these designs were multi layer tanks primarily for the purification of mussels. (Ref. 1-4). These designs involve the stacking of containers up to six high, each container holding 15 Kg of mussels, a depth of 80mm. These designs save a considerable amount of floor space compared to the traditional, single layer, purification tanks and therefore enable them to be housed in a building more conveniently. However, these systems are still fairly labour intensive due to each of the small containers having to be filled with mussels and then placed into the purification tanks. Having considered the Dutch approach to degritting mussels, using large bins with an upwelling or downwelling water flow through mussels in a layer up to 1.2 meters in depth, Seafish decided to investigate the possibility of purifying mussels in a deep layer. Seafish considered such a system would have a number of advantages over the multi-layer approach, for a business handling large quantities of mussels, many pallet bin modules can be 'plugged in' as and when required to a common seawater supply and return circulation system. The system would make use of mechanised handling and without the need for a large number of containers therefore lends itself to reduced direct handling of the mussels and hence labour. This would also reduce the physical shock to the mussels which is beneficial especially prior to purification.

It was decided that the work would be carried out in three phases. The first trials were to determine if mussels could open and filter in a deep layer and to ascertain the physiological parameters that are required to ensure that the mussels purify. The second was to prove that mussels would purify in a deep layer pallet bin and to develop the bin concept into a workable practical unit. The third was to work with a processor in a collaborative project to develop a complete operational system.

This system is not intended for the purification of other species of molluscs which do not naturally grow and function in deep layers.

2. Objectives

The broad aim of this work was to assess the viability of purifying mussels in a deeper layer than the 80mm currently allowed under MAFF conditions of approval for mussel purification.

2.1 To investigate this ability of mussels to open, filter and eliminate faecal bacteria in varying layer depths using either downwelling or upwelling water flows.

2.2 To investigate the water flow rates required to maintain dissolved oxygen (DO) levels above 50% saturation in a deep layer system, across the normal working range of temperatures.

2.3 To develop a pilot scale bulk bin purification system and check its efficacy.

2.4 To further develop the bulk bin system into a full scale commercial mussel purification plant and confirm its ability to purify mussels.

3. Trials Sequence

This work was conducted in three phases:-

Phase 1	August-November 1991	**Trials 1-9**	Laboratory trials
Phase 2	September 1992- March 1993	**Trials 10-20**	Pilot trials of bulk bin
Phase 3	September-December 1993	**Trials 21-26**	Commercial development

4. Trials Sites

Phase	Trials	Location
1	1-8	Seafish Fish Laboratory, Hull
	9	J and J Shellfish, Kings Lynn, Norfolk
2	10-14	Myti Mussels, Port Penthyn, Bangor, Gwynedd
	15-17	Seafish Fish Laboratory, Hull
	18-20	J and J Shellfish, Kings Lynn, Norfolk
3	21-26	Myti Mussels, Port Penthyn, Bangor, Gwynedd

5. Trials Equipment

5.1 Monitoring Equipment

Dissolved oxygen (DO) monitoring was conducted using a 5 channel Oxyguard oxygen monitor with data logger and an Oxyguard hand held oxygen meter. Temperature was monitored with an Escort data logger. Temperature was also measured with a Kane and May digital electronic thermometer. Salinity was measured with a hydrometer.

5.2 Phase 1 - Laboratory Trial Purification Equipment

During all but one of these trials plastic cylindrical containers were used to hold the mussels in a vertical column. These containers were either sat on top of a small scale purification tank or placed inside a pallet box (Figure No. 1). The purification tank or the pallet box acting as a sump from which the water was circulated. Circulation was by a submersible pump in either an upwelling or downwelling configuration. The cylindrical containers used were Paxton type HR110 and HR160. These have a diameter of 425mm and heights of 438 and 641mm respectively. In the later phase 1 trials, the base was removed from an HR160 and it was placed on top of a second to double the depth achievable to 1.2 metres. During these trials no UV sterilization was used.

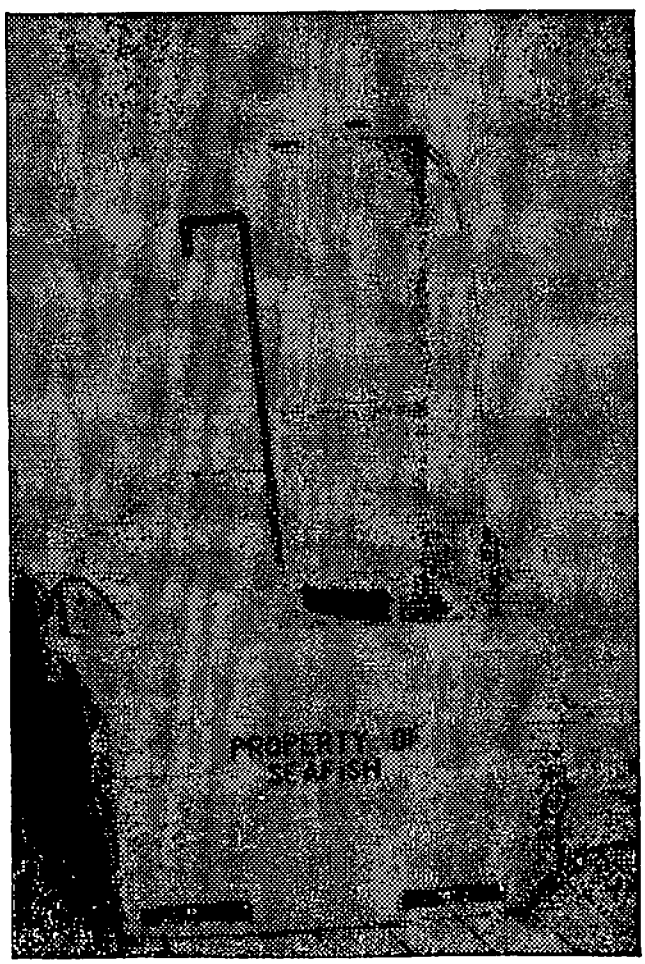


Figure 1 - Phase 1: 1.2m Down Welling Column

In the final trial in this phase a perforated pallet bin was used to hold the mussels. This was an Allibert type 21667 container with a capacity of 650 litres and external dimensions of 1000 x 1200 x 765 mm. This container was placed into a large-scale Seafish designed purification tank in a cross flow situation.

5.3 Phase 2 - Pilot Bulk Bin Purification System

These trials were on a larger scale than the laboratory scale trials and used a modified pallet bin to hold the mussels (Figure Nos. 2 to 5). An Allibert pallet bin (Type 21626) with external dimensions of 1000 x 1200 x 765mm was fitted with a false floor made from Allibert floor tiles (Type 61300) cut to fit the internal shape of the bin. The floor tiles were suspended on slot-in feet (Type 00103) which held the floor tiles 200mm above the bin floor. This left 300mm depth for the mussels and a further 100mm for water to cover the mussels. Water flowed into the bin from above, down through the mass of mussels and into a matrix of pipes situated below the false floor. This matrix had a number of holes drilled into the pipes to take in the water in a distributed fashion and was connected to a pair of vertical stand pipes which exited through the side of the bin just below its top edge. This arrangement ensured a water level of at least that of the exit pipes and a downwelling flow through the mussels. Drainage of the bin was through a 25mm fitting in the base of the bin, which was fitted with a screw cap. For the trials the bin was placed on top of another unmodified bin to gain some height and a third bin was used as a sump.

Water was circulated by being drawn out of the sump bin by a submersible pump and passed through a titanium in-line water chiller, 2 x 30 watt UV light sterilizers and a flow control valve to feed into the bin by a spray bar just above the water surface. The water cascaded from the mussel bin outlets back into the sump tank. During the trials there were a number of modifications made to the bin floor and to the water inlet and outlet pipes. These are detailed in the trials methodology in Section 10.

5.4 Phase 3 - Commercial Purification System

This is described in detail in Section 11.

5.5 Small Scale Standard Purification Tank Used as a Control

During many of the trials a small-scale purification tank of known performance was run alongside the experiment as a control measure of the effectiveness of the experiment. This tank was based on an Allibert type 21250 container with a capacity of 250 litres and external dimensions of 1000 x 600 x 662mm. The tank is designed to hold shellfish in a single plastic mesh tray which is located clear of the tank base on 15mm plastic battens. The tray used was a Allibert type 41042 measuring 750 x 450 x 175mm externally with a capacity of up to 15 Kg of mussels. The tank was fitted with a pump, UV sterilizer, flow meter, control valve, suction bar and spray bar to allow a range of MAFF technical specifications for purification tanks to be met. (Figure No. 6). It was operated in the standard conditions for a single shallow layer of mussels.

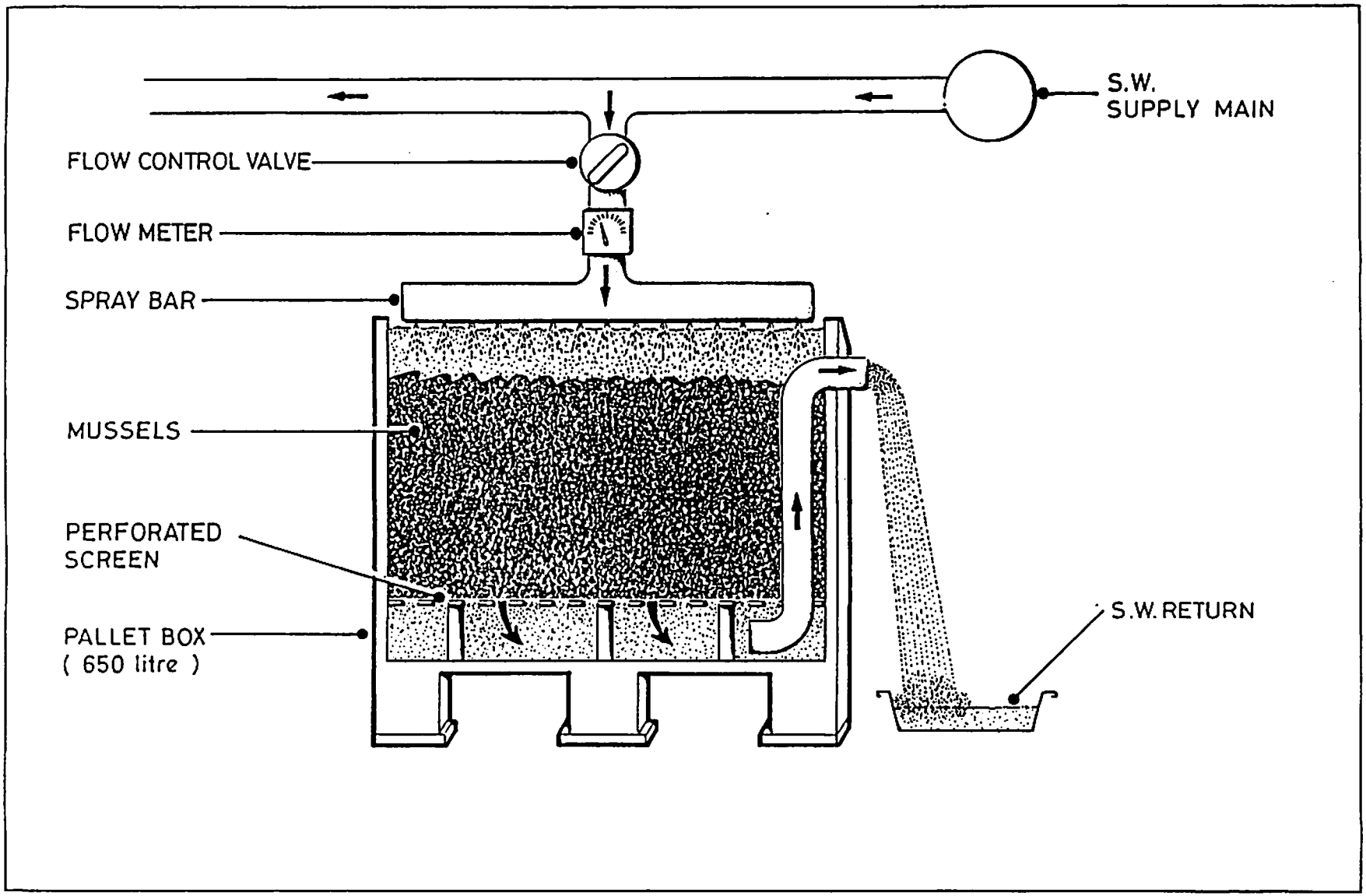


Figure 2 - Phase 2 Bin Design

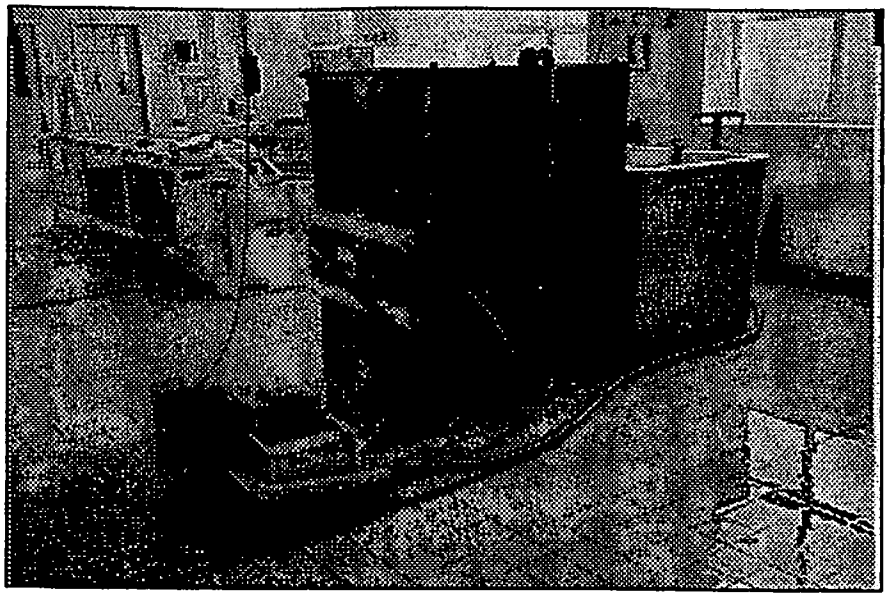


Figure 3 - Pallet Bin Trial, as setup in Seafish Fish Laboratory

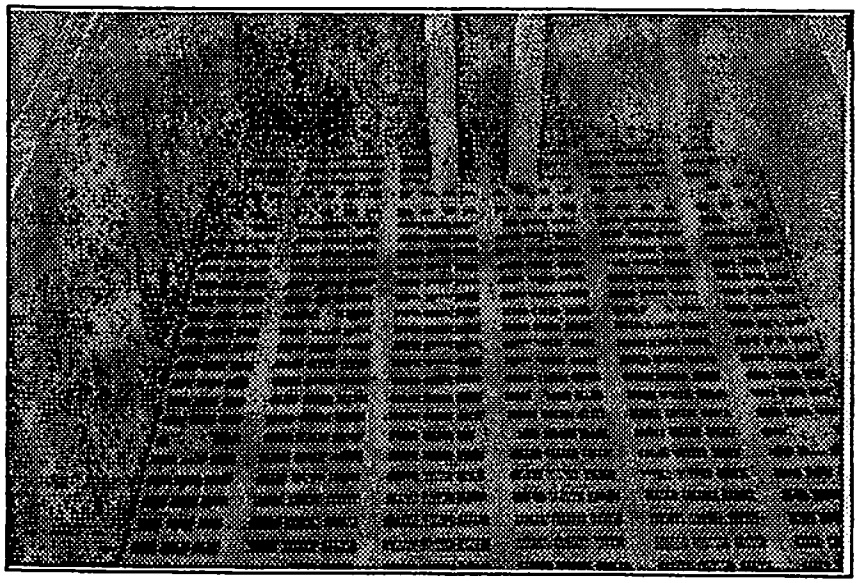


Figure 4 - Allibert floor tiles making raised false floor.

