Mussel Purification, Development of a Medium-Scale Deep Stack Purification Tank

MAFF Commission

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SEA FISH INDUSTRY AUTHORITY
Seafish Technology

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Seafish Report No. 387
Project Code No. FT4
MAFF Commission NBC16

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SUMMARY

Following on from the development of a large-scale deep stacking purification plant at Heiploeg and Lynn, King's Lynn, Seafish have been engaged in studies of purification plant for mussels as part of a programme to improve standards and thereby increase the value of this important resource. The tank is aimed at the small scale purification or co-operative.

The tank which was already under development by M. Butler and D. Coulson, at Boston was designed to hold 750kg of mussels in each cycle. This tank was not only smaller than the large-scale tank but it also had differences in design, water/mussel ratio and water circulation rates. This necessitated further investigations to ensure that it would meet the necessary purification standards.

Initial trials of the tank were carried out by Seafish in 1989, and are described in Internal Report No. 1413. It was concluded that the tank showed promise but that its operating conditions required to be limited.

This report describes a second, more extensive series of trials of a developed version of the tank which were carried out by Seafish over a three month period in 1989 at the premises of M. Butler. The tank employs high density stacking, a low water mussel ratio and a high water flow rate.

Six purification cycles were made with the tank using artificial seawater to which approximately 4% make-up was added after each use. The trials experimented with high water flow rates and differing stacking densities. Each trial was carefully monitored. For water temperature, pH, dissolved oxygen, salinity, ammonia and nitrite build up. The bacterial quality of the mussels was assessed before and after purification.

It is concluded that the tank operates satisfactorily provided that certain operating criteria are met. A maximum tank loading, minimum water flow, limited operating temperature range and limited water re-use are recommended, in the report. The water exchange rate required is far higher than that previously recommended by the MAFF operating procedures for mussel purification tanks.

The tank has now been granted an operating license by the Department of Health.

The work has been carried out under MAFF Commission NBC16. Seafish would like to acknowledge the help given by the following during the trials :-

M. Butler and D. Coulson, Boston
Environmental Health Department, Boston
Public Health Laboratory, Lincoln
J. Williamson, J J Shellfish, King's Lynn
Eastern Sea Fisheries Joint Committee

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1. INTRODUCTION

The existing criteria upon which existing large-scale mussel purification is based requires mussels to be spread in large shallow tanks with access to a supply of clean seawater. This results in a purification plant that requires a lot of land, is labour intensive and generally remote from the source of mussel supply. To make purification more cost effective, Seafish are developing multi-layer systems with partial re-use of artificial seawater. These plants have a reduced land and labour requirement enabling them to be housed in a building, thereby offering much improved control of environmental and hygiene conditions. It also enables the plants to be sited near to the source of mussel supply, thus reducing transport costs. At an early stage Seafish carried out successful mussel purification trials demonstrating the principle of both multi-layer stacking (Ref. 1 & 2) and the re-use of artificial seawater (Ref. 3 & 4).

Seafish is now developing a series of standard plant designs to cater for the varying needs of industry.

Trials on the large-scale high density mussel purification tank at Kings Lynn successfully demonstrated the application of these principles of multi-layer stacking of mussels and partial re-use of artificial seawater in a commercial plant. The capacity of the tank at 1500kg was considered, however, to be too large for some operators. A scaled down version of the Kings Lynn tank was considered but a smaller multi-layer tank of 750kg nominal capacity, already under development at Boston, was worthy of further investigation. Following the limited success of initial trials in March 1989 of this medium-scale deep stacking purification tank (Ref. 13) which had been developed over three years by M. Butler and D. Coulson. Seafish and M. Butler have developed an improved version of this medium-scale deep stacked purificiation tank. The most significant of the improvements was a substantial increase in the pump capacity and hence flow rates.

The trials of this tank incorporated three changes to the standard MAFF purification criteria: multi-layer stacking up to five boxes deep, a maximum water flow based on up to five tank changes per hour, and boxes filled up to 150mm deep. The new tank differs from the large-scale tanks at Kings Lynn as it relies on a more rapid water circulation and a lower water/mussel ratio. However, the effective water flow rate is not controlled by the number of water changes alone but relates also to the tank length, and the Boston tank is relatively short. A series of monitored purification trials have been carried out by Seafish with this more developed tank to assess its effectiveness.

A small single layer Control Tank used in previous trials and operating to existing MAFF criteria was run alongside the experimental tank throughout the trials to obtain comparative data.

2. OBJECTIVES

To develop and prove a standard design of medium-scale deep stacked mussel purification tank, and in particular:-

2.1 To investigate the effect on purification of increased water flow and reduced ratio of water to mussels.

2.2 To investigate the effectiveness of purification with increased mussel density in the boxes.

3. OUTLINE OF TRIALS SEQUENCE

The sequence consisted of six consecutive purification trials in the deep stack tank over a period of 10 weeks in December 1989 - February 1990, re-using the artificial seawater. Comprehensive bacteriological, physical and chemical monitoring was carried out.

The first trial was restricted to initial investigation of various water flow rates and subsequent levels of dissolved oxygen, and no bacteriological analysis was made.

In the second trial the flow rate was set to its maximum and the quantity of mussels in each of the boxes was increased significantly to increase the total loading of the tank. In the third trial the high level of loading remained and the flow rate was decreased but had to be returned to its maximum level during the trial when dissolved oxygen levels dropped as tank temperature rose. Thereafter the flow rate was left at the maximum.

For the fourth trial the number of boxes in the tank was reduced but their individual loading was further increased to investigate the effects of a deeper layer of mussels. High water temperatures were becoming a problem and an alternative water input system was experimented with.

Up to this point the water had been re-used with a small percentage make up after each trial. However, in trial 4 there appeared to be problems caused by deteriorating water quality and so the old water was discarded and a fresh batch of artificial seawater was made up for trial 5 and re-used for trial 6.

In trial 5 the number of boxes was returned to the original number and the loading per box was increased slightly from that in trials 2 and 3. In an attempt to overcome the problem of low *E. coli* counts in the mussels prior to purification a method of artificial contamination was investigated.

In trial 6 the tank loading was returned to that of trials 2 and 3 but in view of the problems in trial 4 the loading of the control tank was increased to further investigate depuration in a deeper layer of mussels.

Following the trials the Department of Health granted an operating license for the tank conditional on certain operation criteria.

4. TRIALS SITE

The trials were conducted within the premises of M. Butler at the Riverside Industrial Estate, Boston.

5. TRIALS EQUIPMENT

5.1 Purification Boxes

50 Allibert type 11037 stack nest boxes of 37 litres capacity and external dimensions of 600mm x 400mm x 236mm were used. This is a significantly deeper box than those usually used for purification but forms part of an integrated handling system from fishing ground through to purification.

5.2. High Density Purification Tank

The tank is of wooden construction with an external wooden frame and lined with G.R.P. It has internal dimensions of 2.44m x 1.2m x 1.2m. It was painted with a 2 pot epoxy paint. General views of the tank are shown in Figure 1.

Pipework and fittings are in 1.5 inch ABS and UPVC plastics. P.V.C. sheet is used inside the tank for flow control screens.

Diagramatic views of the tank and water flow system are shown in Figure 2. The tank has its own circulation pump and U.V. sterilizer (2 x 30 watt tubes). Flow rate can be monitored by a flowmeter and can be adjusted by means of a valve. Water is delivered across the width of the tank at one end via a spray bar mounted above the water surface (see Figure 3). Two types of flow control screen have been used at the input end of the tank. One is a solid screen with a 150mm gap underneath. The other is a perforated screen with 140-10mm holes in rows of 10 holes. The water suction consists of a pair of ABS tee's joined in the centre of the tank (see figure 4). The tank is filled and emptied through two, 3 - way valves into a reservoir tank.

The tank is designed to hold 50 boxes stacked 5 deep and to be loaded and unloaded manually. At the maximum 75mm depth of mussels that MAFF has required for purification each box holds approximately 11.5kg of mussels and in this condition the capacity of the tank is 575kg. However, the tank is designed with high water flow rates in the hope of achieving a 100mm depth

of mussels to give a nominal 15kg per box and capacity of 750kg. At this nominal capacity there is approximately a 100mm gap above each layer of mussels which permits water circulation and prevents embyssment of the top layer of mussels on the box above.

At the nominal capacity the tank holds approximately 2.6m^3 of water and this corresponds to a water/mussel ratio of 3.5 litres/kg which compares with 6.1 litres/kg for the large-scale tanks at King's Lynn and 6.6 litres/kg for the control tank. The maximum output of the pump is 13.5m^3/hr which corresponds to an exchange rate of 5.1 times per hour at nominal capacity which compares to the MAFF previously recommended range of 1-2 times per hour and one change per hour in both the large-scale and control tanks. At maximum pump output and nominal capacity the water flow rate through the tank is approximately 12.5m/hr which compares with 6m/hr for the large-scale tank and 0.180m/hr for the control tank.

The bottom boxes sit on ribs moulded on the base of the tank which raise the boxes approximately 50mm from the base. The base slopes toward a drain for flushing out. When the purification water is pumped to the reservoir approximately 4% remains in the bottom purification tank with the detritus (mud, shell, faeces, etc) and is then washed away to prevent contamination of the next purification cycle. This 4% of lost water then has to be made up with new artificial seawater for the next purification cycle.

Provision was made in the design of the tank for a water chiller unit but it was not used during these trials as it was desired to investigate any water temperature problems without resorting to refrigeration.

5.3. Artificial Seawater Reservoir Tank

A G.R.P. lined tank of similar construction to the purification tank is used both as a reservoir and for the mixing of artificial seawater.

5.4. Control Mussel Purification Tank

Seafish installed on site a small Control Tank (Ref. 3 and 4), capable of holding a single tray of mussels (see Figure 5). The tank has its own U.V. sterilizer, pump, control and aeration and is designed to operate within the existing MAFF criteria for mussel purification.

5.5. Environmental Chamber used for Storage Trials

Manufactured by Cee-Tel Thermal Equipment Limited, the test chamber installed at IDU premises, Hull, has a 1000mm x 1000mm x 1000mm chamber and any required temperature profile between -30°C to 100°C can be maintained. The chamber was used for holding live mussels at 15°C. The accelerated spoilage so induced can be related to storage at lower temperatures and other comparable trials data.