Figure 5 - Allibert bin tipped to show sediment after purification and outlet pipe configuration

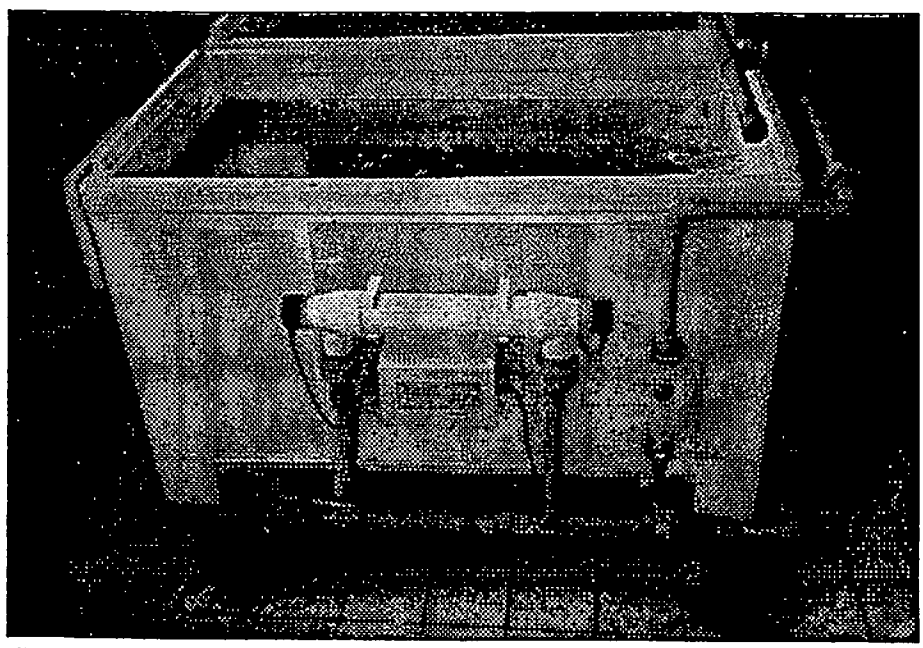


Figure 6 - Small Scale Control Tank

6. Seawater Supply

For trials carried out at Myti Mussels seawater was sourced from their seawater supply for their existing purification tanks, which is pumped ashore at high water. This is adjacent to a Class B mussel growing area. For all other trials, a five part artificial seawater (ASW) mix was produced in accordance with MAFF Laboratory Leaflet No. 39 (Ref. 8).

7. Mussel Supply

Seafish are grateful to the following people for their help in supplying mussels for these trials.

Kim Mould	Myti Mussels, Bangor
John Williamson	J and J Shellfish , Kings Lynn
Terry Large	Brancaster

8. Bacteriological Analysis

Bacteriological analysis was carried out by Hull Public Health Laboratory with the mussels extracted from the shells at the Seafish Fish Laboratory and delivered in sterile 150ml containers. Bacteriological analysis was carried out using a 5 tube 3 dilution M.P.N. (Mean Probable Number) method as approved by MAFF. (Ref . 9).

9. Artificial Dosing of Bivalve Molluscs with *E. coli*

In England and Wales the purification of bivalve molluscs in purpose built tanks requires a bacteriological test as part of the approval procedure to demonstrate satisfactory operation. Using the bacteria *Eschericia coli (E. coli)* as an indicator of faecal pollution, initial high levels in bivalve molluscs placed in a tank must reduce to below prescribed levels within 42 hours.

Problems with obtaining naturally or artificially contaminated bivalve molluscs with sufficiently high levels of *E. coli* has resulted at times in considerable cost and inconvenience. The main problem with artificial dosing was the unpredictability associated with attempting to dose all bivalve molluscs in the purification tank at once. MAFF and Seafish developed a new approach to dose only a small quantity in a separate tank and place these molluscs at defined sampling points in the purification tank instead. (Ref. 10).

The procedure used to artificially dose the shellfish with *E. coli* is given in Appendix I.

10. Phase 1: Laboratory Scale Trials, Methods and Results

The purpose of this first phase of the trials (Trials 1 to 9 inclusive) was to determine if mussels could open and filter in a deep column and to ascertain the physical parameters that are required to ensure that the mussels purify. Trials 1 to 6 were carried out with water circulating from the top of the container used to the bottom (downwelling). For trials 7 and 8, the flows were reversed (upwelling) and for trial 9 a conventional horizontal flow was used with different equipment. Table No. 1 details aspects of the trials methodology and the physical parameters recorded during the trials, including immersion time, water and mussel data. Table No. 2 details the bacteriological data, although bacterial analysis was not carried out for all trials.

10.1 Downwelling Trials

Trials 1 and 2

Trials 1 and 2 were conducted in the type HR110 container, with mussels to a depth of 25cm. The mussels were active and byssed up well. After trials 1 and 2 mussel samples were held in chilled storage and mortality rates monitored. (Figure Nos. 7 and 8). No significant difference was noticed between the mussels from the deep stack column and the control tank. In trial 1 a marginal difference between top and bottom of the deep stack column was noticed.

Figure 7 Trial 1 Mussel Mortalities During Storage at Chill Temperature

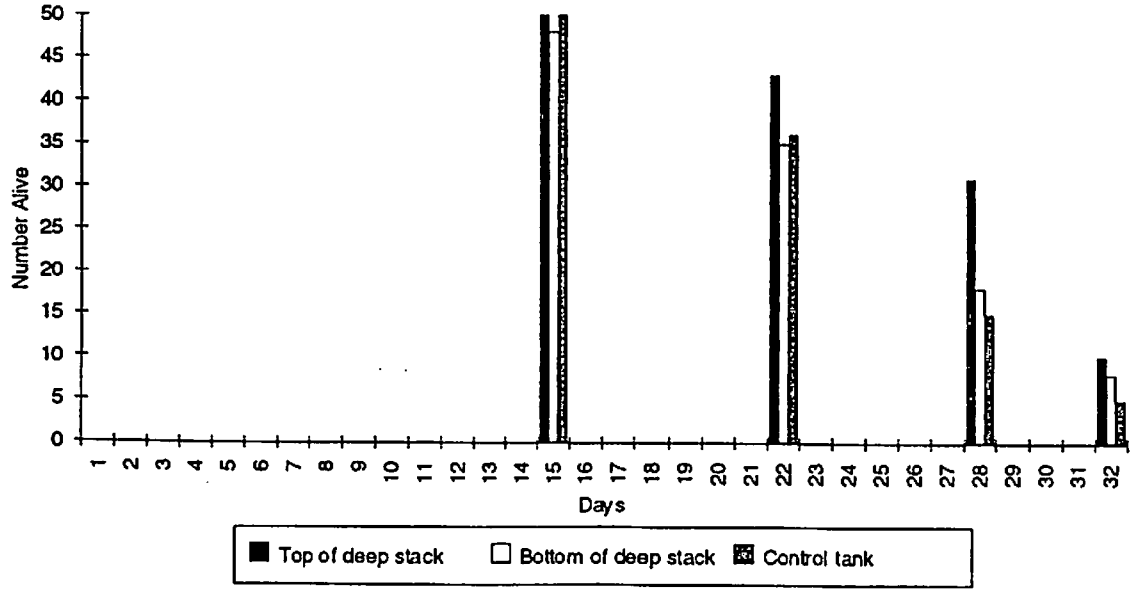
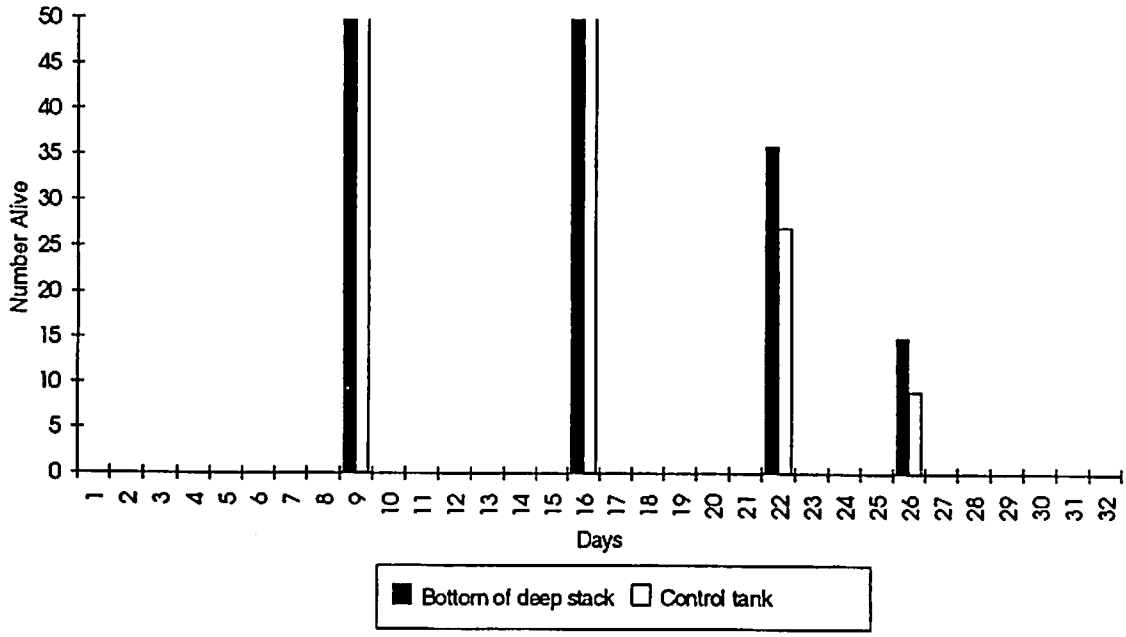


Figure 8 Trial 2 Mussel Mortalities During Storage at Chill Temperature

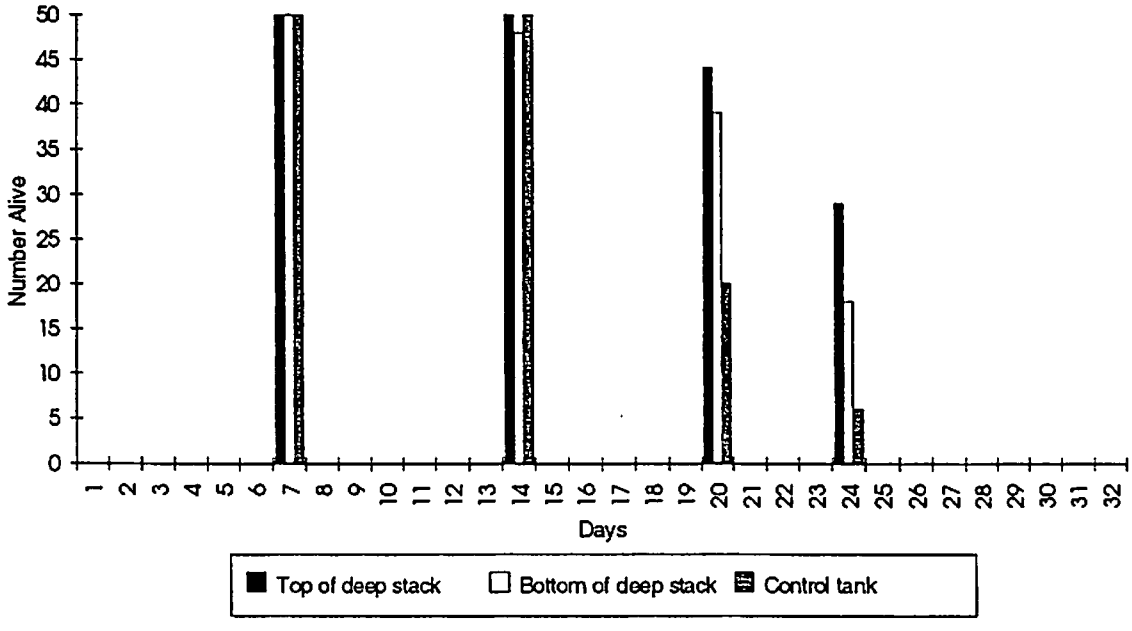


Trial 3

Trial 3 was similar to trials 1 and 2 but used the type HR160 container with a 50 cm high column of mussels. A perspex window was fitted near the bottom of the container, through which it was possible to see the mussels at the bottom open and filtering when immersed. At the relatively lower water flow rates in relation to the mass of mussels compared to trials 1 and 2, the DO levels dropped below the required 50% saturation (Table No. 1).

A similar check on mortality rates during subsequent storage was conducted and it showed no difference in mortality until 14 days after removal from the water. (Figure No. 9). As in trials 1 and 2, at 20 days and over the mussels in the control tank were dying off somewhat faster than those in the deep stack.

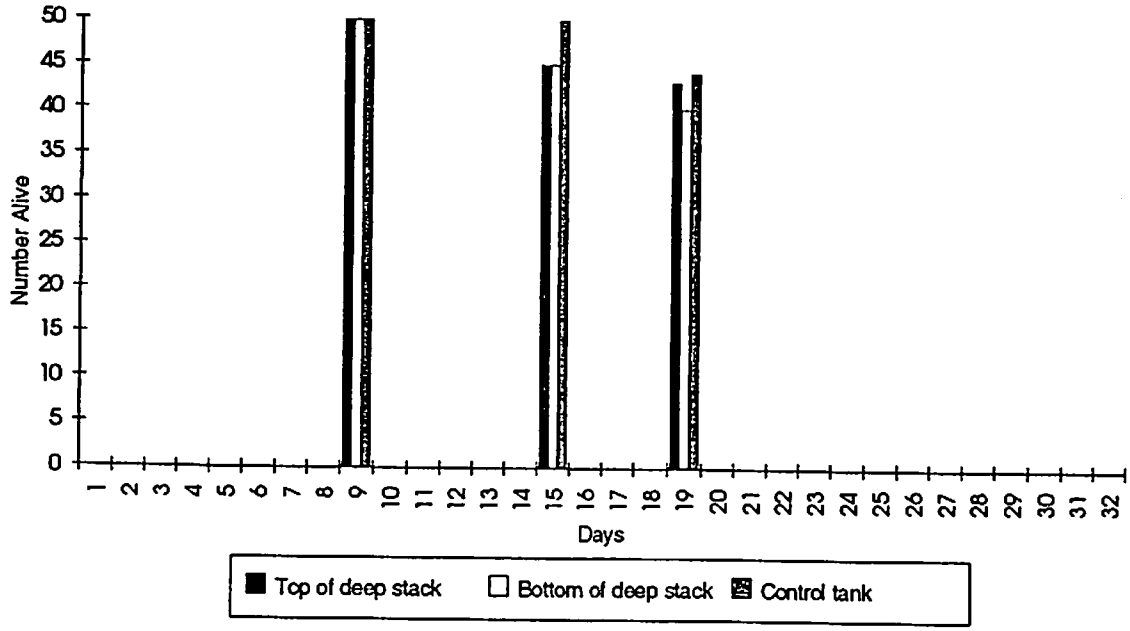
Figure 9 Trial 3 Mussel Mortalities During Storage at Chill Temperature



Trial 4

Trial 4 was conducted with the two HR160 containers stacked one on top of the other. The base was cut out of the top container to make a column 1.2m high that was filled with mussels (Figure No. 1). The perspex window permitted visual observation of the mussels at the bottom and the mussels were seen to be active and filtering. During subsequent chilled storage, which was curtailed at 19 days, no significant difference was noticed between the mussels from the top and bottom of the column or the control tank. (Figure No. 10).

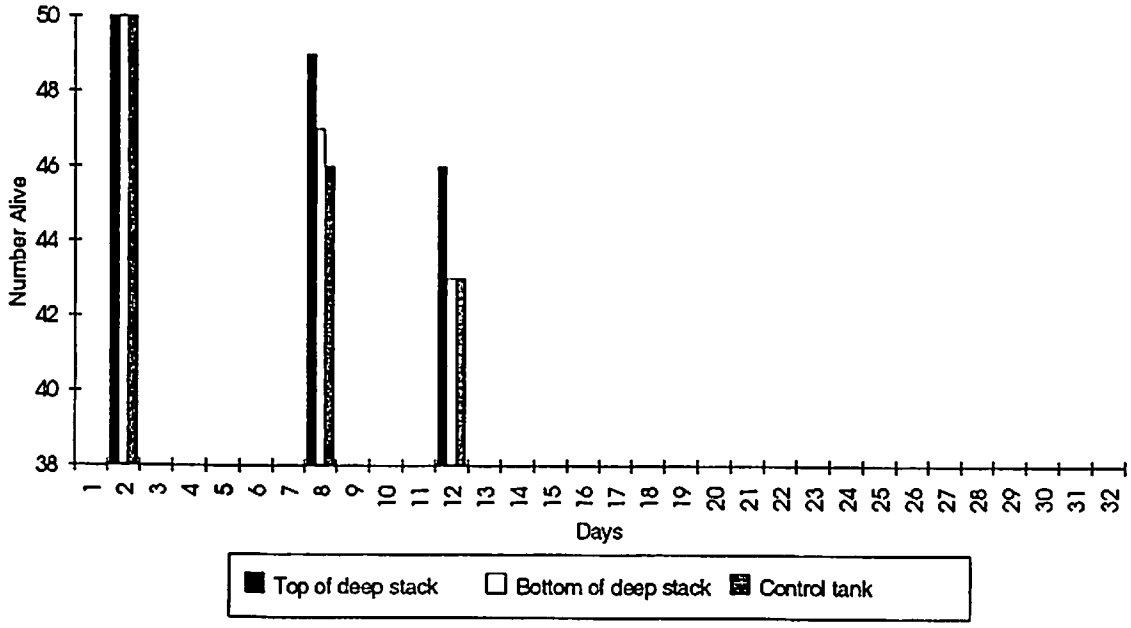
Figure 10 Trial 4 Mussel Mortalities During Storage at Chill Temperature



Trial 5

Trial 5 was set up similarly to Trial 4. The mussels observed through the lower window did not seem to be as visually active in this trial. Bacteriological samples were taken on this trial and are shown in Table No. 2. The results show a reduction in levels from a low category B level production area to an undetectable level. Chilled storage was curtailed after 12 days and there was no significant difference in mortality between the mussels from the top and bottom of the column or the control tank. (Figure No. 11).

Figure 11 Trial 5 Mussel Mortalities During Storage at Chill Temperature



Trial 6

Trial 6 was set up with a single type HR160 container filled to 60cm with the same mussels as used in trial 5. The same batch of ASW was used also. The mussels observed through the lower window were visibly more open and active. This may have been due to the greater water flow to mussel mass ratio.

10.2 Upwelling Trials

Trial 7 and 8

The water was pumped in at the bottom of the type HR160 and allowed to flow up through the mussels and cascade over the rim of the container at the top. In trial 7, 90 Kg of mussels were placed into a 1.2m column to a depth of 1.1m, with previously used ASW. No activity was observed in the mussels so the trial was stopped and the water disposed of.

Trial 8 saw the same batch of mussels re-immersed in a fresh batch of ASW after cleaning out the old water and sediment. The mussels showed visible signs of filtration activity. On draining down at the end of the trial it was evident, by looking through the perspex window, that mud and faecal material which had settled in the system during upwelling was

disturbed by draining down in the opposite direction. This led to re-suspended material flowing downwards past the immersed mussels which may have been re-ingested before the water drained completely and the mussels closed. This could lead to re-contamination and so no further trials were carried out using upwelling flow. Bacteriological samples were taken on this trial and are shown in Table No. 2.

10.3 Horizontal Flow Trial

Trial 9

This trial was conducted to find out if the traditional side to side water flow could penetrate through a mass of mussels in a deep layer and provide suitable conditions for purification activity to occur. The trial involved filling a perforated bin with 400 Kg of mussels and placing it into a large-scale standard design purification system.

The mussels at the edges and bottom of the pallet bin were observed to be active and byssed up, but the mussels in the core of the mass away from the outer edges did not open and produce byssus. It was observed during the trial that the water in the tank had a tendency to go around the mass of the mussels and not through them thereby starving them of oxygen.

This approach was not pursued any further.

10.4 Discussion

These initial trials allowed the basic physical parameters for deep layer mussel purification to be defined. The downwelling water flow was considered to be the only method suitable because:-

a) Draining down the upwelling water flow method was found to disturb the detritus which had settled out during upwelling and the contamination may then be re-ingested by mussels lower in the column.

b) The horizontal flow used in standard systems may not be capable of simple adaptation for deep layers because of the problem of ensuring that the water flow goes through the mussel mass and not around the sides of the container in the tank. We did not consider that this work would allow the depth of mussels in a container in a standard system to be increased from that already allowed.

The depth of mussels, as far as the trials went (1.2 m), did not appear to prevent mussels from opening to filter and to put out byssus threads. The limiting factor was considered to be maintaining DO levels above 50% saturation within a large mass of mussels, especially when coupled with higher water temperatures. The relationship between the water flow rate, temperature and the mass of mussels in the system has been shown to be important. At a temperature of 14°C a flow rate of 18 litres per hour per kg of mussels is just able to maintain 50% oxygen saturation.

Bacteriological analysis from trial 5 has shown a reduction in counts of *E.coli*, although from a fairly low level of 400-800/100g. Mortality trials have shown no significant differences between top and bottom of the deep layer and the control tank.

11. Phase 2: Pilot Scale Pallet Bin Trials, Methods and Results

Having determined in phase 1 that mussels remained active in a deep layer, given that the environmental conditions were correct, the purpose of this phase was to prove mussels would purify in a deep layer pallet bin, and to develop the bin concept into a practical working unit. These trials were conducted with a single pallet bin set up with an experimental re-circulation system as described in Section 5.3. (Figure Nos. 2 to 5).

A fresh batch of mussels was harvested for every trial and a fresh batch of seawater was used. In some trials a small quantity of grossly contaminated mussels was obtained along with a batch of normal (category B) mussels. The contaminated mussels were placed loosely into sacks with a large mesh. These mesh sacks were placed at strategic locations in the bin and used for bacteriological analysis purposes.

The physical and bacteriological data are shown in Tables 1 and 2.

11.1 Trials at Port Penrhyn

Trials were carried out at Myti Mussels due to their enthusiasm for the project and the convenient access to mussels and seawater.

Trial 10

The Allibert pallet bin was filled 25cm deep, with unwashed mussels which were very muddy and full of shell etc. We estimate 150kg of mussels in a total mass of 200 kg. All the mussels actively filtered and produced byssus. There was a lot of mud and shell on the base of the bin under the raised floor after the trial. The DO levels measured were above 50% saturation. (Table No. 1). The bacteriological results were of little significance due to low initial counts (Table No. 2).

Trial 11

Prior to this trial starting the false floor was modified by lowering the feet to give a height of 100mm above the bin base. This allowed an increased depth of 38 cm of mussels to be put into the bin leaving 12 cm for water over the top mussels. This modification was then retained for the further trials. Fairly clean (externally) mussels were put into the bin. In the middle of the mass of molluscs at 18°C, DO levels were below the 50 % saturation level . (Table No. 1). The flow of 16 L/hr/kg should therefore be increased to increase these levels. All the mussels were well byssed up and were visibly active. After the trial there was a lot of mud under the false floor. The bacteriological results were again of little significance due to low initial counts (Table No. 2).

Trial 12

The bin was filled with mussels on the quayside, straight out of the boat's dredge and then they were washed in the bin. The trial was conducted at the same flow rate as the previous trial.(16 L/hr/kg) The DO level in the middle of the mass at 17.7°C were close to the 50 % saturation level. (Table No. 1). The bacteriological results were again of little significance due to low initial counts (Table No. 2).

Trial 13

Before filling the bin on this trial the matrix of pipework attached to the two outlet pipes, under the false floor, was removed leaving the pair of vertical stand pipes open at the bottom. This was done to enable an increased water flow out of the bin. In addition, the water spray bar was cut off leaving a 'T' piece input pipe which was lowered to just below the water surface. All aeration therefore resulted from the cascade to the sump tank. This stopped any foaming on top of the bin of mussels. These modifications were then retained for the further trials. (Figure No. 2).

The bin was filled with mussels which were fairly clean (externally) before putting into the bin. The flow rate was altered throughout this trial to identify changes in DO levels which are shown in Figure No. 12. At 2880 L/hr (9.6 L/hr/kg) and a temperature of 16°C, the DO levels were too low. At 4500 L/hr (15 L/hr/kg) a DO level of 50 % saturation was just maintained in the middle of the bin. A flow of 6000 L/hr (20L/hr/kg) maintained DO levels at greater than 50% saturation. The bacteriological results were significant with initial counts of 1400-2300 *E.coli* /100g reducing to non- detectable levels throughout the mass of mussels in the bin. (Table No. 2).

Trial 14

Before filling the bin on this trial the mussels were washed and graded using a declumper/rotary grader to remove shells, mud, crabs etc. The bin was then filled full with mussels to a depth of 42 cm. (approx. 350kg mussels compared to 300Kg at 38cm depth). There were a few damaged mussels in the bin due to the action of the declumper. The morning after the trial commenced the sump tank was covered in foam, as is normal for mussel purification systems, especially when the mussels are active and in only a small volume of recirculating water. To remove the foam a pint of milk was added, a practice not recommended for purification but sometimes used for vivier systems, this removed the foam. This was seen to coincide with a reduction in DO levels. (Figure No. 13) It is thought unlikely that the milk alone could have caused such a rapid reduction, and is thought to have been a result of increased mussel activity stimulated by the milk as a food source. The DO levels reduced to about 40% saturation at a temperature of 15.3 °C then over the next few hours, gradually returned to the levels before the milk was added. This was with a water flow/mussel mass ratio of 18 L/hr/kg. The bacteriological results were significant with initial counts of 800-3000 *E.coli* /100g reducing to non-detectable levels. (Table No. 2). The filling of the bin to a depth of 42cm was shown to be unsuitable as the top mussels ended up out of the water. This is because the mussels open and move during the purification cycle and their effective volume increases. Subsequent trials returned to 38cm depth.

Figure 12 Trial 13 Variation in Dissolved Oxygen Levels with Water Flow, at 16°C

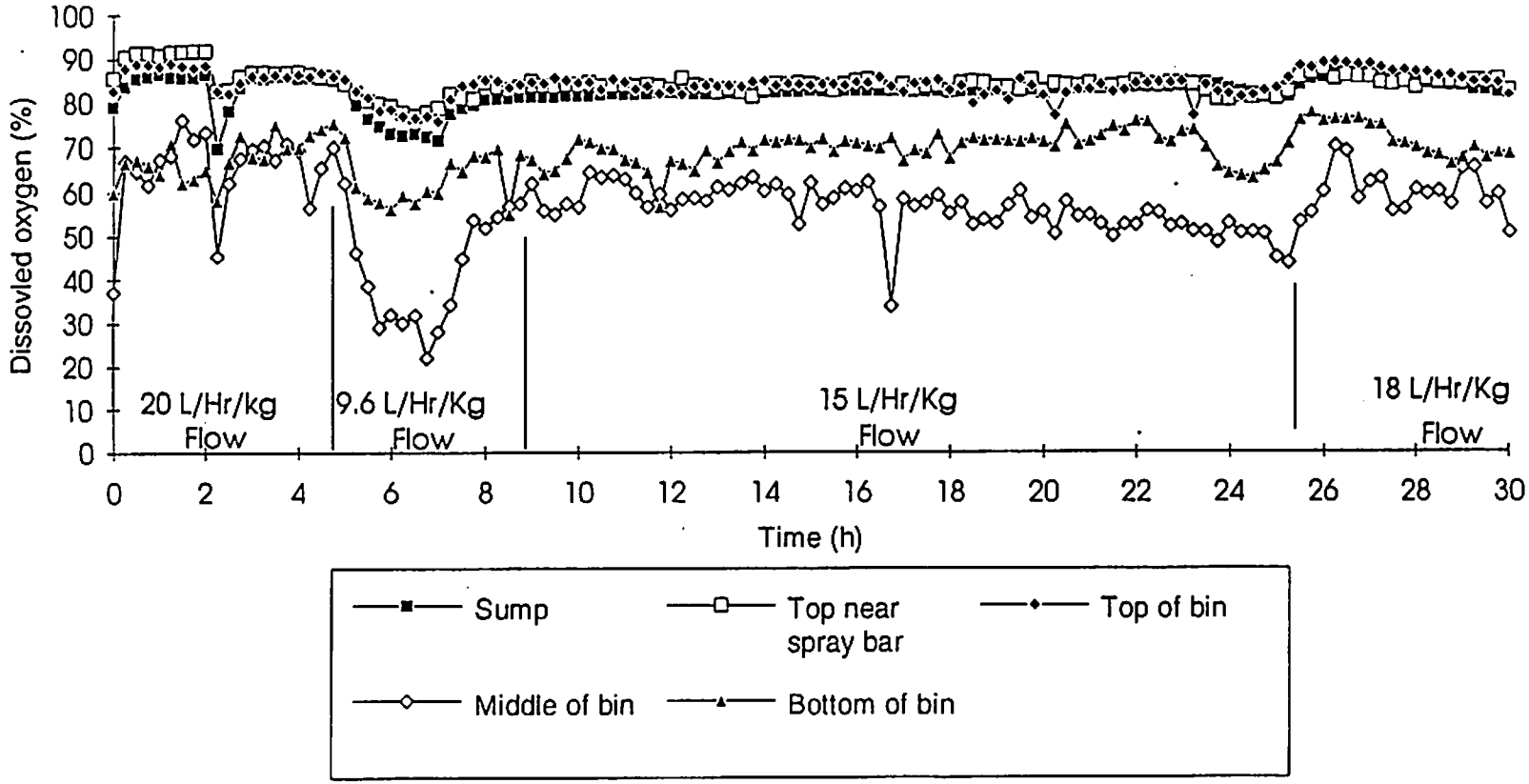
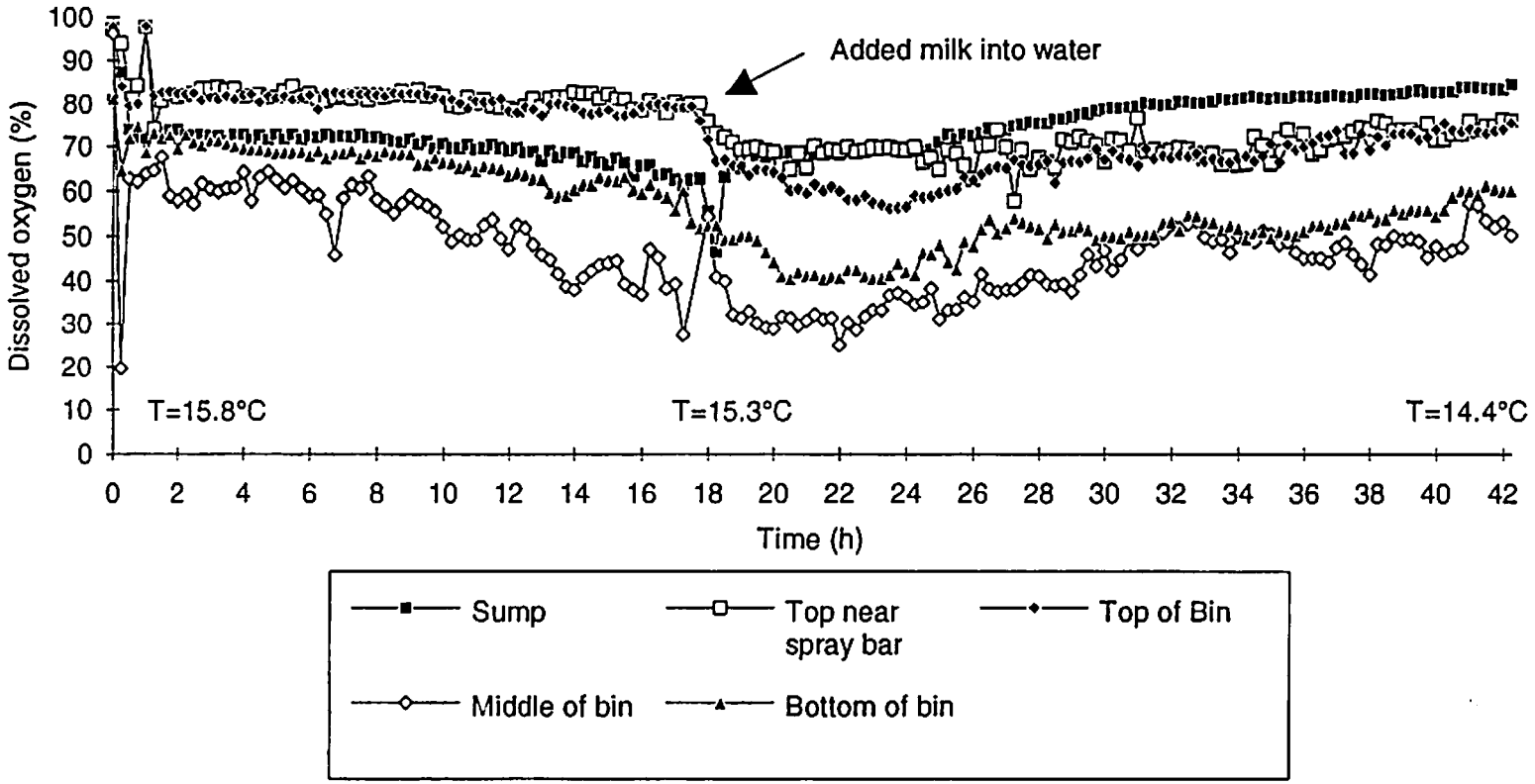