6. INSTRUMENTATION

6.1. Temperature

Comark 9001 digital thermometer with probe.

6.2. pH

Jenway portable PH meter with temperature compensation.

6.3. Oxygen

Oxyguard Handy portable oxygen meter with temperature compensation.

6.4. Salinity

Dryden portable salinity meter.

6.5. Ammonia

Merck Aquaquant colourmetric test kit. Ammonia measured in the range 0.2 to 8.0 mg/l but can be extended to 80 mg/l.

6.6. Nitrite

Merck Aquamerck colourmaster test kit. Nitrite measured in the range 0.02 to 20 mg/l.

7. ARTIFICIAL SEAWATER

The artificial seawater (ASW) was made up using the five basic salts, as defined by MAFF Laboratory Leaflet No. 39, (Reference 5). Details are given in Appendix 1.

The salt content or salinity of seawater is usually expressed as number of parts by weight of salt in one thousand parts of weight of water. The unit 'parts per thousand' is indicated by the symbol °/oo. Salinities of 22°/oo, 27°/oo and 30°/oo are specified, all using five basic salts. The salinity of 27°/oo was chosen for re-use even though a lower salinity of 22°/oo is satisfactory. This was to allow a working margin for error (in a commercial environment) and avoid the danger that if salinity fell too low the mussels would not purify.

8. MUSSEL SUPPLY

D. Coulson supplied the mussels for trials 1,2,3,5 and 6.
An alternative source at Kings Lynn supplied the mussels for the fourth trial. All came from The Wash.

9. BACTERIOLOGICAL ANALYSIS

Several quantitative methods exist in the United Kingdom for the examination of bivalve molluscs for sewage contamination. These include roll tubes, pour plates and most probable number (MPN) techniques but there is apparently no national standard method. For the purposes of this trial an MPN method specified by P.A. West and M. R. Coleman was used (see Ref. 6). The Lincoln Public Health Laboratory were contracted to take samples throughout the trials. At the start of each trial three or four mussel samples were taken and at the end a further five with a water sample as well. Mussel and water samples were examined for Escherichia coli (E. coli) and Group D faecal streptococci. Faecal streptococci were to be used as an indication that purification had occurred in the absence of sufficient numbers of E. coil.

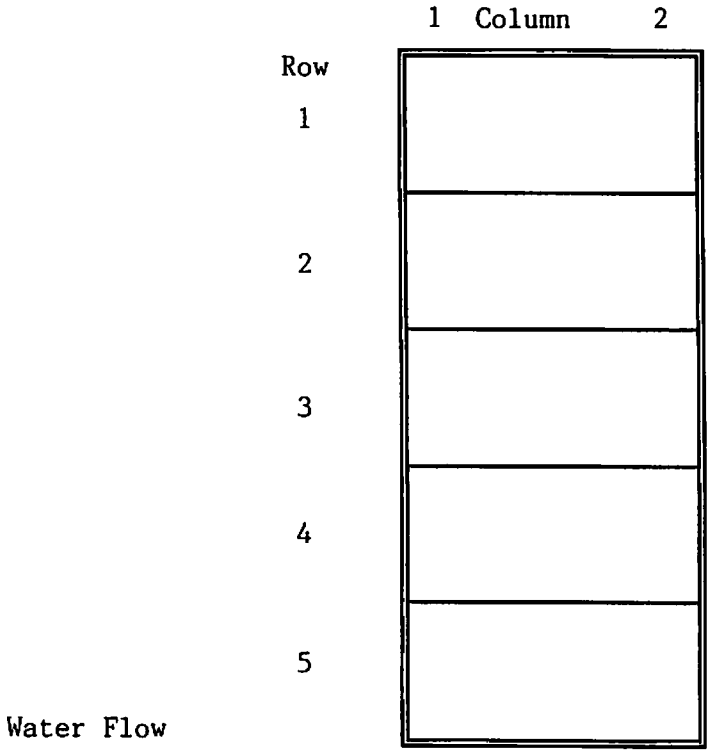
At end end of purification, counts of E. coli should all be less than 230 E.coli/100g mussel flesh and in water less than two E. coli/100ml. Counts of streptococci in purified mussels are not well defined but more than 1000/100gm would be suspicious.

9.1 Mussel Sampling

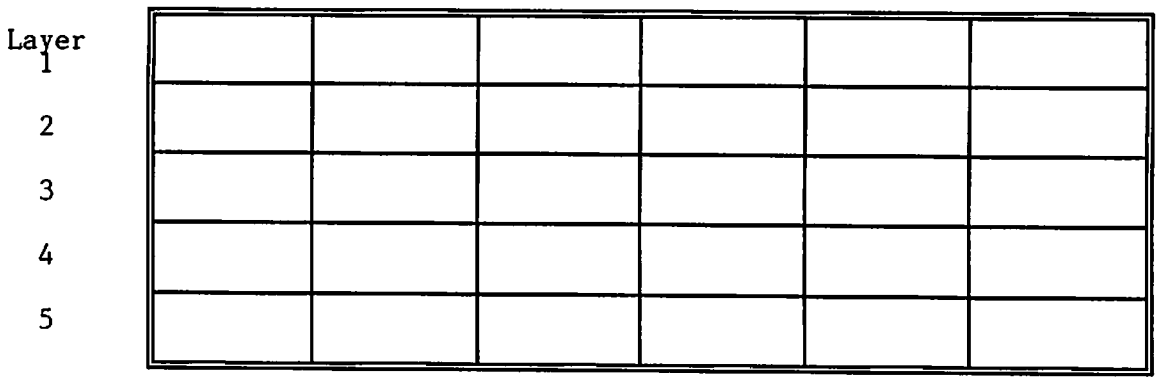
Each sample for bacteriological analysis contained a sufficient number of mussels to provide 50gm of shelled mussel meat (usually 12 to 15 mussels) and was taken from an individual box. Mussels were taken from the four corners and centre of the box and placed in a clean, coded bag and sealed. The coding system used for the location within the tank of the boxes sampled is shown overleaf.

TRIALS 1,2,3,5 AND 6

Plan View of Tank

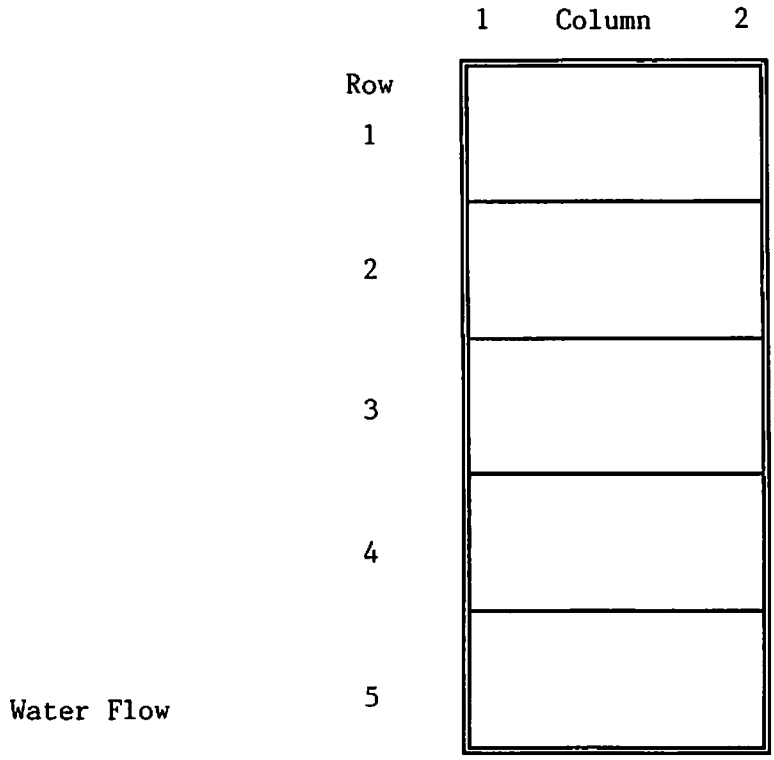


Side Elevation of Tank

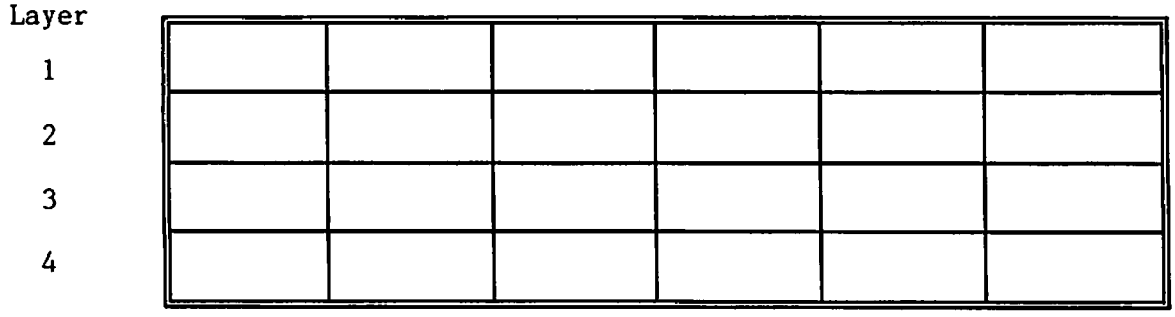


TRIAL 4

Plan View of Tank



Side Elevation of Tank



9.2 Mussels Samples - Pre-purification

Samples were taken from a variety of locations throughout the tank for each trial. Details of the locations are given in Table 1.

9.3 Mussels Samples - Post Purification

Samples were taken from a similar variety of locations and from the control tank as detailed in Table 1.

The bottom of the tank at the suction end is considered the most likely area for any possible problems as it represents the area where we might expect to see the lowest oxygen levels coupled with the maximum effect of detritus falling down through the tank.

9.4 Water Samples

A single water sample was taken just before the end of each purification cycle at the suction end of the tank.

10. TRIALS PROCEDURE

All trials involved the operation by Seafish staff of both multi-layer and control tanks with each purification cycle being monitored.