Figure 13 Trial 14 Dissolved Oxygen Levels at 18L/Hr/Kg and about 15°C.



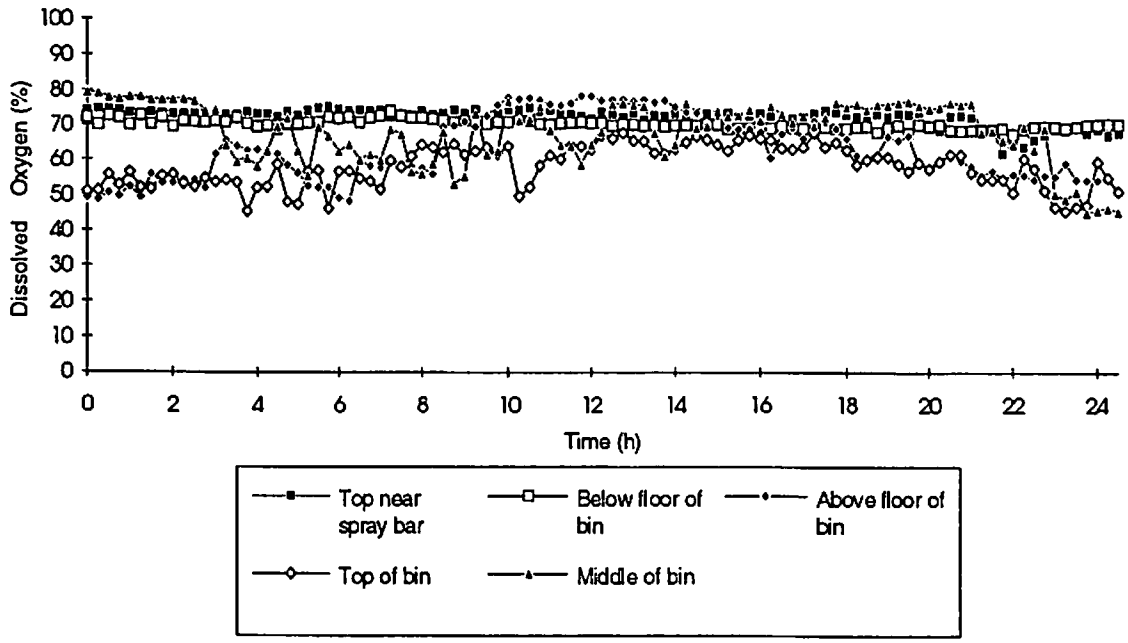
11.2 Trials at Hull

Trials were continued in the laboratory to obtain further bacteriological results using the artificial contamination technique detailed in Section 9. In these trials ASW rather than natural seawater was used.

Trial 15

The bin was filled with fairly muddy, unwashed, mussels. The trial showed that a flow of 6500 L/hr (22L/hr/kg) was suitable to maintain greater than 50% saturation DO levels at 18°C, higher than the normal working temperature range recommended for mussels. (Figure No. 14). An attempt was made to artificially contaminate mussels before they went into the bin, but this was not successful and the bacteriological results were of little significance due to low initial counts. (Table No. 2).

Figure 14 Trial 15 Dissolved Oxygen Results at 22L/Hr/Kg



Trial 16

This trial was a repeat of trial 15 with a further attempt to artificially contaminate the mussels before they were put into the bin and again this was not successful and the bacteriological results were of little significance due to low initial counts. (Table No. 2).

Trial 17

Trial 17 was again a repeat of trial 15 with yet another attempt to artificially contaminate the mussels before they were put into the bin. The bacteriological count in the water during the contamination process went from 3000 *E. coli*/100ml to 400 *E. coli*/100ml in one hour at a temperature of 11.3°C. However, the process was only partially successful with counts in the mussels reaching 700 *E. coli* /100g.

The bin was filled with very muddy mussels, with the artificially contaminated ones in loose bags with a large mesh situated on top of the false floor and on the top of the mussels in the bin. A further sample was placed under the false floor, where under normal circumstances there would not be any mussels, to establish the worst case scenario of mussels sat in the faecally contaminated detritus.

The bacteriological results showed initial counts of 80-700 *E. coli*/100g reducing to <200 *E. coli*/100g. Due to some confusion at the laboratory, with the sample dilution strength, the minimum level monitored for was <200 not <20.

11.3 Trials at Kings Lynn

Trials were moved to Kings Lynn to take advantage of some 'dirty' mussels which were obtainable for trials purposes (not for human consumption) from a prohibited area. This was necessary due to the lack of success using the artificial contamination method. For trials 18,19 and 20 the deep stack purification equipment was taken to Kings Lynn and set up in J & J Shellfish's purification plant. Mussels were sourced from two places for each trial, the mussels for bacteriological analysis were obtained from the barrier wall at the mouth of the River Ouse near Kings Lynn, whilst the bulk of the mussels were obtained from Brancaster.

Trial 18

The deep stack bin was placed on top of a large-scale purification tank, which was used as a sump.(Figure No. 15). The pumping, UV and chilling arrangements were the same as in previous trials. Two tonnes of artificial seawater was used. The salinity was initially 22 PPT, but was increased to 31 PPT as the Brancaster mussels which are used to higher salinities were not filtering. The bin was operated at a water flow of 6500 L/hr (22 /hr/kg). The mussels for bacteriological analysis were placed in loose bags with a large mesh on the top of the bin, above the false floor, and below the false floor. The bacteriological results were of little significance due to low initial counts. (Table No. 2).

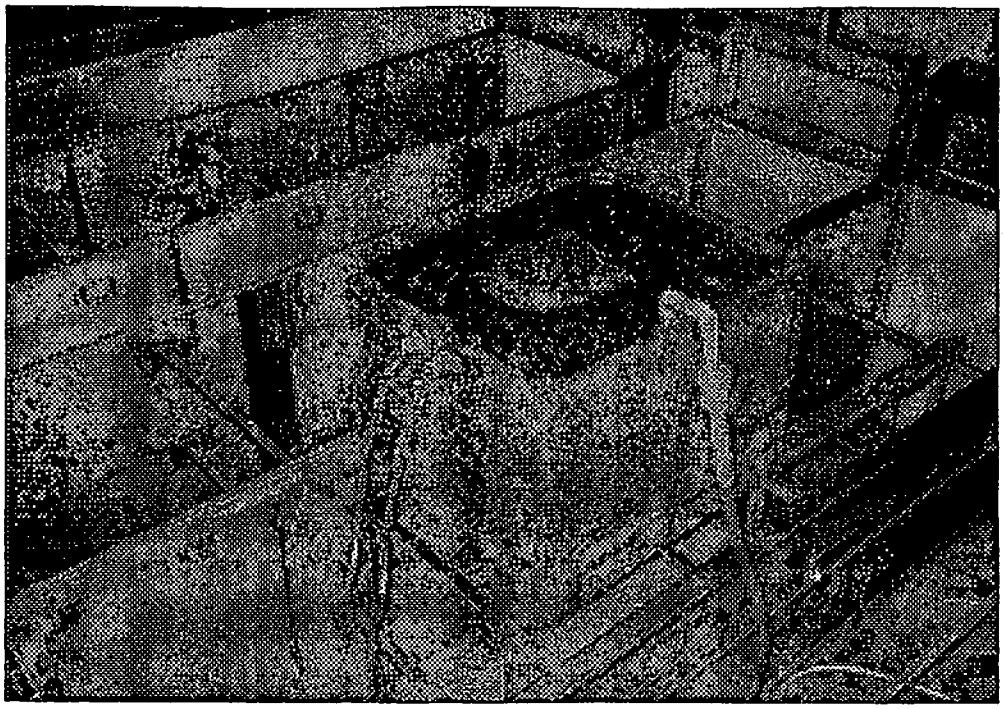


Figure 15 - Allibert Bin suspended over large scale multi-layer purification tank.

Trial 19

This was a repeat of trial 18, but the barrier wall mussels in this trial were very weak with a lot of dead mussels in the batch provided for the trial, although the dead mussels were removed before the trial. This trial used the same ASW as the previous trial. The obvious weakness in the mussels was shown in the bacteriological results with initial counts of 1100-1300 *E.coli* /100g reducing only to 40-130 *E.coli* /100g. Although permissable of product standard we would have expected levels to be lower as achieved in earlier trials. The counts of mussels in the control tank reduced only slightly to 700- 950 *E. coli*/100g indicating that the conditions in the bins were more suitable.

Trial 20

This was a further repeat trial using mussels from the barrier wall that were in good condition, following discussions with the fisherman. The ASW was replaced by a fresh batch because of concern about the previous batch of mussels. Before this trial all the mussels were well washed in a rotary riddle. The bacteriological counts pre purification were very high with pre levels of >18000 *E.coli* /100g reducing to a similar level of 70-250 *E. coli*/100g in both the working area of the bin and the control tank. Although these results are again not as low as would normally be expected the initial levels were extremely high and to have achieved a permissable product standard at all is considered to be a good result. The count of the mussel sample taken from the bin floor had reduced considerably but remained high and highlights the need to keep molluscs clear of the detritus.

11.4 Discussion

The apparent unreliability of the artificial contamination process is thought to be caused by variations in the quality (i.e. viability) of different batches of the freeze dried *E. coli*. This may be due to their manufacture or incorrect subsequent storage conditions.

These pilot scale trials have shown that a standard size pallet bin, which can handle commercially useful quantities of mussels, can be used to purify mussels successfully. These bins were used as they are cheap, easy to modify and readily available. They are also a suitable size for moving around by standard mechanical handling methods such as pallet and fork lift trucks. Due to the bins capacity, a depth of 38cm was the maximum depth practical. The depths achieved in phase 1 trials, up to 1.2 m, may still be possible given an appropriate container and the correct water/shellfish mass ratios.

The trials were considered successful even though there were initial problems of obtaining sufficiently 'dirty' mussels. It is considered that the bacteriological data on trials 13, 14, 19 and 20 demonstrated effective purification, with the possible exception of the suspect mussels in trial 19. The mussels always appeared active and well byssed up, which is a good sign of activity.

With the improved technology available it is now possible to measure DO levels in amongst the mussels and under the perforated false floor to get a better picture as to what is happening in the bins. The results from these show:-

1. A reduction in DO levels between water input and output points indicating that the mussels are filtering. This is related to water temperature and hence mussel activity.

2. A maintenance of above 50% oxygen saturation in the water amongst the mass of mussels within the bin throughout the recommended purification temperature range.

The trials again demonstrated the importance of water flow to maintain DO levels above 50% saturation. Flows of 16 and 18 L/hr/kg were just able to maintain 50% saturation at 15-16°C. This was considered insufficient for commercial operation where temperatures in excess of 15°C can occur at certain times of the year. A water flow of 22 L/hr/kg has been shown to be suitable to maintain good filtration activity and keep DO levels above 50% saturation at temperatures as high as 17-18°C and is our recommended flow for the system. If a system was operated whereby consistently lower temperatures were the norm, a lower flow rate could be specified.

The trials results were discussed with MAFF, Fish Diseases Laboratory, Weymouth, and they approved the method in principle and a nominal bin specification was agreed. This was a downwelling design with a water flow of 20-23.3 L/hr/kg and a temperature range of 5-15°C. The mussels to be well washed and graded gently. The capacity for each bin to be 300kg.

12. Phase 3: Commercial Development

A purification system based upon the use of bulk bins requires a supply of clean seawater to be supplied to each bin, from a common sump and a means of directing water back to the sump for re-oxygenation and re-circulation. The system should be housed in a building to provide environmental control.

Following the MAFF approval in principle of the bulk bin method, the next stage in its development was for Seafish to work with a processor, in a collaborative project to develop a complete operational system. Myti Mussels, who had assisted Seafish in the pilot scale trials, already had a number of traditional outdoor concrete purification tanks for purifying mussels and wished to replace them with an improved system. This was considered an ideal opportunity to progress the work to completion and so Seafish and Myti Mussels worked together in the design, development and testing of this new system up to its formal approval by MAFF and the local Food Authority. The following sections describe the system layout, modifications to the bin design following the phase 2 trials, a series of trials to confirm the systems effectiveness and system operation.

12.1 System Layout

For this first commercial plant an agricultural type building was erected over the site of an existing outdoor purification tank in order to house two independent 24 bin mussel purification systems each with a nominal capacity of 7,200kg of mussels. The bins would be filled at the quayside directly from the harvesting vessel, and taken the short distance to the purification building by fork lift truck. A hand pallet truck would then be used to manoeuvre the bins within the building. After purification the bins would be taken by fork lift truck to an adjacent building used for washing, sorting and packing. Each 24 bin system was to have its own sump and seawater circulation comprising pump, UV sterilization and pipework. To simplify the initial setting up and proving trials it was considered appropriate to split each system into two units of 12 bins and develop a single 12 bin unit first which could then be replicated to create a complete 24 bin system, using a common sump. The bins to be arranged in two parallel rows of 12 with a space between the rows in which a drainage channel was built into the concrete floor. (Figure Nos. 16 and 17). The drainage channel was approx 200mm wide and 200mm deep, with a raised lip above the floor to prevent floor drainage entering it, and directed the seawater into a large concrete sump tank. This was one of the existing seawater holding tanks used as part of the old system and was situated outdoors (which is not ideal) at the end of the building. The sump dimensions are 6m x 4.3m x 1.5m internal with a maximum water capacity of 38,700 litres. In use the sump would normally only be filled to 28,000 litres and this would give a water to mussel ratio of 3.8:1 with all 24 bins operating.

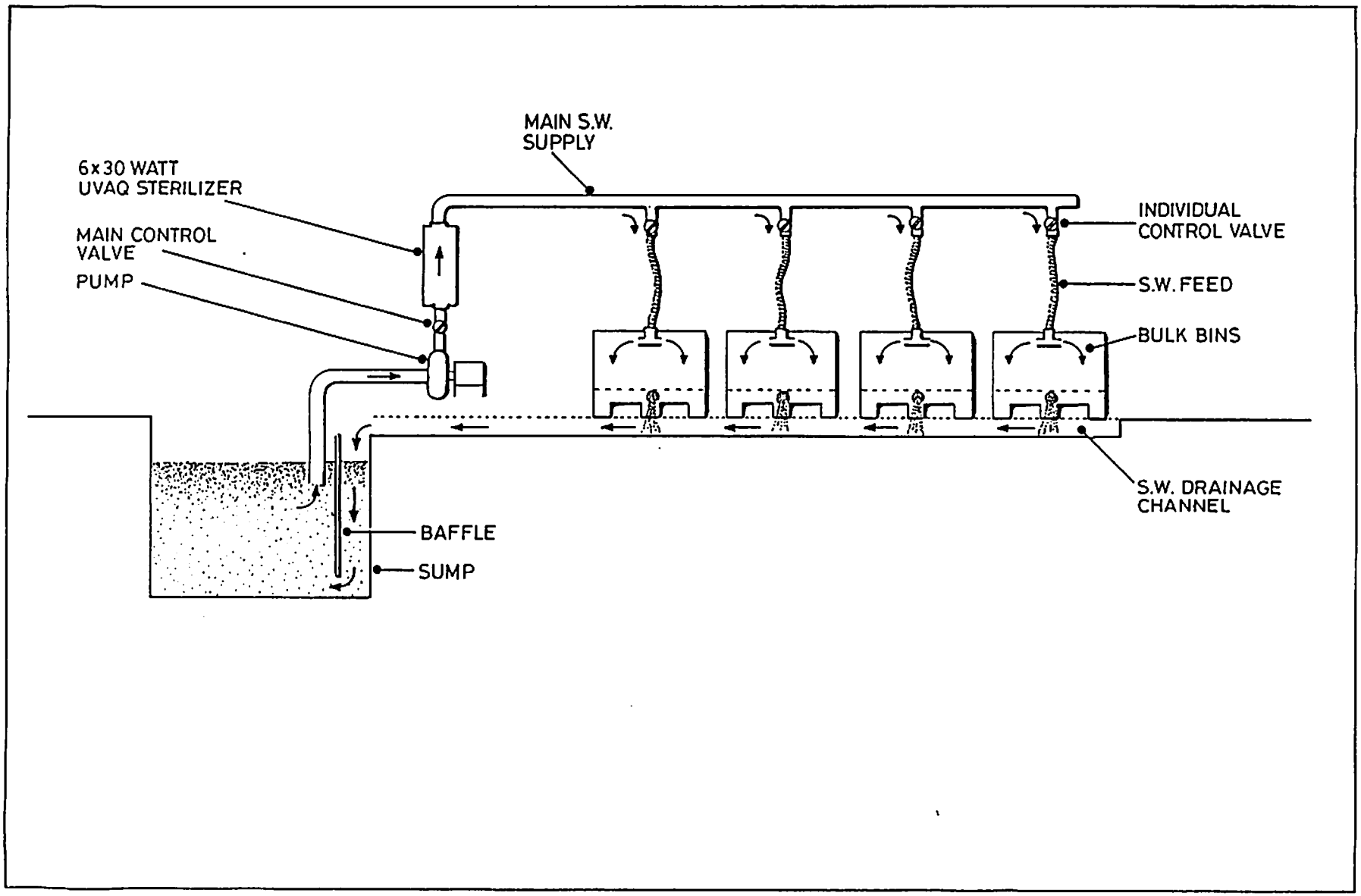


Figure 16 - Pipework and Bin Configuration as Installed at Myti Mussels

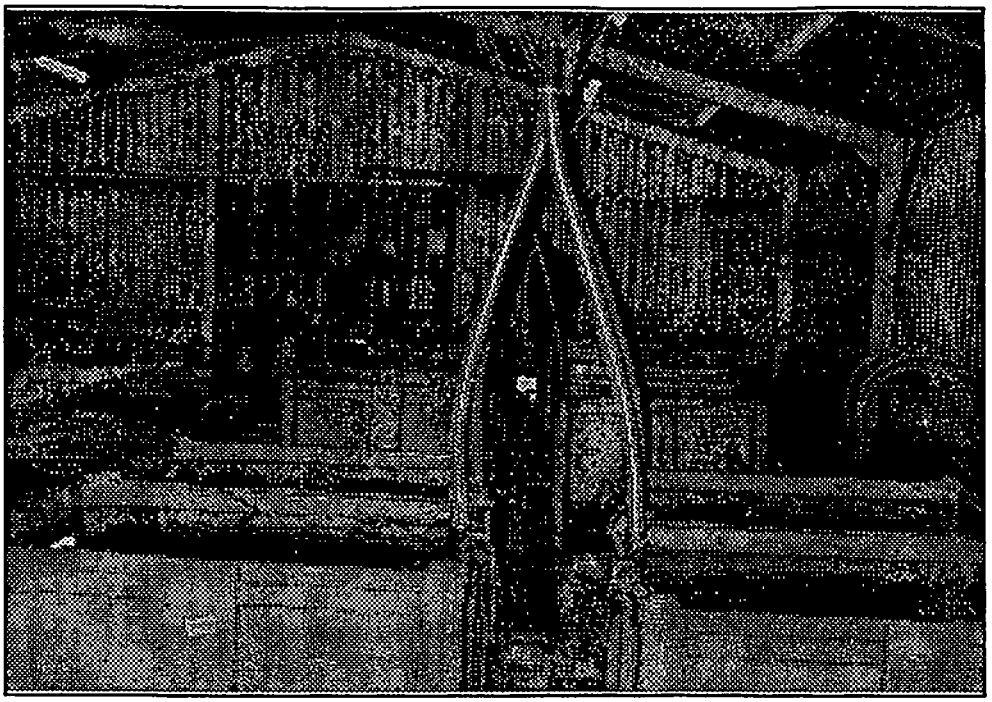


Figure 17 - Twelve Bins in operation

The water was re-circulated out of the sump tank through a 4 inch pipe, via a basket filter, by a 3Kw Desmi pump . The water then flowed into a 3 inch manifold which splits the water into three separate pipes each feeding into a UVAQ 630/8P water sterilizer. (Figure No. 18). Each sterilizer has 6 x 30 watt UV tubes and from each steriliser the water flows through a 3 inch pipe feeding four 1.25 inch flexible down pipes to the bins each down pipe being fitted with a control valve, and ending in a Tee. These flexible pipes are placed one in each bin and the water flow to each being controlled individually with the valves to the required rate. (Figure No. 19).

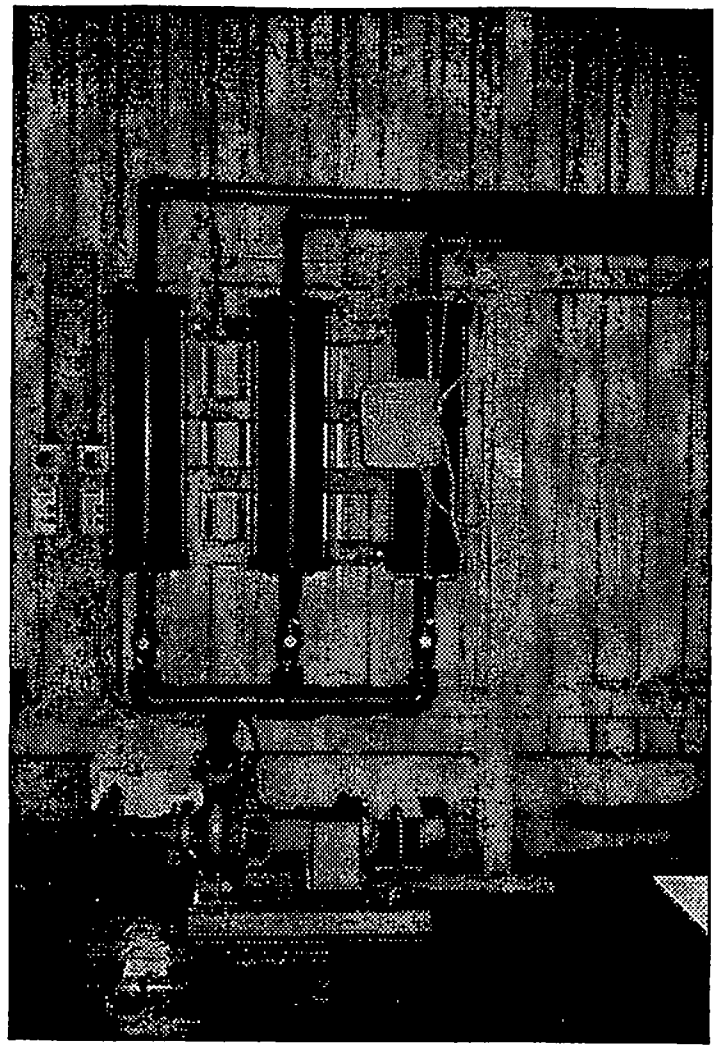


Figure 18 - Pump and UV Sterilizer configuration at Myti Mussels

Figure 19 - Flexible Feed Pipes and Control Valves

The rule of thumb for the existing MAFF requirement for UV sterilization in purification systems is based on one 30 watt tube for each 2,200 litres of seawater in the system. If this system were to be run at full sump capacity of 38,700 litres, 18 tubes would then be required, however the installed UV sterilization capacity with all 24 bins will in fact be 36 tubes, just over double the minimum. With only 12 bins and only 18 tubes installed the UV capacity in relation to the sump is halved but still meets the requirement even with a full sump. In practice the sump is not to be filled completely and it is intended that the water volume used will be 28,000 litres. This apparent doubling of the recommended UV capacity was to enable the required flow rate to be physically passed through the UV units. UV capacity is discussed further in Section 13.3.5.

12.2 Bulk Bin Design

Following the phase 2 trials and prior to the construction of the commercial plant the type of bin used and its modification had to be re-considered. The Allibert bin had steel and wooden inserts as an integral part of its moulding which had to be drilled through in order to fit the outflow pipes. This presented us with the problem of sealing the holes against ingress of water and so it was decided to change from using the Allibert bin to an identically sized GPG DOLAV bin. This bin was made totally from plastic. The GPG bin also had a cambered floor which helped with bin drainage. GPG assisted in the supply of a fabricated plastic, perforated, false floor for the bin which was a good fit to the bin sides and a big improvement on the Allibert floor tiles where two tiles had to be slotted together