Each trial/purification cycle consisted of loading the boxes of mussels into the tank, filling the tank with water then circulating the water through the tank and UV for a minimum of 42 hours. The water was then returned to the reservoir, the boxes of mussels removed and the tank flushed out.

10.1. Summary of Tank Loading

Trial Number	No. of Boxes	Mussels per Box	Total Loading
1	50	11.5 kg	575 kg
2	50	14.3 kg	715 kg
3	50	14.3 kg	715 kg
4	40*	16.7 kg	668 kg
5	50	15.7 kg	785 kg
6	50	14.3 kg	715 kg

* This would correspond to 835 kg if 50 boxes were used

The control tank was filled with 12-14 kg of mussels in a 75mm layer in single box except for trial 6.

10.2. Filling Purification Tank with Artificial Seawater

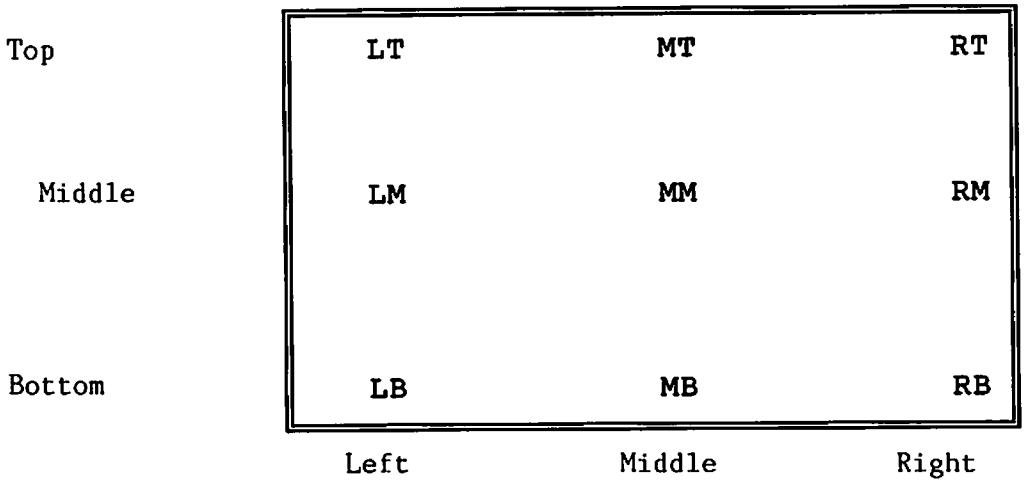
For the first trial 100% freshly made artificial seawater was transferred from the reservoir into the purification tank through the U.V. until the top layer of mussel boxes were covered. For trials 2,3, and 4 the tank was filled with 96% from the previous trial and 4% freshly made. The water was then replaced totally with fresh ASW on run 5 as there were problems with mussel activity on run 4 probably caused by water quality. The control tank was always filled with freshly made ASW.

10.3. Monitoring Trials

pH, ammonia and nitrite levels were measured at the end of each trial, with salinity checked at the start. Water temperature and dissolved oxygen levels were monitored throughout each trial. Dissolved oxygen levels were measured between the boxes and flow screens at either end of the tank, as this was the only accessible place for the DO_2 probe. See diagram below for the DO_2 measurement locations across the cross section of the tank.

OXYGEN PROBE LOCATIONS

Flow Screen



10.4 Emptying Purification Tank

Each trial operated on a 48 hour cycle which included filling and emptying the tanks. The minimum immersion time of the mussels in water was 42 hours.

In the high density tank, having first taken the water sample, the artificial seawater was pumped back to the reservoir.

After the tank was drained, the mussel samples for bacteriological assessment and holding trials were taken out. The remaining boxes were then mannually removed and re-bagged for disposal as unpurified mussels, bait or dumped on a tip.

10.5 Holding Trials

After trials 2 and 4, samples of mussels were taken from the deep stack tank and from the control tank. These were taken back to the Seafish laboratory in Hull. Here they were put into shallow trays and held at 15°C, whilst kept moist, in the Environmental Chamber. Mortality in each sample was then recorded (Ref 7).

10.6. Details of Individual Trials

10.6.1. Trial 1

This initial trial was to investigate the flow rates at which the tank could operate. The fifty boxes were filled with an average of 11.5kg (at the MAFF specified depth of 75mm) to give a total mussel mass of 575kg. This gave a water/mussel ratio of 4.6:1. Water flows of 13.5m^3/hr, 7.5m^3/hr, 8.2m^3/hr and 5.5m^3/hr were tried during the trial. The solid front flow screen was tried at 9inch and 6 inch clearance off the tank floor to assess any differences in flow patterns. Monitoring concentrated on measurement of dissolved oxygen levels throughout the tank to assess the adequacy and uniformity of flow. Bacteriological analysis was not carried out.

10.6.2. Trial 2

In this trial the fifty boxes were filled with an average of 14.3kg per box (nearly to the nominal capacity) to give a total mussel mass of 715kg. This gave a water/mussel ratio of 3.7:1. During this trial the flow was left at 13.5m^3/hr. The spray bar was modified during the trial to a "T" configuration from its original end feed to get more even water distribution. The spray bar holes were also enlarged from 10mm to 13mm.

10.6.3. Trial 3

In this trial the boxes were filled as in Trial 2. The water flow initially was only 8.5m^3/hr. However, by the second day this was increased to 13.5m^3/hr because oxygen levels were falling alarmingly low as water temperature increased.

10.6.4. Trial 4

In this trial only forty boxes were used. They were filled to an average weight of 16.6kg giving a total mass of 666kg in only 2.2m^3 of water. This gave a water mussel ratio of 3.33:1. Some of the boxes were filled to a depth of 150mm.

The spray bar on this trial was raised by 4 feet in an attempt to lower the water temperature. It was thought that the high water temperatures found in the tank would drop to the ambient air temperature with a larger water drop allowing the water droplets to cool in the air. The maximum water flow rate possible at this spray bar height was 12.5m^3/hr, this maximum rate was used henceforward during the trials.

There were problems during this trial as the mussels showed little sign of activity. Mussels taken out of the tank after the purification cycle and then put into fresh A.S.W. started to respond rapidly. This lead to the conclusion that the re-used water quality was suspect and therefore it was dumped.

10.6.5. Trial 5

The number of boxes was returned to 50. They were filled to an average weight of 15.7 kg giving a total mass of 785kg. The water mussel ratio was 3.38:1.

Due to low E.coli counts pre-purification in the previous trials, it was decided on this trial to try artificially dosing mussels to in E. coli rich water prior to purification. A fresh batch of A.S.W. was made up into which we poured an E. coli culture of nominally 2.5×10^7 E. coli. This would dilute down to 1000/m.p.n. per 100ml of A.S.W. This water was then pumped into the purification tank holding the mussels and circulated for two hours without the U.V. lights on. It was hoped this would be adequate to boost the E. coli levels.

The contaminated water was drained down and dumped and mussel samples taken out for bacteriological analysis. The tank was then refilled with fresh A.S.W. and the purification process started.

During the trial the solid flow screen at the front of the tank was replaced by a perforated flow screen to compare oxygenation patterns between the two types.

The boats from Boston were not fishing during this period and so an alternative source from King's Lynn was employed. It turned out that these mussels had extremely high levels of contamination prior to artificially dosing.

10.6.6. Trial 6

In this trial the original source of mussels at Boston was employed and the boxes were filled the same as in Trial 2, approximately 715kg. This gave a water/mussel ratio of 3.7:1.

For further investigation of purificaton in deep layers the box was removed from the control tank and the entire tank base between the flow screens was filled with 41 kg of mussels laid out in a sloping layer increasing in depth from 3 inch at the input end of the tank to 6 inch at the suction end.

11. RESULTS AND DISCUSSION

11.1 Bacteriological Results

The bacteriological results are shown in Table 1 overleaf.

In order to show that satisfactory purification has occurred it is necessary to have high initial counts in the mussels before purification and final counts of less than 230 E. coli per 100gms. Although in general initial results were low, 7 out of the 17 pre-purified samples were over 230 E. coli per 100gms and of these four were over 1000 E. coli per 100 gms.

Satisfactory results were obtained from 17 of the 19 post purification counts. The two poor results are both from Trial 5 where levels pre-purification were very high at 16,000 E. coli per 100gms. It is assumed that one of these results must have been from a dead mussel as it gave a reading of 5400 E. coli per 100gms. The other result was just above the permitted level at 270 E. coli per 100gms. Counts of faecal streptococci can be used as an indicator of purification when E. coli counts are low. The faecal streptococci showed higher initial counts with 12 out of 17 pre-purified samples giving readings into the thousands. The reduction required to indicate purification is a factor of 10. This is apparent in all cases. In 17 out of the 19 post-purification samples the levels dropped to zero.

Considered overall, the bacteriological results indicate that purification was occurring.

The artificial dosing of the mussels in trial 5 did not lead to any significant increase in the levels of E. coli. Further reading indicates that the dosing level should have been 10 times greater and for a period of six hours (Ref 11 and 12).