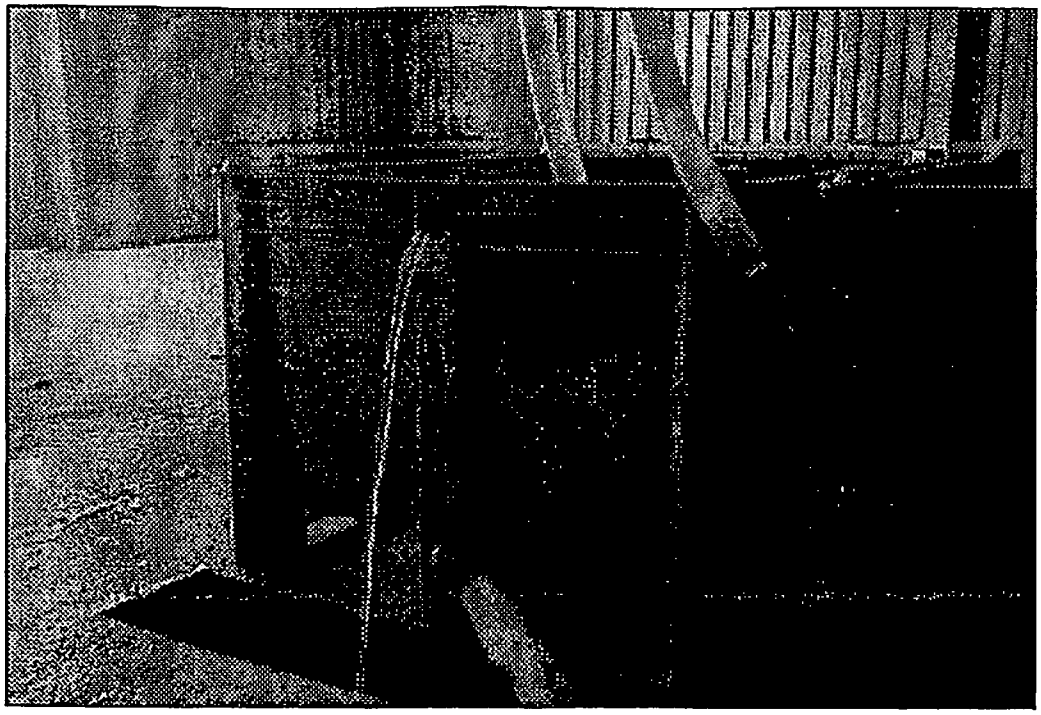


Figure 20 - GPG Bin showing upper and lower outlet pipes

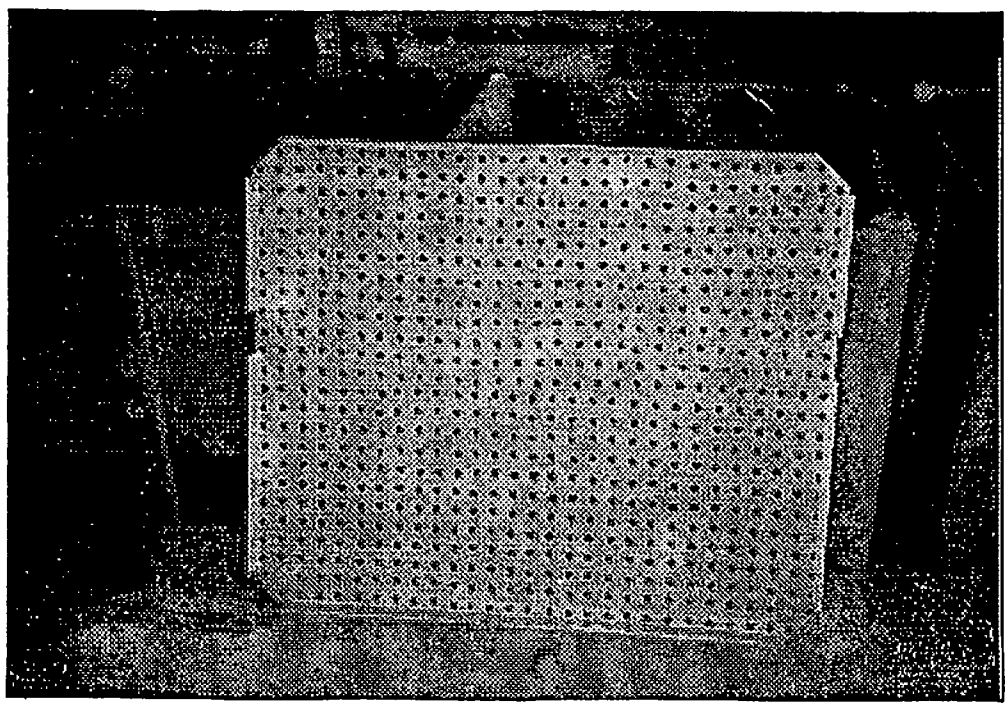


Figure 21- GPG Bins and fabricated false floor

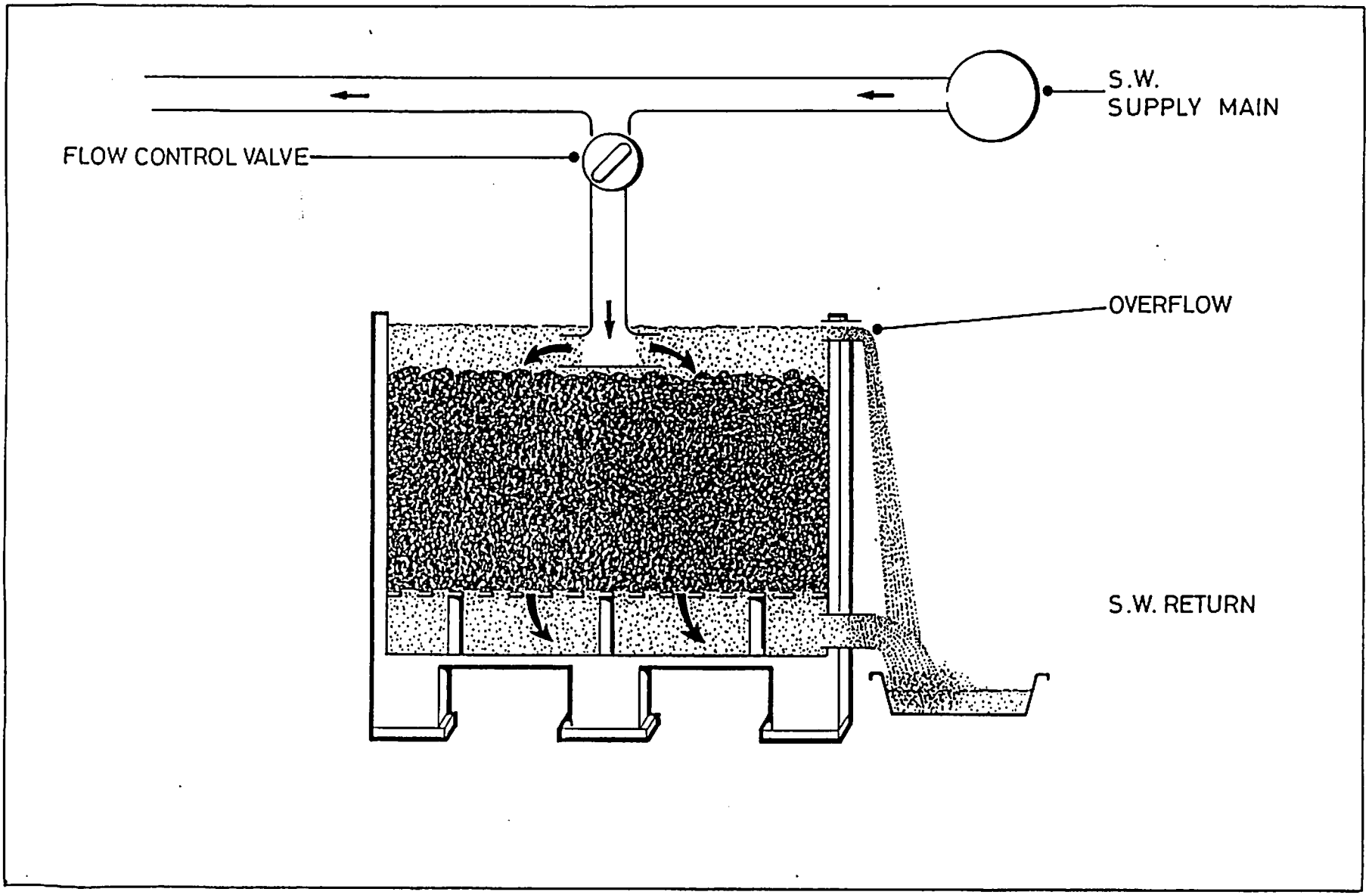


Figure 22 - Phase 3 Bin Design

then cut to fit the shape required. The bins were thus each fitted with a false floor which was 80mm deep and sat on the base of the bins, above the outlet pipe. (Figure Nos. 20 and 21).

It was also considered necessary to alter the bin water outlet pipe design as there had been some operational problems with the outlet pipe configuration used:-

1. The bins required a drainage point additional to the outlet pipe, as the outlet pipe was situated near the top of the bin to maintain the water level. The original drain plug in the bin base was difficult to access.

2. The bins would each need a flow meter to ensure that the correct flow of water was being delivered to each bin, as this is essential for maintaining the DO levels.

3. The outlet pipes passed up through the false floor, and this created a hole through which mussels might fall and end up in detritus on the bin base.

4. If a pump failed or the bins were not drained down promptly (by undoing the difficult to access drain plugs) the mussels would be sat in stagnant water in the bins, which would be detrimental to their subsequent survival.

The solution to these problems was to install an outlet pipe directly through the side of the bin near to the base and to size the outlet to give the required flow rate when the bin was filled with water to the correct level. (Figure No. 22). The upright stand pipe to set the level was discontinued. This solves the problems as :-

1. The bins would drain automatically when the pump was turned off or if the pump fails.

2. There is no requirement for flow meters because if the water flow rate to the bins was not correct the water level would change and this would be apparent. If the water flow was too low the mussels would become uncovered. If the water flow was too high the water would overflow the top of the bins.

3. There is no need to cut holes in the false floor. To provide some flexibility in flow control an overflow pipe was fitted through the side of the bin near the top to direct a small level of overflow into the drainage channel.

This new design required that the outlet pipe hole size was calculated to maintain the required head at the required flow rate. (Ref. 11). Using the flow rate of 6,000 litres minute determined necessary in the phase 2 trials, to ensure a DO level of greater than 50% saturation for 300kg of mussels at 17-18°C, it was calculated that a 32mm diameter hole was required for the given flow rate and head using the following equation.

$$Q = C (Pi\ D2/4\ ) (2gH)^{0.5}$$

Where:
Q = Flow required
C = Non dimentional Coefficient (0.6-0.7)
D = oriface diameter
g = gravitational constant
H = Head

This fortunately was the internal diameter of standard type 'T' 1.25 " BSP pipe. The bins were fitted with a 1.25 " BSP threaded outlet pipe 25mm above the bin base and a 1.25" overflow pipe near the top of the bin.

The bins are drained down after purification simply by turning the supply off and allowing seawater to drain out through the outlet pipe. The phase 2 trials had been conducted using a 50cm long lower outlet pipe which extended to the centre of the bin floor, However, due to the convex shape of the bin floor, it was found that more water could be drained out of the GPG bins if a shorter,12cm, pipe was used instead due to the convex shape of the bin floor. Therefore, as part of the phase 3 trials, experiments were conducted to show that this short outlet pipe did not have a detrimental effect on the water flow through the pallet bin.

12.3 Trials Methods and Results

More than one bin was usually used for each trial. The bins were loaded with molluscs on the quayside directly from the boat and then moved to the building by forklift truck. They were positioned correctly in the building with a hand pallet truck. The water sump tank outside had been filled with a fresh batch of seawater on the previous high tide. The water inlet pipe was positioned in each bin then the pump and UV were turned on. The water filled the bins and only fine tuning of the flow rate was required using the flow control valve on the inlet pipe to each bin. (Figure No. 19). The system was then left running for 42 hours, with routine monitoring occurring 2-3 times per day. The physical and bacteriological data is shown in Table Nos. 1 and 2.

Trial 21

This trial compared the two different outlet pipe lengths. In this trial two pallet bins were filled with mussels. One bin was fitted with a long outlet pipe and the other with the short outlet pipe described in Section 12.2. A large mesh bag was loosely filled with mussels dosed with *E.coli* using the artificial dosing procedure described in Section 9 and placed in the middle of each bin. Dissolved oxygen probes were located in the following places:

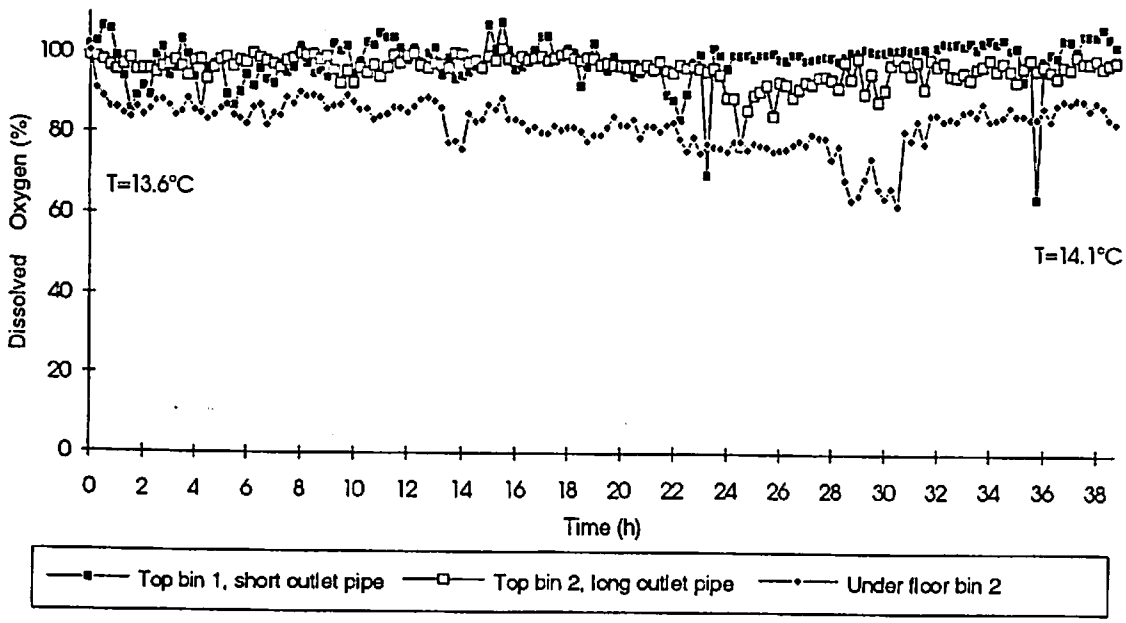
1. On top of bin 1
2. Under the floor on bin 1 (probe failed to work correctly)
3. On top of bin 2
4. Under the floor on bin 2

The DO results are shown in Figure No. 23. Although the DO probe under the floor of bin 1 was faulty, readings were taken at intervals during the trials at the water outlet pipe. (Table No. 1) and showed no significant difference between the bins.

After purification it took 10 minutes for each bin to drain fully.

The bacteriological analysis showed initial counts of 170-500 *E.coli* /100g reducing to <20-40 *E.coli* /100g. (Table No. 2).

Figure 23 Trial 21 Dissolved Oxygen Levels at 22L/Hr/Kg and about 14°C.



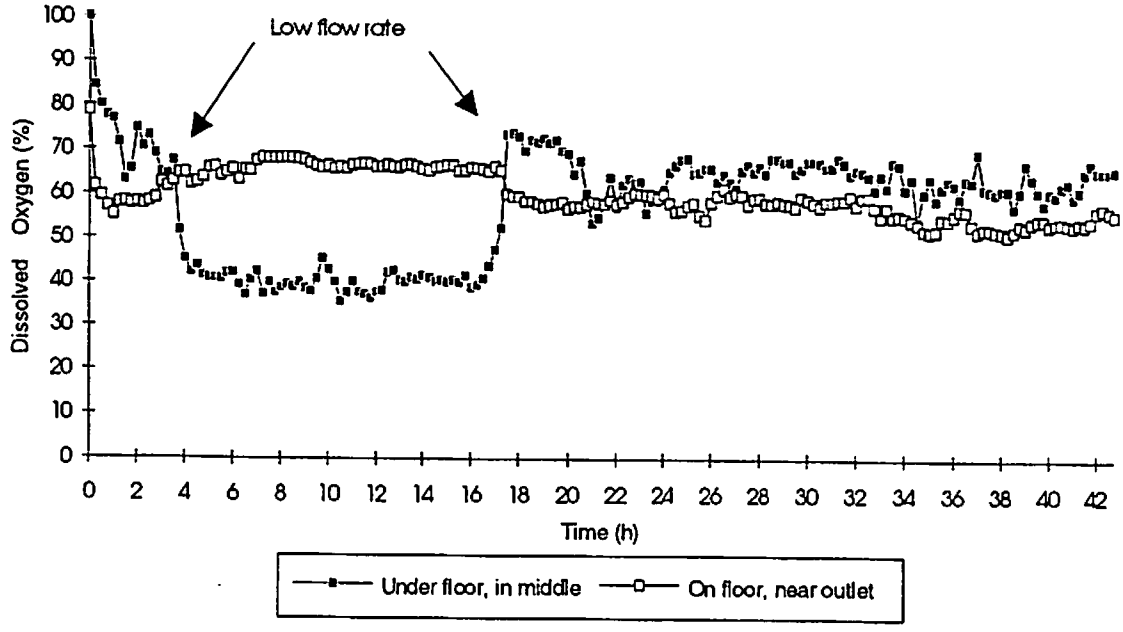
Trial 22

This trial was to investigate more closely DO within the bin when using the short outlet pipe. Only the one bin was filled with mussels. Dissolved oxygen probes were located in the following places:-

1. Under the floor in the middle of the bin
2. On top of the floor away from the outlet pipe (probe failed to work correctly)
3. On top of the floor near outlet pipe
4. In the middle of the mass of mussels (probe failed to work correctly)

The DO results are shown in Figure No. 24. This trial began late in the day and appeared satisfactory when it was left in the evening. However, the following morning the loaded bin was only half full of water due to the flow being reduced. The flow was increased to the loaded bin by turning off the water flow to some of the other bins and the water level recovered. This is reflected in the DO levels. Later that day it was found that the pump suction pipe in the sump had become blocked, by sucking up the basket filter fitted on it's end. This was modified to prevent further blockage by ballasting down the suction pipe filter basket. The bacteriological results were of little significance due to low initial counts. (Table No. 2).

Figure 24 Trial 22 Dissolved Oxygen Results at 22L/Hr/Kg (nominal) and about 14°C.



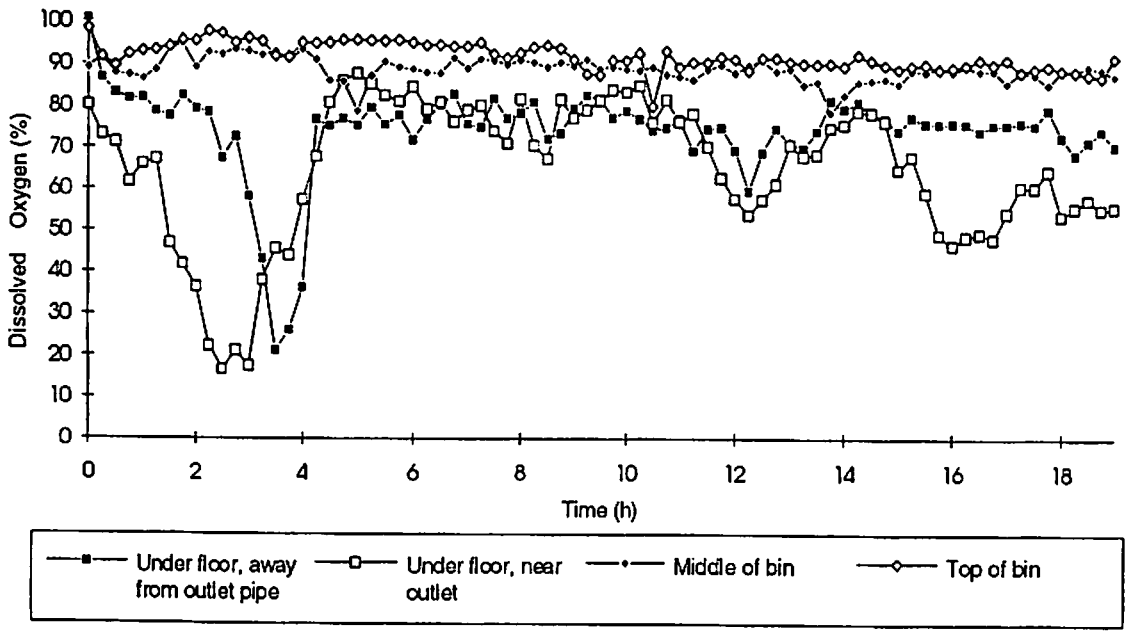
Trial 23

In this trial all 12 bins were filled with mussels and running. In one bin a large mesh bag was loosely filled with artificially dosed mussels and placed on top of the false floor. That bin was set up with a long outlet pipe and dissolved oxygen probes were put in at the following places:-

1. Under the floor away from the outlet pipe
2. Under the floor near the outlet pipe
3. In the middle of the mass of mussels.
4. On top of the mussels

The DO levels are shown in Figure No. 25. The low DO levels at the start of the trial were discovered after completion of the trial when the data was printed out from the data logger. This clearly indicates a reduction in water flow from the lower outlet pipe. The exact reason for this is not known but it is suspected that a plastic disc was left in the bin during construction when the outlet holes were drilled and this for a time, caused a partial blockage to water flow. However, the bacteriological results were significant with initial counts of 800-2400 *E.coli* /100g reducing to non-detectable levels.

Figure25 Trial 23 Dissolved Oxygen Results at 22L/Hr/Kg (Nominal) and about 13°C.



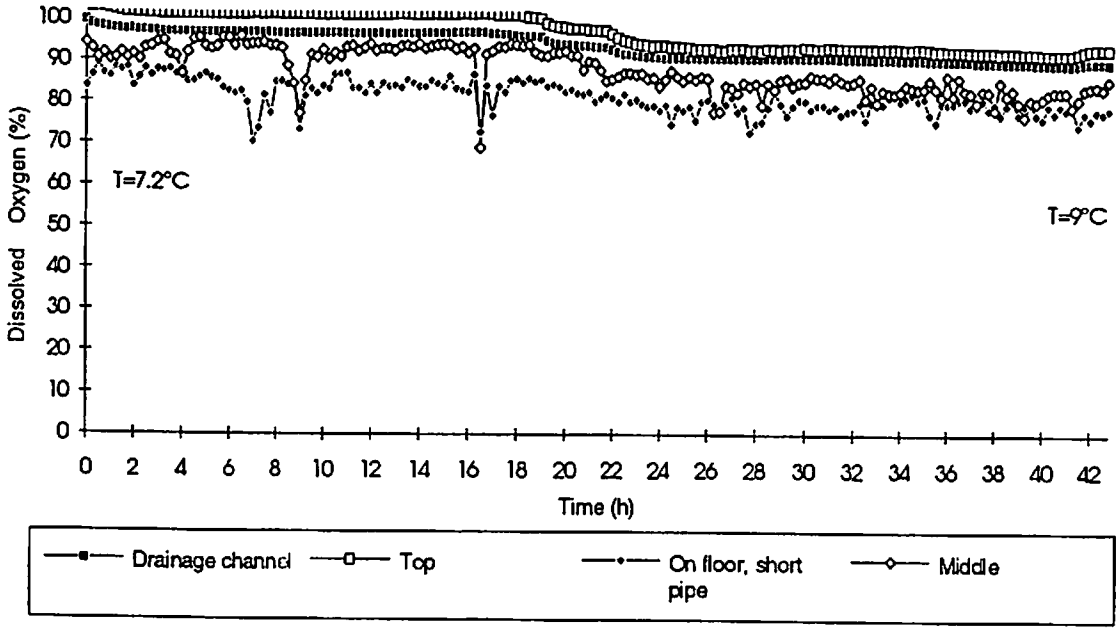
Trial 24

In this trial all 12 bins were filled with mussels and running. Dissolved oxygen probes were put in one bin, set up with a short outlet pipe, at the following places:-

1. On top of the mussels
2. On top of the floor near the outlet pipe
3. In the middle of the mass of mussels

In addition, a further probe was placed in the drainage channel next to the last bin before the water flows into the sump tank. The DO results are shown in Figure No. 26. Levels were high due to the low operating temperature of 7-9°C. The bacteriological results were of little significance due to low initial counts. (Table No. 2).

Figure 26 Trial 24 Dissolved Oxygen Results at 22L/Hr/Kg and about 8°C.



Trial 25

Seafish arrived on site to find that all 12 bins had been filled the previous day by the employees of Myti Mussels, and all looked well except that one bin was low on water and a few were slightly overfilled with mussels, and hence needed increased water flow to maintain a higher head than that set by the overflow pipe. Two bags of artificially dosed mussels were then put into a bin set up with a short outlet pipe. One bag in the middle and one bag on top of the bin. The water level in the sump tank was lowered as it had been filled to the brim and hence was not allowing any additional aeration to occur when water cascaded from the drain channel into the sump tank. The bacteriological results were of little significance due to low input initial counts. (Table No. 2).

Trial 26

Following off site discussions with MAFF there was some concern that faecal material falling through the false floor might be drawn out of the bins by the localised high flow created in the immediate area of the water outlet. It was thought that this could be re-circulated via the sump tank past the UV back into the top of the bins and so a trial was carried out to investigate the discharge of particulate matter. Three bins were modified. Two had modifications to the false floor and one had a perforated suction bar fitted to the outlet pipe on the inside of the bin.

The two modifications to the false floors were:

1. A solid 200 x 200mm plastic sheet above the outlet pipe.
2. A semi circular, perforated, plastic sheet of 150mm radius attached vertically onto the bottom of the false floor in the area of the suction pipe.

Mesh sieves of about 300mm diameter fitted with cloth sheet filters were placed under the outlet pipes of the three modified bins and to one unmodified bin with the short outlet pipe. (Figure No. 27).

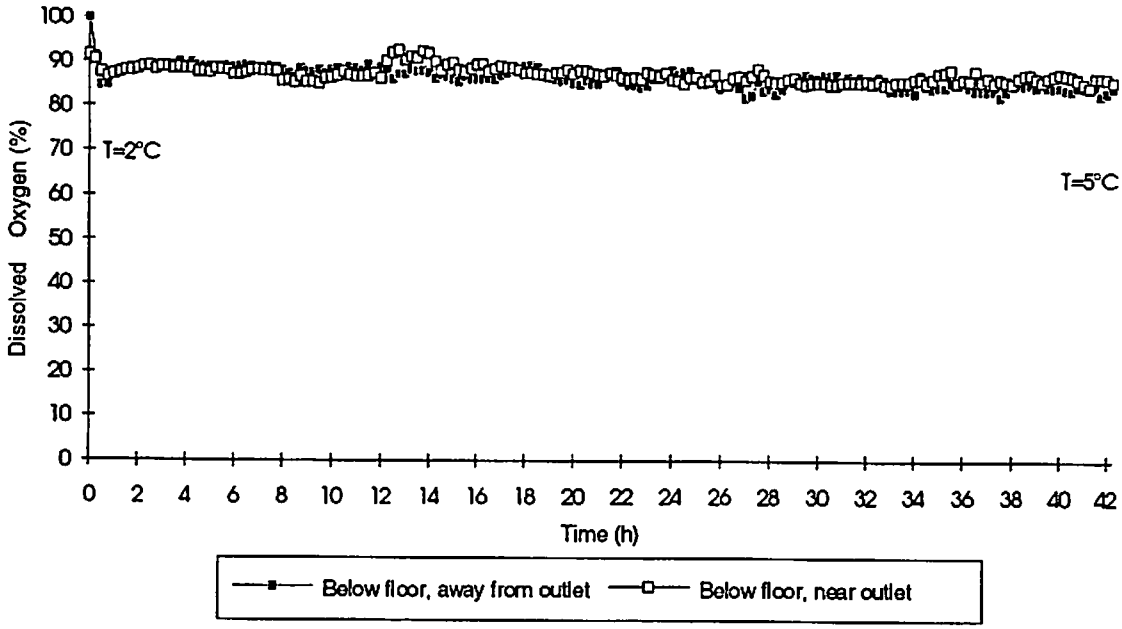
Figure 27 - Outlet Pipes feeding into filters over floor channel

All 12 bins were filled with mussels. The mussels were muddy and deliberately unwashed to exacerbate any particulate discharge and filters were changed after one hour and 27 hours. Two bags of artificially 'dosed' mussels were placed in the unmodified bin, on the top of the false floor and on top of the mussels. DO probes were put in the unmodified bin at the following places:-

1. Below the false floor away from the outlet
2. Below the false floor near the outlet

The DO results are shown in Figure No. 28 and showed no difference in levels near to and away from the water outlet, indicating a uniform flow of water through the mussel mass although at the low temperature (2-5°C) mollusc activity would have been low. The bacteriological results were of little significance due to low initial counts. (Table No. 2).

Figure 28 Trial 26 Dissolved Oxygen Results at 22L/Hr/Kg and about 4°C.



At the end of the trial the bins were emptied and the filters brought back to Hull for analysis. The filters were then dried. On examination of filters taken after one hour of purification, all four showed a thin layer of silt, which is hardly suprising due to the unwashed and muddy nature of the mussels. Filters covering 1-27 hours purification time and then 27-42 hours, however did show quite substantial differences between the bins. The unmodified bin had a number of shell pieces and some small crab and silt although there appeared to be little evidence of faecal strand material present. The bins with modified false floors had less shell (smaller pieces), no crab and less silt and no evidence of faecal material. The bin with the suction bar had only silt and at a reduced level compared to the other bins. Again there was no evidence of faecal material. Unlike the other methods of water removal from the bins the suction bar draws water from the full width of the bin floor and not at a single central point. This results in a reduction in localised high flow conditions and hence of material drawn in.

12.3.1 Operation of the System

For the system to work there must be sufficient water flow to all the bins, with enough back pressure to maintain flow control via the valves. If the water flow to one or more bins is inadequate this is easily observed by simply checking that the bins have water coming out of their overflow pipes. If excessive then the bin could ultimately overflow but this is unlikely and not necessarily disastrous. Once all the control valves have been set it should not be necessary to make any further adjustment.

With the water flow turned off the bins drain down automatically over a period of about ten minutes. Drainage is not total as water continues to run off from the mussels for some hours. Nevertheless this is not a problem as the mussels are held clear of the bin floor by the false floor. When tipped out of the bin some of the silt and mussel feacal material may fall away from the bin base, but is contained by falling on to the underside of the false floor. In any case this is not considered a problem as the mussels are closed at this stage and some material is inevitably held within the mussel mass in any purification system and is removed by subsequent washing.

This system is not really suitable for the small scale operator due to the requirement for mechanical handling equipment and the need for a large separate sump tank.

12.3.2 Bin Design

The modifications made to the standard bins have proved simple. Two holes are drilled to fit the upper and lower outlet pipes and the false floor, fabricated by GPG, sits on the bin base. The external length of the lower outlet pipe, fitted with a 90° elbow to direct the water into the floor channel, proved to be a slight problem as it is prone to being knocked. If the floor channel was wider the outlet pipe would not require a 90° elbow and therefore the pipe length could be shorter.

It was suggested during the trials that a smaller diameter outlet pipe and hence lower flow rate could be used when the water temperature is cooler and hence mussel activity is lower. Technically this is possible but it would require a higher degree of control to prevent the system being used in the lower flow configuration when the temperature rose. If the water temperatures were controlled this would not be a problem.

The suction bar fitted to the bin outlet clearly caused a reduction in the amount of debris being drawn out of the bin but as there was little evidence of faecal material being drawn out with any of the designs used, including the open pipe, we are by no means certain that this is necessary. If there is a settlement area within the sump and the sump inlet and outlet pipes are suitably located to aid this settlement, any particulate matter discharged from the bins will settle out in the sump and will not be recirculated into the bins. Following the site visit by MAFF this was in fact the approach adopted as discussed in Section 12.

However, it is wise to try and keep as much of the contaminant in the bins as possible. Thus the installing of the suction pipe modification onto the bin outlet pipes in any new installations is recommended.

12.3.3 DO Results

The inlet and outlet DO levels taken where the water enters the bin from the down pipe and exits through the outlet pipe are similar locations to those where monitoring is carried out in conventional purification systems. The results, shown in Table No. 1, are very good with the lowest output reading recorded being 74 % saturation at 13.6°C, shortly after the purification trial was started. Localised lower levels were found in the mass of mussels but still maintained at above 50% saturation at the design flow.

No significant differences in DO levels, and hence the water flow pattern, near to or away from the outlet pipe with both the long and short outlet pipe configurations were recorded. (Figure Nos. 25 and 28). A near total oxygen replenishment was found in the drainage channel, due to the water cascading out of the bin outlets, before the water exits into the sump tank. (Figure No. 26).

12.3.4 Bacteriological Results

The bacteriological data is limited as high initial counts were obtained only in trial 23 where counts of *E. coli* were reduced to <20/100g after purification. However, account must be taken of the bacteriological results of the pilot scale trials where some very high initial counts of *E. coli* from naturally contaminated mussels were reduced to permissable levels. On the basis of this data, approval of the design was given subsequently by MAFF together with the operating criteria for the bins.

12.3.5 UV Sterilization

One draw back of this system as it stands is that the high water flow rates, required to maintain adequate DO levels to the mussels, necessitate a large amount of UV sterilization capacity under the current MAFF requirements. These requirements are that all the water must pass through the UV sterilizer on every pass with a dose of not less than 10 $mJ/cm^{-2}/s^{-1}$, with a minimum water circulation rate of once per hour, which generally equates to the rule of thumb of one 30 Watt UV sterilizer per 2.2 m^{-3} of water in the system. By circulating the water five times per hour to maintain DO levels and having to pass all the water through the sterilisers results in a sterilising capacity five times greater than in some traditional purification systems. We consider that if the UV sterilizers were operated on a by-pass flow system where 1/5 of the water passed through the sterilizers on each pass, ie an equivalent flow rate of one change per hour, coupled with an increase in the UV dose per pass to drinking water levels of 25-30 $mJ/cm^{-2}/s^{-1}$, an adequate cumulative kill rate would be achieved within the mass of the water with less UV capacity. This theory is to be investigated by Seafish.

12.3.6 Costs

For a company wishing to operate on a medium to large scale this system should accrue cost benefits. The construction costs are reasonable assuming that the construction of a low technology sump tank and floor channel is readily achieved. The bins, pipework and pump are all relatively inexpensive. However, the cost of the UV sterilizers can be a major cost burden at £2000 per three to four bins. The power consumption of the pump and UV sterilizers work out very similar to the Seafish designed medium-scale tank at 1 Watt per hour per Kg of mussels, which equates to 42 kW/Hrs per tonne of mussels per purification cycle. With mechanical handling few staff are required to operate a large purification system. A forklift and mechanical tipping devices are required.