Table 1 — Bacteriological Results

Trial and Sample	Pre E.coli	Post E.coli	Pre F.strep	Post F.strep
T2 1-1-1	60	<20	600	0
T2 3-3-2	110	<20	200	0
T2 5-5-1	50	<20	400	0
T2 5-1-1		<20		0
T2 Control		<20		0
Mussel Mass	715KG			
T3 1-1-1	170	<20	5700	0
T3 1-2-2	170	<20	400	0
T3 3-2-1	130	<20	3100	0
T3 5-5-1	490	<20	2000	0
T3 Water		<1		
T3 Control		<20		0
Mussel Mass	715KG			
T4 1-1-1	20	<20	3000	0
T4 3-3-1	60	20	5800	350
T4 4-2-2	70	20	1400	0
T4 5-4-1	80	20	200	0
T4 Water		<1		
T4 Control	70	20	1400	0
Mussel Mass	666KG			
T5 1-1-1	9200/16000	150	3100/3300	0
T5 3-3-1	16000/16000	5400	11000/6200	0
T5 3-2-2		270		50
T5 5-5-1	16000	220	9100	0
T5 Water		<1		
T5 Control	16000	<20	3100	0
Mussel Mass	784 KG			
T6 1-1-1	1100	<20	1300	0
T6 3-3-1	490	40	1400	0
T6 5-5-1	330	<20	1600	0
T6 Water		0		
IControl top		<20		0
Control Middle		<20		0
Control Bottom		<20		0

Sample position code | Row - layer - Column |

11.2 Mortality

Trials by Seafish have shown a relationship between mussel storage temperature and mortality. Storage for one day at 15°C approximates to three days storage at 5°C and three and half days storage at 0°C. Samples of mussels from both control and multi-layer tanks were taken after trials 2 and 4 and held at 15°C in the environmental chamber at Hull. The incidence of mortality is shown in Figure 6. The results from trial 2 show a small difference in the mortality of mussels from the deep stack and control tanks. Mortality results typically show variations from trial to trial depending upon the handling and condition of each individual sample of mussels (the control sample came from a single bag of mussels) and given the weight of evidence already accumulated on deep stock purification this particular result is not considered significant. Trial 4's results show that there was a large and significant adverse effect upon the mussels in the deep stack tank during that trial, probably caused by poor water quality although testing for salinity, pH, ammonia and nitrates did not reveal exceptional levels. It is assumed that some biological action had occurred after trial 3 resulting from the high water temperature at the end of that trial, 16.2°C, and the water being left still for 5 days in the reservoir tank.

11.3 Water Temperature

Purification relies upon the shellfish filtering naturally. Mussel filtration activity is effected by water temperature and will reduce significantly once temperatures fall below 5°C (Reference 7). MAFF recommend a minimum water temperature of 5°C (Reference 8). It was observed during the trials that mussel activity appeared to increase at water temperatures of 10°C or more.

Unfortunately as water temperature rises, its ability to hold oxygen decreases and consequently a maximum water temperature

needs to be specified. As discussed in 11.4 below, a maximum water temperature of 10-15°C is recommended.

The temperatures in the deep stacked tank, control tank and ambient air for each trial are shown in Figure 7. The straight lines are between plotted points and do not represent a constant rate of temperature change between readings.

High water temperatures in the deep-stack tank during the six trials proved a problem with the temperature rising to a maximum of 16.2°C. The temperature rise during was due to the high flow rates and an inefficient type of pump transferring heat into the water. The insulative property of the G.R.P. lined wooden tank prevented the temperature from dropping back to ambient. Increasing the height of the input cascade had only a minor effect. Fitting a more efficient pump with a less direct heat path from the motor to the impellor would improve the situation. Using a refrigeration system to chill the water would no doubt be effective but at additional cost and complexity.

11.4 Oxygen

Dissolved 0_2 levels (mg/l) in the multi-layer purification tank are shown in Figure 8. The points represent average oxygen levels during each trial at the particular location stated.

Monitoring of oxygen levels is a good indicator of the effectiveness of tank design in producing an even flow rate throughout the mass of mussels of water sufficiently oxygenated to permit natural filtration activity. Low levels of oxygen in the water will inhibit filtration activity, a level of 5mg/l or above is recommended at tank water temperatures of 10°C.

This tank had a major design difference in respect to how it achieved an adequate oxygen flow through the tank, compared to the large scale purification tank system designed by Seafish (Reference 2). In this tank the means of maintaining adequate

dissolved oxygen levels and even flow is to pass the water through the input cascade and tank at a high rate without using an aeration system in the tank.

Figure 8 shows that there is little difference in oxygen levels throughout the cross-section of the tank. This is an indication that an even flow is present and that stratification is not occurring. This is essential if the tank is to purify mussels successfully. Both the solid flow control screen with gap at base and the perforated flow control screen were effective in this respect.

In Trial 1 there was a large variation in oxygen levels as the water flow rate was altered from 5.5m^3/hr to 13.5m^3/hr. Below 7.5m^3/hr the water flow rate was unable to sustain oxygen levels above 5.0mg/l.

In Trial 2 the oxygen level in the water was high at a sustained 13.5m^3/hr flow rate despite the increased loading of the tank, the levels of dissolved oxygen reducing along the tank on average to 7.5mg/l at an average temperature of 10.9°C.

In Trial 3 the oxygen levels in the water were again lower at a reduced flow rate of 8.5m^3/hr and higher temperature, the levels of dissolved oxygen reducing on average to 5.0mg/l at an average temperature of 11.9°C.

In Trials 4,5 and 6 the oxygen levels were high even though the temperature rose up to 16.2°C. The water flow rates were at a maximum of 12.5-13.0m^3/hr. Trial 4 had levels of dissolved oxygen reducing on average to 7.2mg/l with an average temperature of 13.3°C (although in this trial the mussels were not very active because of the water quality). Trial 5 had levels of dissolved oxygen reducing on average to 6.5mg/l with an average temperature of 10.9°C. Trial 6 had levels of dissolved oxygen reducing on average to 6.5mg/l with an average temperature of 11.8°C.

It is known that mussel activity increases with water temperature, with the mussels becoming particularly active at temperatures greater than 10°C (Reference 2). If dissolved oxygen levels above 5mg/l are to be maintained to ensure optimum purification, then a maximum water temperature must be specified. An increase in mussel mass will also increase oxygen consumption thus a maximum mussel density must also be specified.

To make it unlikely that oxygen levels will drop below 5mg/l and thus possibly affect mussel purification, it is recommended that a maximum temperature of 15°C is allowed. This should be in conjunction with a maximum mussel mass of 750kg. The tank must have a water flow of at least 8.5m^3/hr at temperatures of 5 to 10°C and a water flow of at least 12.5m^3/hr at temperatures of 10-15°C if adequate oxygenation is to be maintained. It is important to stress that the system will not work at MAFF specified flow levels of one change per hour. Specification of flow in changes per hour is not necessarily relevant to all types of system, particularly when the water/mussel ratio is low.

11.5 Water Analysis

11.5.1. Ammonia

Figure 9 shows the ammonia levels at the start and finish of each purification cycle, expressed in terms of ammonium concentration in parts per million (PPM). During the trials the ammonia level rose, then reduced before the following trial one week later. The rises in the deep stack tank were up to 20 ppm dropping to 0.2 ppm before the next run.

The water/mussel ratios used in this tank are significantly less than in the large-scale tank at King's Lynn and this leads to a more rapid ammonia increase in the water after two or three re-uses. After a third re-use in the large-scale tank ammonia levels rose to 5 ppm compared to up to 20 ppm in the Boston tank after two re-uses.

If the water was re-used on a longer term basis the ammonia level would rise until it reached a stabilised level. This occurs when any additional ammonia is being broken down by bacteria into nitrites and subsequently nitrates in the water. Laboratory trials have shown some evidence of higher than 40ppm of ammonia starting to effect mussel mortality (Reference 10). This would be unacceptable in a commercial situation.

A greater build up of ammonia might be seen with a more prolonged use under normal operating practice where the tank could be used up to three times per week. A maximum re-use of three times is recommended for this tank.

11.5.2 Nitrite

Figure 10 shows the nitrite levels at the beginning and end of each purification expressed in terms of total nitrite in ppm. The level is seen to rise during the trials and then fall before the next trial.

The build-up of nitrates is due to the effect of nitrifying bacteria attached to the boxes and surfaces of the tank, breaking down the ammonia. Any subsequent drop in nitrites is due to other nitrifying bacteria breaking the nitrite into nitrates. However nitrates were unable to be measured with the test kits available. Nitrate is toxic to mussels but at what level is unclear.

11.5.3. pH

Figure 11 shows the pH levels in the tank. They are all well within the expected region for such a system.

The rise in pH up to Trial 4 (when the water was dumped) and again from Trial 5 to Trial 6 is probably due to the aeration of the tank. The aeration removes CO^2 from the water, which is naturally present in tap water, and thus the pH level rises.

11.6. Deep Box Stacking

Operation at the nominal capacity, at a mussel depth of 100mm, appeared to present no problems.

In Trial 4 some of the boxes were filled deeper but unfortunately the mussels were very clean prior to purification and, in addition, the mussels showed very little signs of activity due to a water quality problem. Thus it is not possible to conclude anything from that trial.

In trial 6 when one end of the control tank had been filled to a depth of 150mm, after 24 hours the mussels had all moved so that there was even layer of 110mm depth across the tank. These mussels purified, dropping from 1100, 490 and 330 m.p.n. *E. coli* per 100gms to <20 m.p.n. *E. coli* in three samples. The faecal streptococci results dropped from 1300, 1400, 1600 to 0.

12. CONCLUSIONS AND RECOMMENDATIONS

1. The purification of mussels in this design of purificiation plant, of high density, low water/mussel ratio, and high flow rate was successful provided that certain operating criteria are satisfied.

2. A nominal tank capacity of 750kg is recommended, this gives a water/mussel ratio of 3.5 litres/kg and a 100mm depth of mussels in each box without apparent harmful effect.

3. A tank operating temperatures range of 5 to 15° C is recommended. To avoid overheating in warm weather, refrigeration of the water or operating environment may be required.

4. At an operating temperature of 10°C a minimum water flow rate of 8.5m^3/hr is recommended to provide adequate oxygen to the mussels but at 15°C this must be raised to at least 12.5m^3/hr. Flow rates as high as 13.5m^3/hr, which corresponds to 5.1 water changes per hour and a flow rate through the tank of 12.5m^3/hr have been tested without apparent harmful effect.

5. It is recommended that for this high water/mussel ratio design the artificial seawater should be completely replaced after three purification cycles.

6. Despite exceeding the previously recommended MAFF general operating criteria, particularly in flow rate, on the basis of the above this particular design of plant has been approved by MAFF and granted an operating license by the Department of Health.

7. It is further recommended, that the water circulation through the U V is continued to inhibit biological action in the water. This is based on the problems of maintaining

the water quality when the plant is left idle at high ambient temperatures and particularly for extended periods. Similar difficulties were encountered at Kings Lynn.

13. FURTHER WORK

1. A trial carried out over a week's commercial operation should be carried out to monitor the ammonia and nitrate build-up which occurs.

2. A trial should be carried out on the large-scale tanks at King's Lynn to investigate replacing the direct aeration by increased flow rates.

3. Further work on the permissable depth of mussel layers in purification plants should be carried out as no technical limitation has been found in the trials to date.

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View of the Boston Tank with type 11037 boxes Fig 1

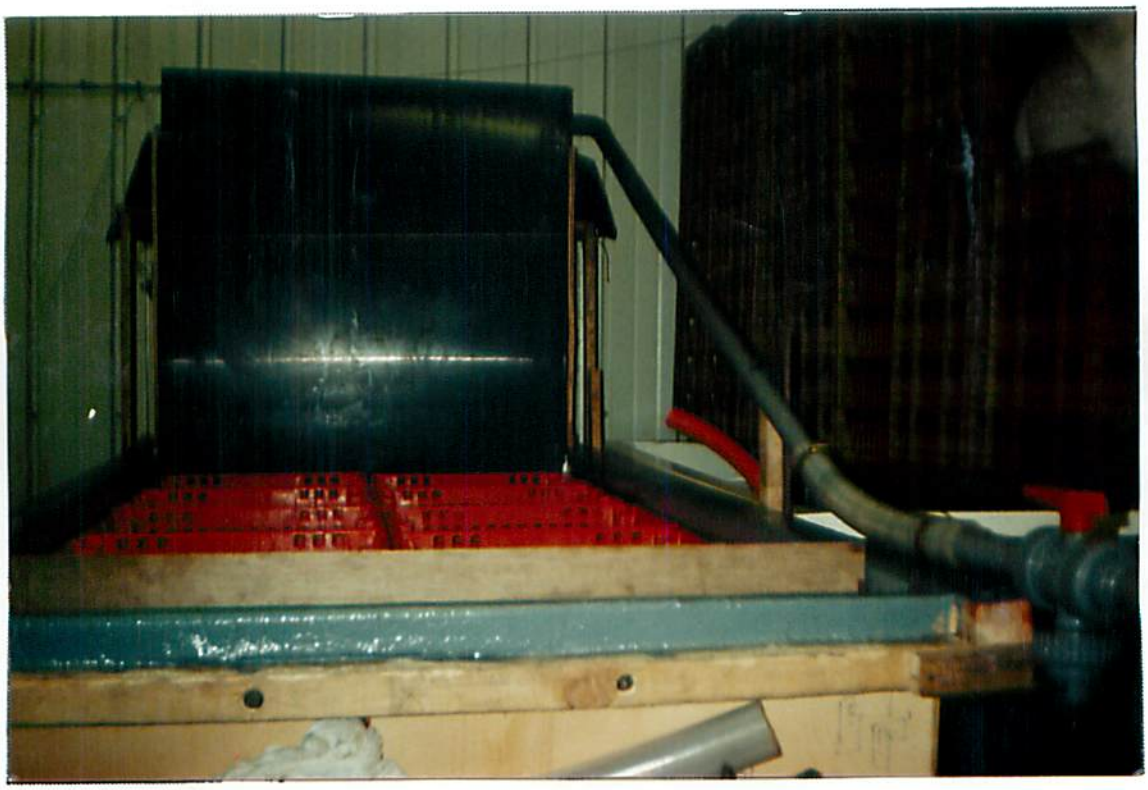
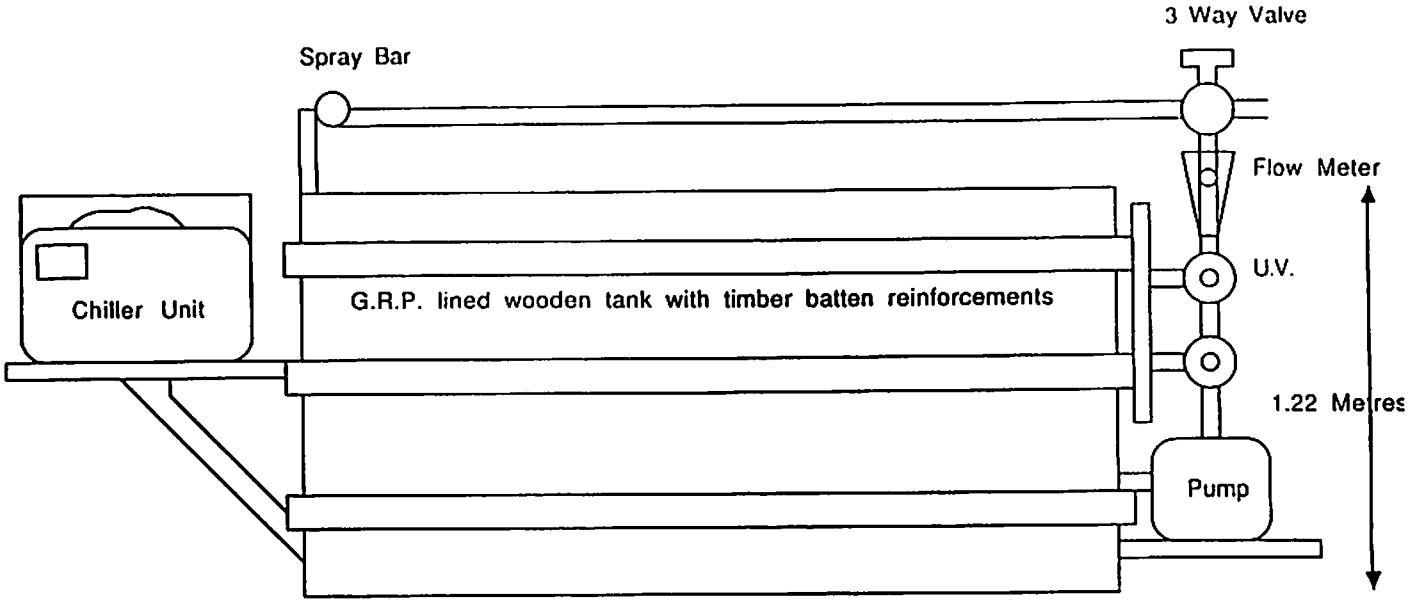