12.4 Outline Recommendations for the Operation of a Pallet Bin Mussel Purification System

1. Wash the bins and false floors prior to filling the bins. Ensure all mussels from previous use are removed.

2. Wash the mussels prior to putting into the bins.

3. Only fill the bins to a maximum depth of 38 cm mussels, approximately 300 kg.

4. Make sure the UV sterilizer is switched on and working.

5. Turn on the pump and make sure that all the bins fill with water to the correct level and with the correct flow rate i.e. a small flow coming out of the overflow pipes. With this system the bin design acts as a flowmeter. If the flow rate to any bin is too low or too high adjust its flow control valve to suit.

6. Check the water temperature and salinity and record the information along with the number of bins full of mussels and their source, and the start time of the purification.

7. Leave for 42 hours to purify, checking periodically that all bins are maintaining the correct flow.

8. After the purification cycle turn off the pump and UV sterilizers. The bins will automatically drain down into the sump tank.

9. Wait until the bins have stopped draining down before moving them.

10. Remove the bins and put the mussels through the post purification washing and grading processes. Mussels should be tipped out and not shovelled out as this can cause unnecessary damage.

12.5 Site Visit by MAFF and Subsequent Approval

A site visit was made by MAFF officials from Fish Diseases Laboratory, Weymouth and the local Environmental Health Officer (EHO) on December 15th 1993. For the purpose of the visit a repeat of the filter trial was carried out. However, on this occasion the differences between the different configurations were not as apparent. It was decided that the best approach was to ensure settlement in the sump and not to modify the bins. It was decided consequently to change the water flow through the sump from surface entry to entry at 2/3 depth and shortening the suction pipe to draw water off from the top 1/3 of the sump, thereby assisting material to settle onto the sump floor. It was required also to cover the sump (which already was the intention of Myti Mussels). A further bacteriological test was carried out by the local EHO with mussels from a 'dirty' area, but again the results were not significant due to low initial counts. MAFF decided however that data from earlier trials was sufficient evidence to demonstrate that the bin system works. MAFF subsequently issued the conditions of approval to the EHO, subject to the sump modifications being carried out. (Ref. 12).

13. Conclusions and Recommendations

1. The basic physical parameters for deep layer mussel purification have been defined by this work and a practical working system has been developed.

2. A downwelling water flow is suitable for purification.

3. An upwelling water flow is not considered suitable for purification.

4. Horizontal water flow tanks have not proved capable of simple adaptation for deep layers of mussels.

5. The trials results have shown, by reduction in *E. coli* levels, the ability to purify mussels in a 38cm deep layer.

6. The trials results have shown that DO levels above 50% saturation are maintained at the correct water flow to mass ratios.

7. A water flow of 22L/hr/kg of mussels is suitable to maintain good filtration activity and DO levels, up to a temperature of 17-18°C.

8. A standard size pallet bin can be easily modified for use as a purification container.

9. The outlet pipe design used in the commercial scale trials has proved practicable and allows for a simple system operation.

10. A 32mm diameter outlet pipe must be used in the bin system developed to ensure the correct water flow rates, if water temperatures are variable.

11. If water temperatures are constant and lower than 12°C then a smaller diameter outlet pipe and hence flow could be used.

12. For a multi-bin system to work there must be sufficient water flow to all the bins, with enough back pressure in the supply main to maintain flow control.

13. A depth of 38cm is considered the maximum depth of mussels practical in a standard pallet bin. This generally equates to about 300kg of mussels per bin.

14. The design of the sump must permit the selling out of detritus, however the fitting of a suction pipe to the bins lower outlet pipe, as discussed in Section 12.3.2 is recommended to minimise the outflow of detritus from the bins.

15. The system has considerable practical operating advantages for large-scale usage with forklift handling and mechanical tipping devices, but is not suited to small-scale operations.

16. We do not consider that this work would allow the depth of mussels in a container in a standard horizontal flow system to be increased from that already allowed, or that other species of molluscs could be purified in deep layers.

17. Further work to investigate the effectiveness of different UV doses and UV configurations in reducing bacteriological indicators is required.

Tables

Table No. 1 Physiological Parameters Data

Trial No.	Fresh Batch of Mussels	Depth of Mussels in Container	Mass	Water Type N or ASW	New Seawater Batch Y/N	Flow Method DW, UW, TF	Water Flow Rate	Water Flow /Mass Ratio	Water Temperature	Oxygen Level Inlet Side of System	Oxygen Level Middle of Mussel Mass	Oxygen Level Outlet Side of System	Immersion Time for Mussels during Trial	Control Tank Present	Comments
1	Y	25	14	ASW	Y	DW	7.5	32	10	70		60	48	Y	
2	N	25	14	ASW	N	DW	7.5	32	10	70		60	96	Y	
3	Y	25	40	ASW	Y	DW	4.0-7.0	8-10.5	11	77-80		66-55	24	Y	
4	Y	120	85	ASW	Y	DW	30	18	14	75		48	20	Y	
5	Y	120	100	ASW	Y	DW	30	16	14	89		70	40	N	
6	N	60	50	ASW	N	DW	30	36	14	81		74	24	N	
7	Y	120	80	ASW	N	UW	30	20	14				19	N	No activity
8	N	120	90	ASW	Y	UW	30	20	14	80		55	27	N	Water changed mussels filtering
9	Y	50	400	ASW	Y	TF	150	23					48	Y	No flow in middle of mass
10	Y	25	150	Natural	Y	DW	80	32	14.6 - 15.4	93-86		75-72	45	Y	
11	Y	38	300	Natural	Y	DW	80	16	14.4 - 15.2	96-87	80-47	62-78	42	Y	
12	Y	38	300	Natural	Y	DW	80	16	15 - 17.7	87-74	63-53	90-81	42	Y	
13a	Y	38	300	Natural	Y	DW	100	20	16				45	Y	See figure 14
13b		38	300	Natural		DW	48	9.6	16				42	Y	See figure 15
14	Y	42	350	Natural	Y	DW	105	18	14.4 - 15.6				42	Y	See figure 16
15	Y	38	300	ASW	Y	DW	108	22	12 - 18.8				44	Y	
16	Y	38	300	ASW	Y	DW	108	22	10.7 - 16.5				48	Y	
17	Y	38	300	ASW	Y	DW	108	22	11.0 - 19				62	Y	
18	Y	38	300	ASW	Y	DW	108	22	9				48	Y	
19	Y	38	300	ASW	N	DW	108	22	9.0 - 13				42	Y	
20	Y	38	300	ASW	Y	DW	108	22	8.0 - 10.5				42	N	See figure 20 Short outlet pipe
21a	Y	38	300	Natural	Y	DW	108	22	13.8 - 14.1	98-95		80-74	42		See figure 20 Long outlet pipe
21b		38	300	Natural		DW	108	22	13.6 - 14.1	98-95		82-76		N	See figure 21
22	Y	38	300	Natural	Y	DW	108	22	14	93		78	42	N	See figure 22
23	Y	38	300	Natural	Y	DW	108	22	13	90		78	42	N	See figure 23 Short outlet pipe
24	Y	38	300	Natural	Y	DW	108	22	7.2 - 9	99		88-91	42	N	Short outlet pipe
25	Y	38	300	Natural	Y	DW	108	22	8.3 - 9.4	89 - 87		87-81	42	N	See figure 25 Short outlet pipe
26	Y	38	300	Natural	Y	DW	108	22	2.0 - 5.0	95		92-90	42	N	

Key:
ASW - Artificial Seawater, DW - Downwelling, UW - Upwelling, TF - Through flow

Table No. 2 Bacteriological Analysis Results

Trial No	Pre Purification Count of E. coli /100g	Source of Contamination	Post Purification Count of E. coli /100g	Post Purification Sample Location
5	400	Natural	0	Top
	800	Natural	0	Middle
			0	Bottom
8	200	Natural	400	Top
	0	Natural	0	Middle
	0	Natural	0	Bottom
10	<20	Natural	<20	Control
	<20	Natural	<20	Top
	<20	Natural	<20	3" Deep
			<20	6" Deep
			<20	9" Deep
11	500	Natural	<20	Control
	220	Natural	<20	Top
	230	Natural	<20	6" deep
			20	12"deep
12	400	Natural	2300	Control
	2300	Natural	200	Top
			<200	Middle
			<200	Bottom
13	2300	Natural	<20	Control
	2200	Natural	<20	Top
	1400	Natural	<20	Middle
			<20	Middle
			<20	Bottom
			<20	Bottom
14	800	Natural	<20	Control
	2300	Natural	<20	Top near water inlet
	3000	Natural	<20	Top away from water inlet
			<20	Middle middle
			<20	Middle edges
			<20	Bottom
15	<20	Natural	<20	Control
	<20	Natural	<20	Top
	<20	Artificially Contaminated	<20	Middle
	<20	Artificially Contaminated	<20	Bottom
	<20	Artificially Contaminated	<20	Top
	<20	Artificially Contaminated	<20	Middle
			<20	Bottom
16	<200	Natural	<200	Top
	<200	Artificially Contaminated	<200	Middle
	<200	Artificially Contaminated	<200	Bottom
	<200	Artificially Contaminated	<200	Control
	<200	Artificially Contaminated		
17	700	Artificially Contaminated	200	Top
	700	Artificially Contaminated	200	Bottom
	700	Artificially Contaminated	200	Below Floor
	80	Artificially Contaminated	200	Control top
			<200	Control bottom

18	400	Natural	<20	Top
	200	Natural	<20	Above floor
	400	Natural	<20	Under floor
			<20	Under floor
			<20	Control
19	1100	Natural	130	Top
	1100	Natural	40	Above floor
	1300	Natural	130	Below floor
			40	Below floor
			950	Control
			700	Control
20	>18000	Natural	220	Top
	>18000	Natural	130	Above floor
	>18000	Natural	200	Above floor
			1100	Below floor
			70	Control
			250	Control
21	340	Artificially Contaminated	40	Middle bin 1
	170	Artificially Contaminated	20	Middle bin 1
	500	Artificially Contaminated	20	Middle bin 2
			<20	Middle bin 2
22	40	Natural	80	Top
	<20	Natural	<20	Bottom
	80	Natural		
23	1300	Artificially Contaminated	<20	On false floor
	2400	Artificially Contaminated	<20	On false floor
	800	Artificially Contaminated	<20	On false floor
24	<20	Natural	<20	Top
	20	Natural	<20	Middle
	110	Natural	<20	Bottom
			<20	Bottom
25	170	Artificially Contaminated	<20	Top
	<20	Artificially Contaminated	<20	Top
	40	Artificially Contaminated	<20	Middle
			40	Middle
26	300	Artificially Contaminated	40	Top
	90	Artificially Contaminated	<20	Top
			70	Bottom
			20	Bottom

14. References

1. **Wilson P.**, Commercial Trial of a Multi-Layer Mussel Purification Tank with Re-Use of Artificial Seawater. 1989. Seafish Report No. 355.

2. **Wilson P., Boulter M.**, Design and Commission of a Mussel Purification Plant at Kings Lynn. 1991. Seafish Report No. 380.

3. **Boulter M.**, Mussel Purification. Initial Trial of a Medium Scale Deep Stack Purification Tank. 1989. Internal Report No. 1413.

4. **Boulter M.**, Mussel Purification. Further Development of a Medium Scale Deep Stack Purification Tank. 1990. Seafish Report No. 387.

5. **Lart W.J., Wood P.C., Wilson P., Denton J.W.**, Biologoical Criteria for the Depuration of the Pacific Oyster (Crastostrea gigas) and the Design of a Prototype Small-Scale Depuration Plant. 1994. Seafish Report No. 433.

6. **Boulter M.**, Repeated Re-Use of Artificial Seawater in a Small-Scale Vertical Stack Oyster Purification Plant under Development by Seafish. 1991. Seafish Report No.

7. **Boulter M.**, M.L. Supplies. Mollusc Purification Plant. Small Scale Purification System. 1992. Seafish Consultancy Report No. 57.

8. **Wood P.C., Ayres P.A.**, Artificial Seawater for Shellfish Tanks. MAFF Laboratory Leaflet No. 39. Lowestoft.

9. **MAFF, DoH, PHLS Working Group**, Bacteriological Examination of Shellfish. PHLS Microbiology Digest 1992; 9(2); 76-82.

10. **Wilson P., Lees D., Doré W.**, Artificial Dosing of Bivalve Molluscs with *E. coli* for the Testing of Purification Tanks. 1992. Seafish Report No. 413.

11. **Huguenin J. E., Colt J.**, Design and Operating Guide for Aquaculture Systems. Developments in Aquaculture and Fisheries Science, 20. 1989. Elsevier.

12. **Boulter M.**, Process Verification Trials of Deep Stacking Purification Tanks at Myti Mussels. 1994. Seafish Consultancy Report No. 78.

Appendix 1

App. I

Procedure for Artificial Contamination of Bivalve Molluscs with *Escherichia coli* for Testing Commercial Depuration Plants

Instructions for Dosing Shellfish with *E. coli* in the Seafish Designed 2 Tray Control Tank

The following general principles should be observed:

Shellfish:
Shellfish should be as fresh as possible and should not have been recently subject to repeated depuration cycles or other forms of stress.

Temperature:
Water temperature should be maintained at 1-2°C above the original ambient temperature of the shellfish waters but should not be less than 10°C.

E. Coli:
E. coli is not a human pathogen but it is a wise precaution to wash hands after dosing the model depuration plant.

Loading Density:
Both trays of the model depuration plant should be loaded with a 3" layer of mussels (15kg per tray).

1. Depurate shellfish to be dosed for 20-24 hours in control tank (CT) with plant in normal working mode and UV on.

2. Remove trays from CT and turn UV off.

3. Score glass *E. coli* vial with diamond scratcher in a complete circle in the area of the cotton plug. Hold vial inside paper hand towel and snap. Fill supplied plastic tube with 5ml of seawater (from CT). Transfer about 0.5ml to vial (using supplied plastic pipette), dissolve white pellet and transfer back to plastic tube. Repeat several times, and pipette the tube contents up and down, to ensure pellet is fully dissolved and well dispersed. Add an accurate volume of this to the CT (volume specified with each batch of freeze dried *(E. coli)* and stir well with stick.

4. Run CT for 1 hour with UV off to ensure complete mixing of *E. coli.*

5. Replace trays and leave shellfish to dose for 4 hours.

6. Remove trays and gently hose down external surfaces of shellfish.

7. After trays have been removed, CT should be run overnight with UV **on** to kill remaining *E. coli.*

8. Take triplicate samples of time zero dosed shellfish (sample size for mussels 15 individuals). Send to laboratory on ice, or with cool blocks for analysis.

9. Transfer rest of dosed shellfish to loosely tied large mesh sacks. Load commercial tank to full capacity with undepurated shellfish with the dosed shellfish in sacks at top, middle and bottom of the bin. Start normal depuration run.

10. Take samples after 42 hours. Send samples to laboratory as soon as they are taken. The laboratory must open and homogenise all shellfish from each sample to ensure a proper test.

11. Any remaining dosed shellfish at end of run should be removed from site and appropriately disposed of. Dosed shellfish **must not** go for human consumption.

12. Laboratory results should show time zero samples to be in the range 2000-5000 MPN *E. coli* per 100g of shellfish flesh and fluid. Duplicate samples should be fairly close for good confidence in test.