Fig. 2

Side Elevation of Boston Purification Plant



Plan View Boston Purification Plant

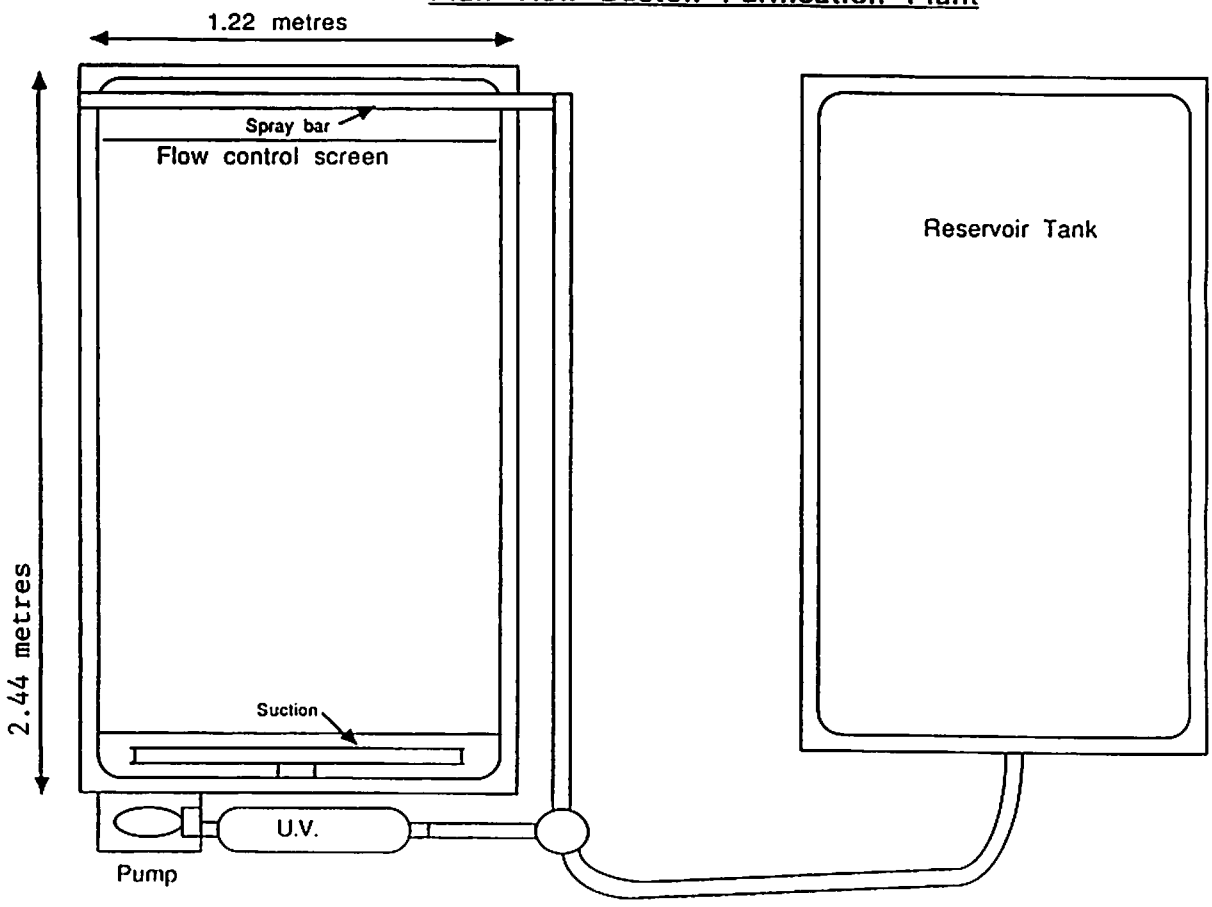


Fig. 3

Tank Spray Bar

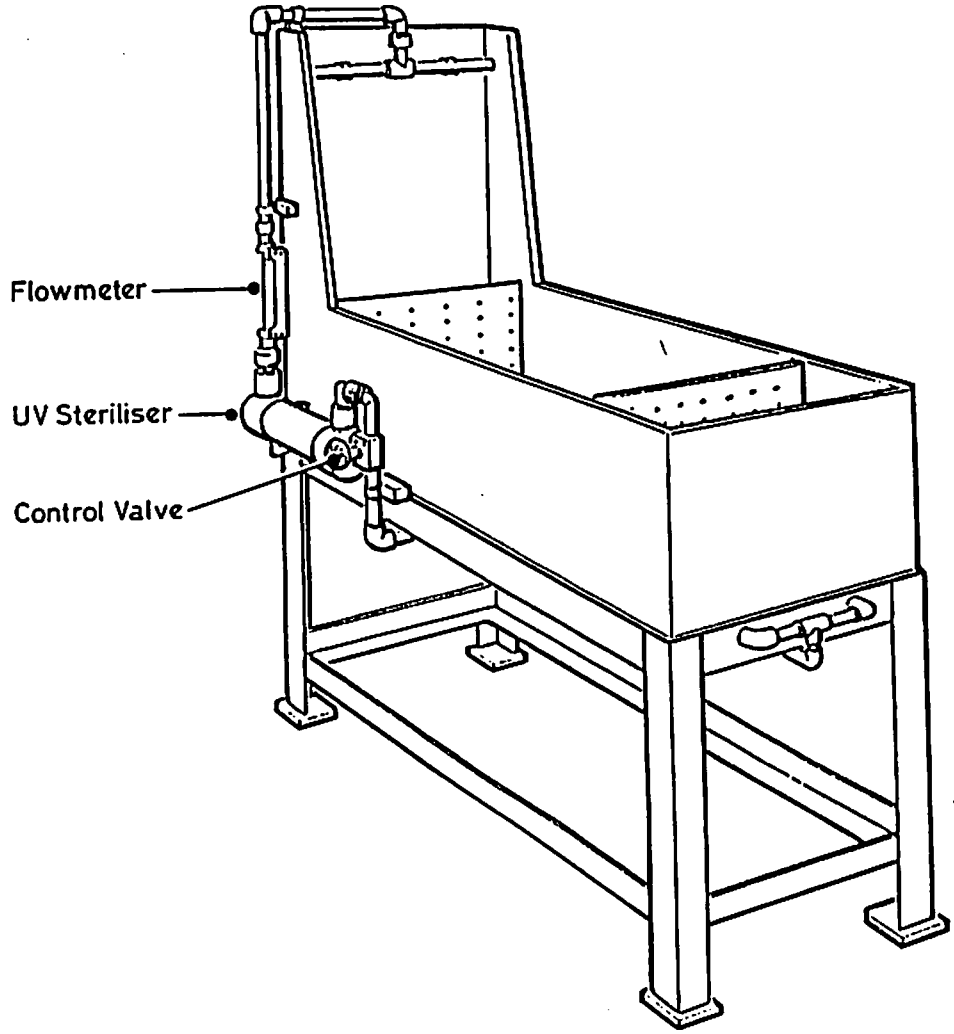
Tank Spray Bar

FIGURE 3

Tank Suction Bar

Fig 4

Figure 5 Control Purification - Equipment Specification



TANK - Constructed in marine ply with light blue epoxy resin surface finish. Mild steel stand.

PIPEWORK/VALVES - ½" A.B.S.

PUMP - Nikkiso Magpan CP03. Centrifugal pump with magnetic drive. Maximum capacity 6 l/min at 1 metre head. Mounted under tank.

ULTRA VIOLET STERILIZER - UVAQ 15/3P with 15 watt tube.

FLOWMETER - Paton PG 1 - 10 l/min.

AERATION - Atlantis B800 air pump mounted on back of tank feeding two 300mm diffuser blocks on tank base.

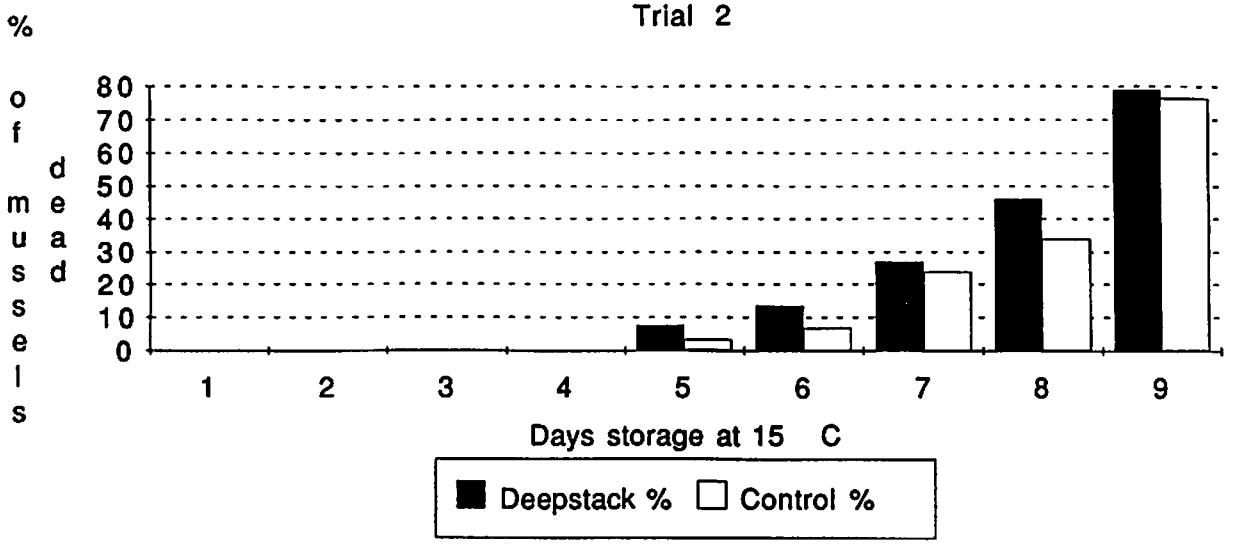
WATER CAPACITY - At working depth of 180mm, 110 litres (24 gallons) and 340mm, 206 litres

TRAY - Up to two Allibert Type 41042 (752 x 448 x 167 mm)

HEATING - 300 watt aquarium heater used with Digistat temperature controller.

Fig 6

Comparison of mortality rate between deep stack and control tank



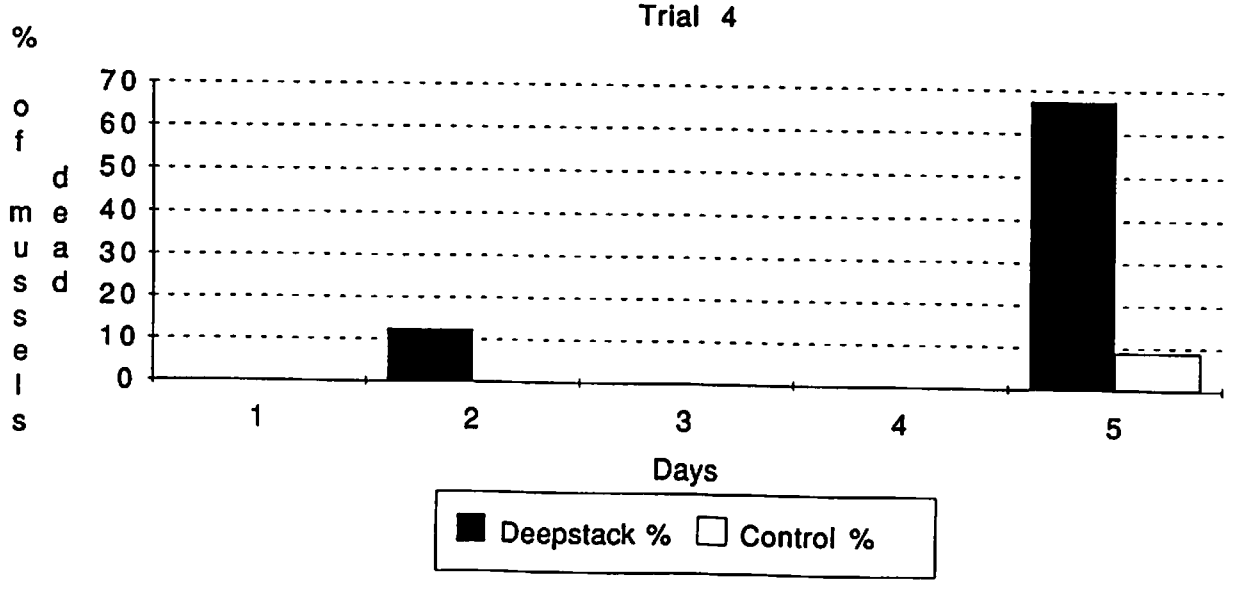
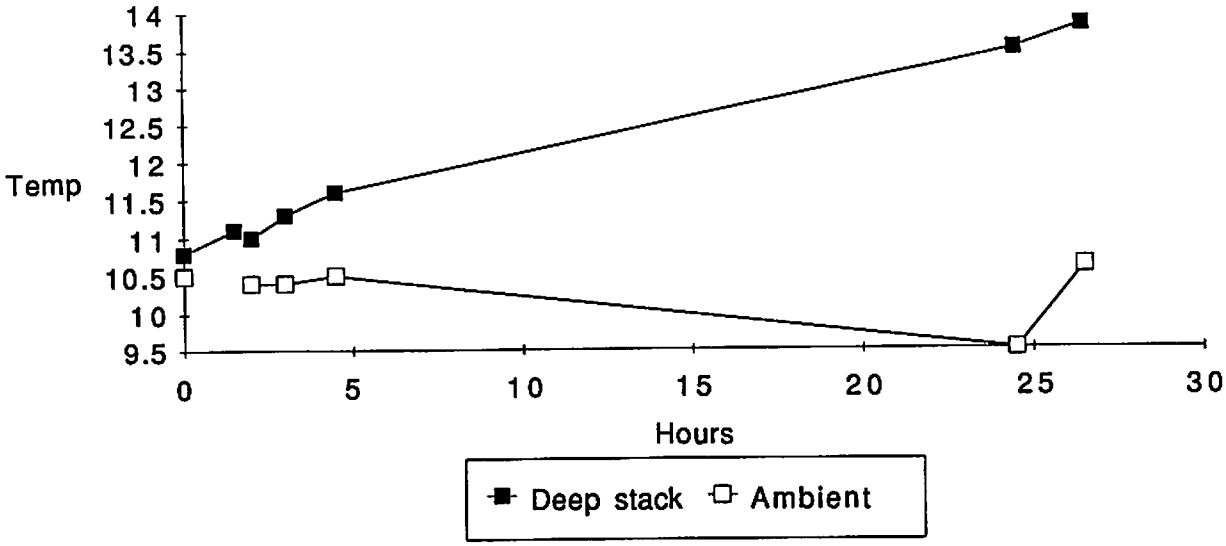


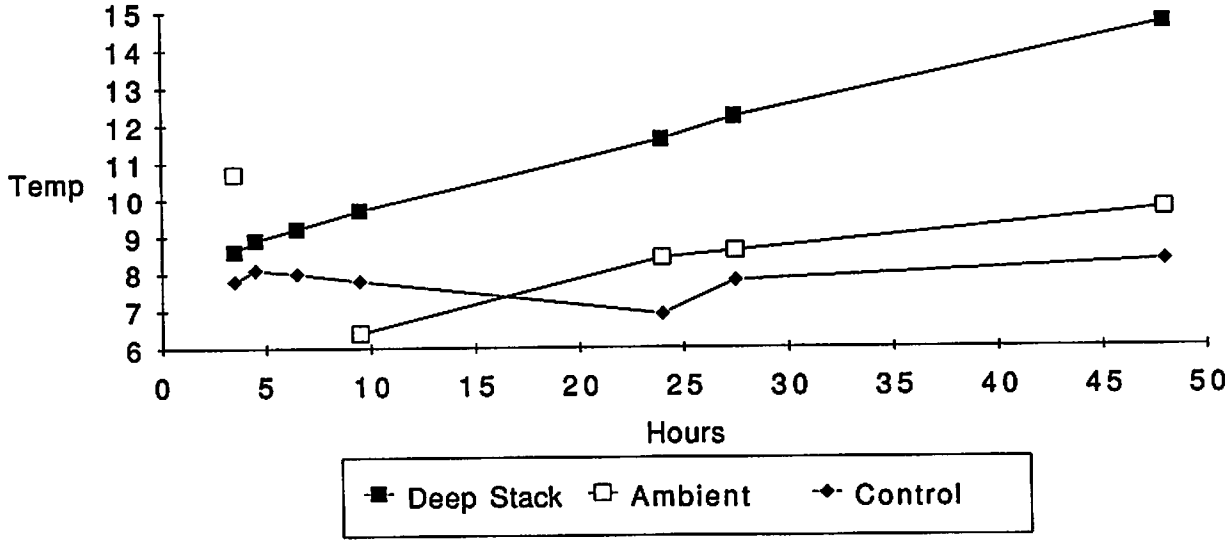
Fig. 7

Temperature changes during purification

Trial 1



Trial 2



Trial 3

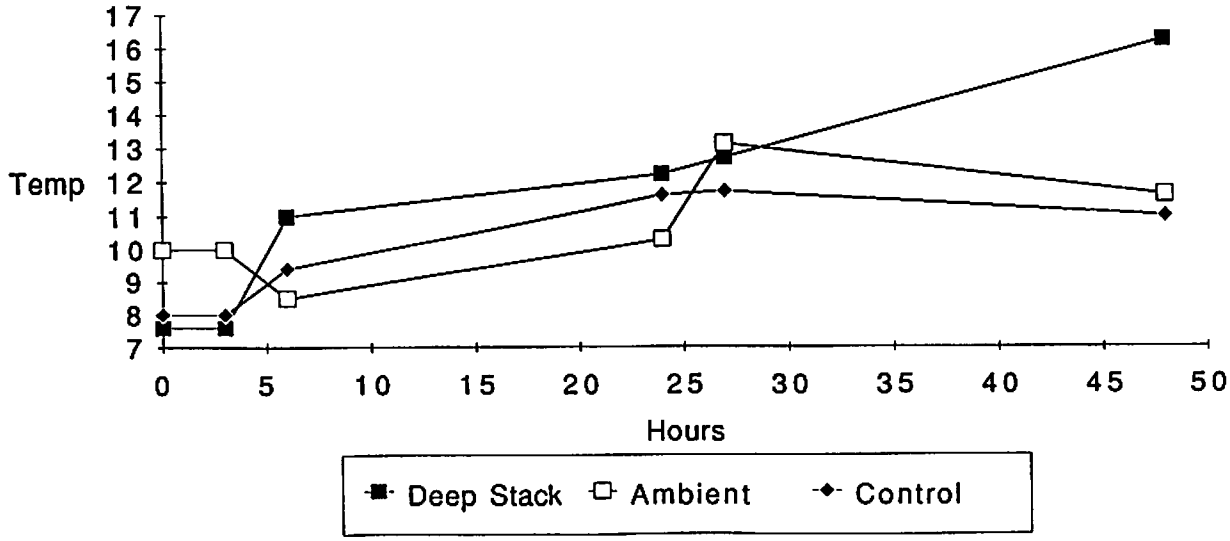
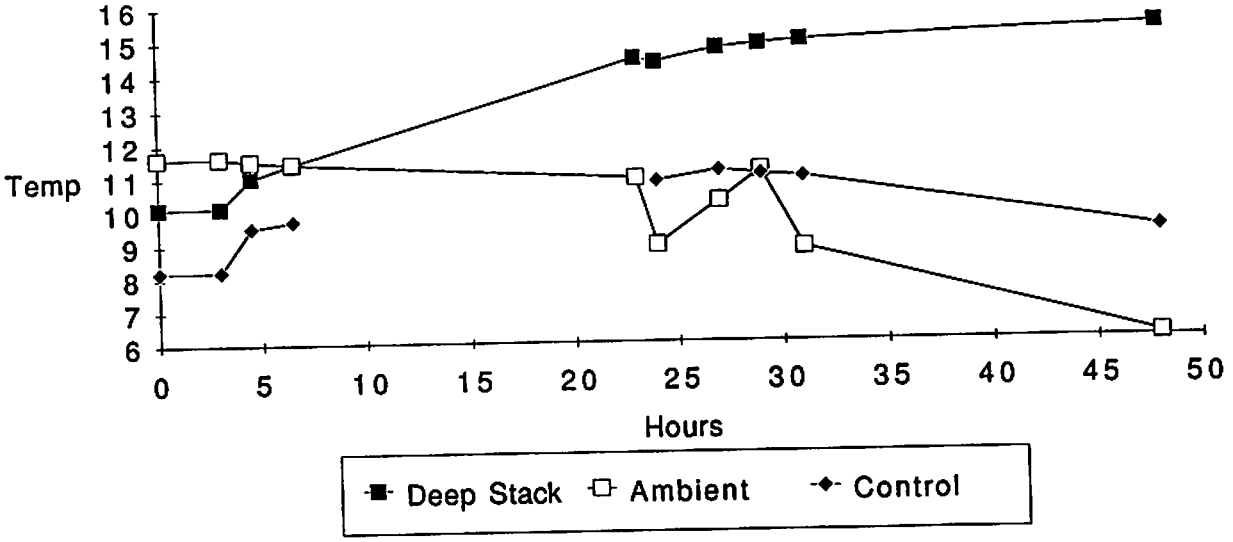
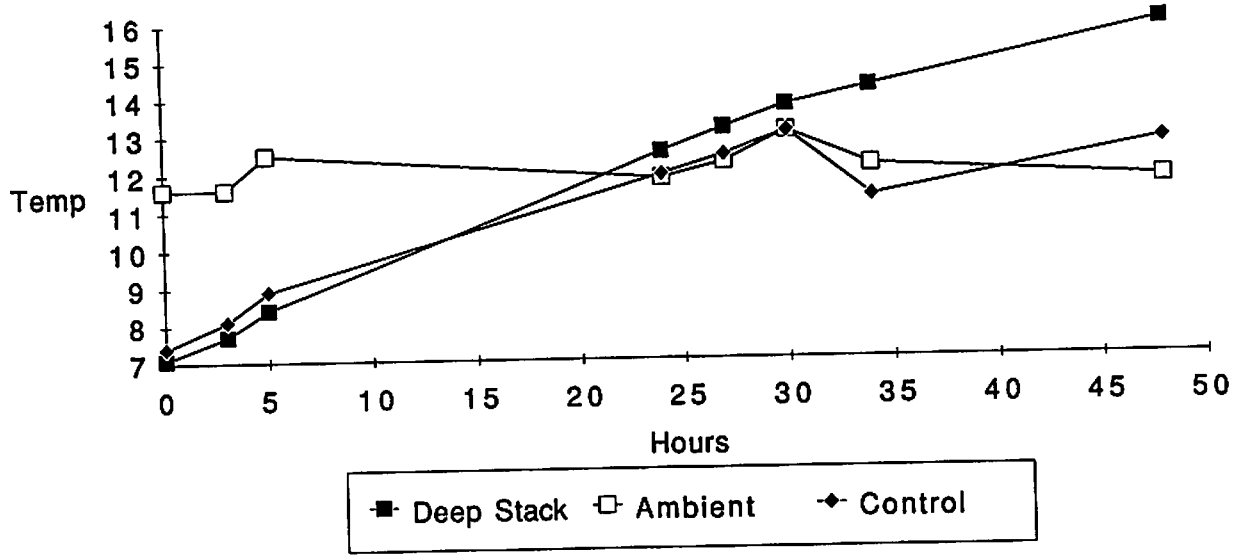


Fig. 7
(cont.)

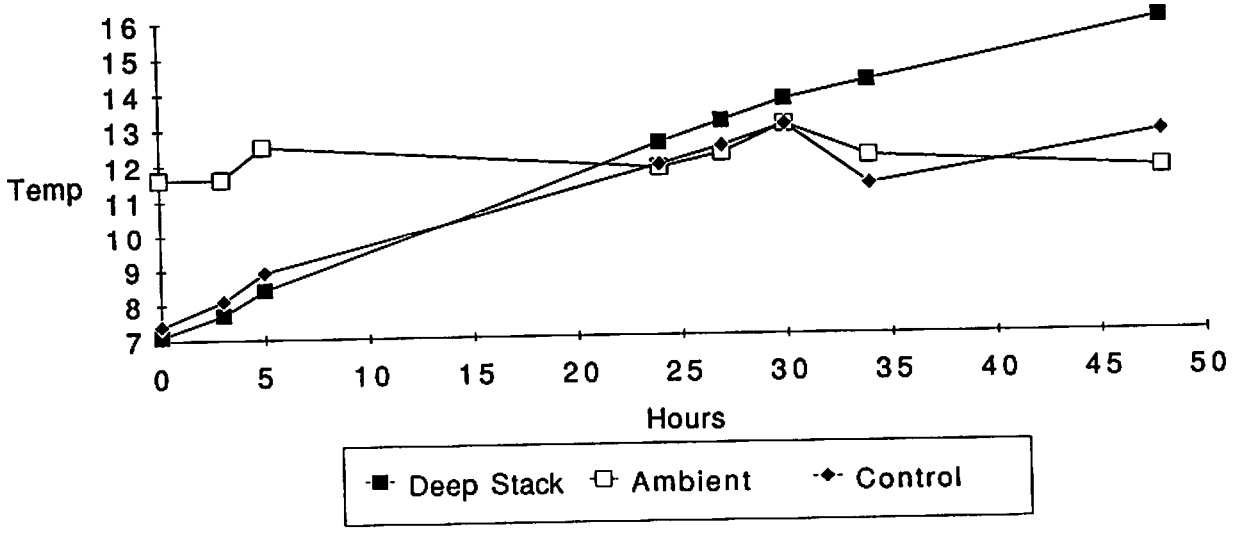
Trial 4



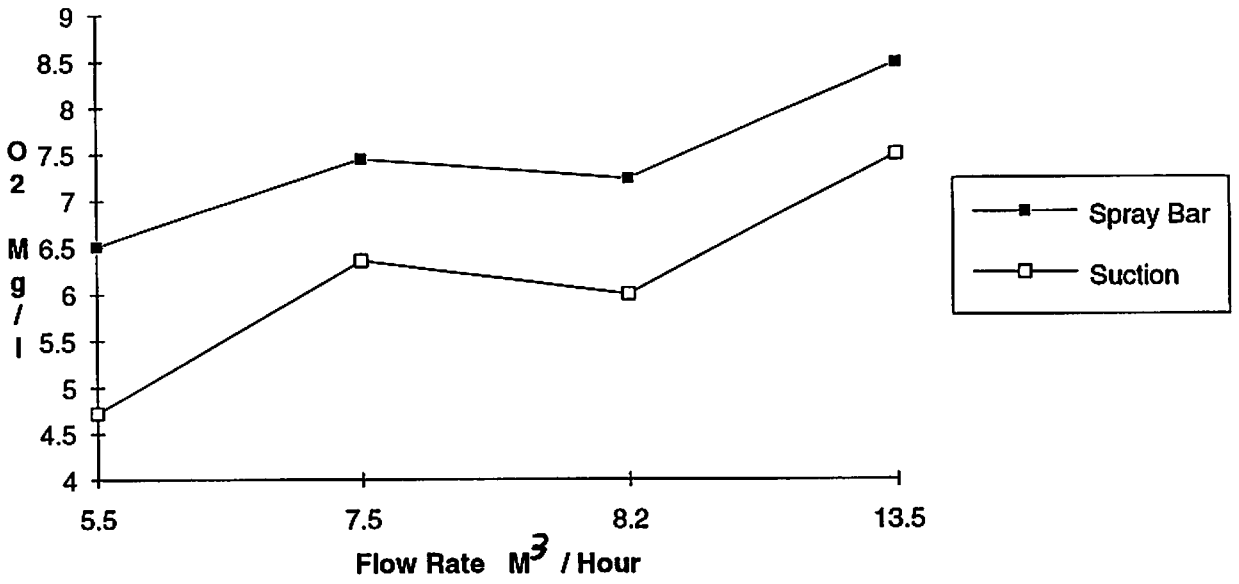
Trial 5



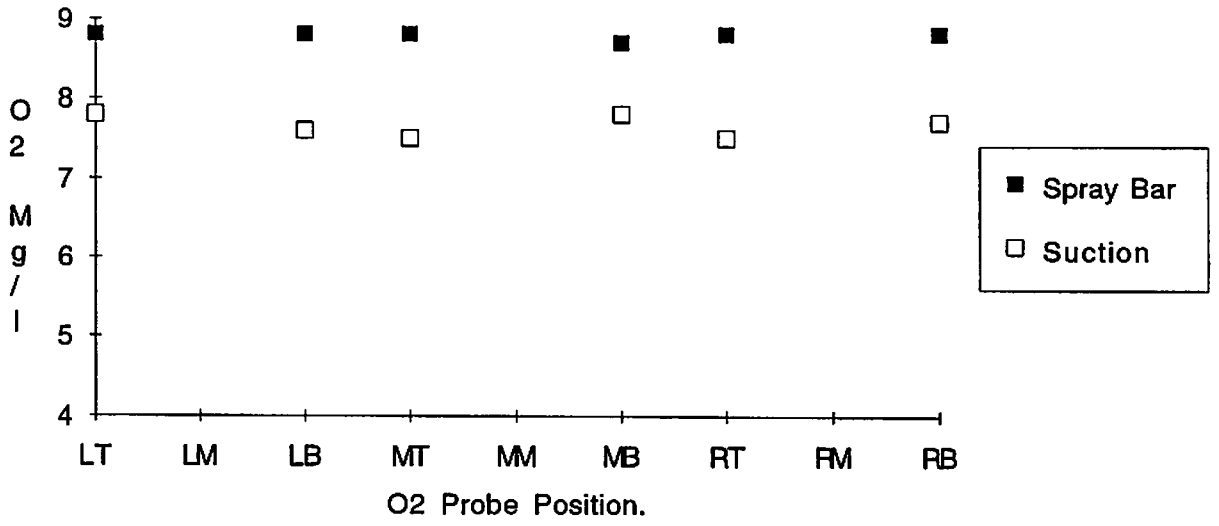
Trial 6



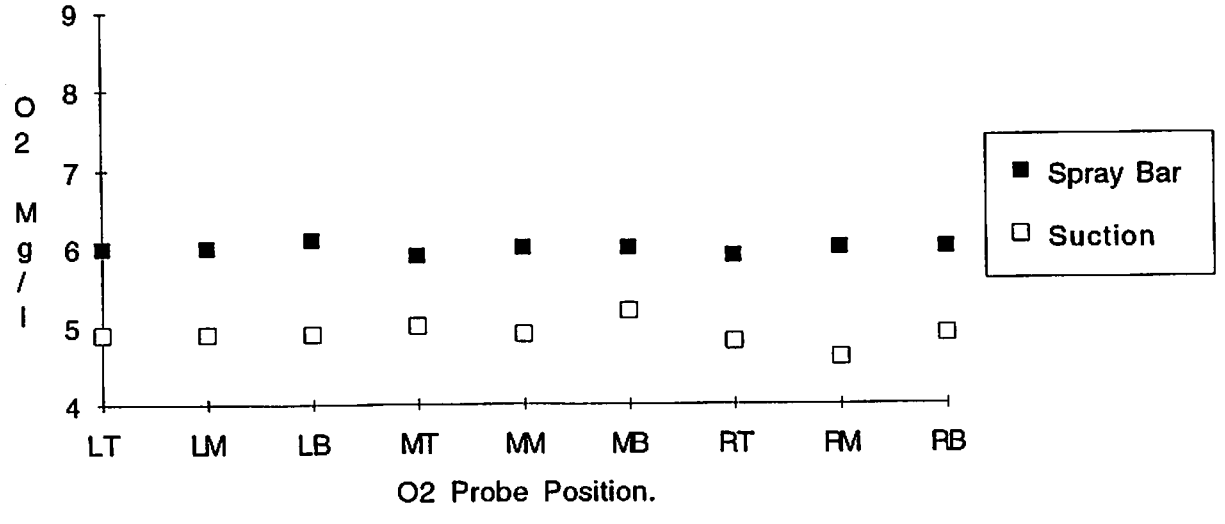
Trial 1 Mean Oxygen Levels During Purification



Trial 2

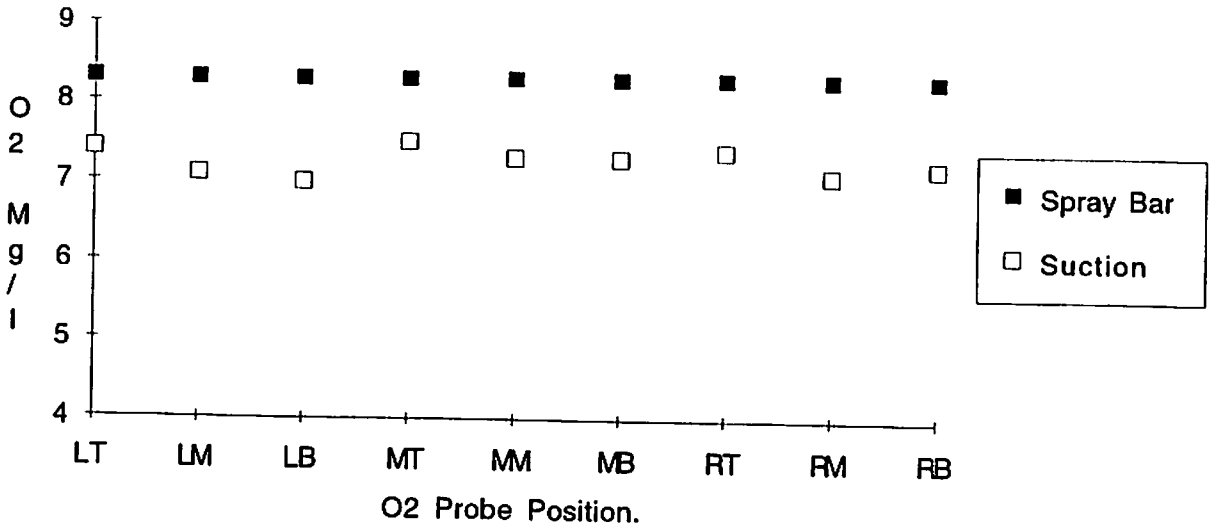


Trial 3



See Section 10.3 for probe position codes

Trial 4



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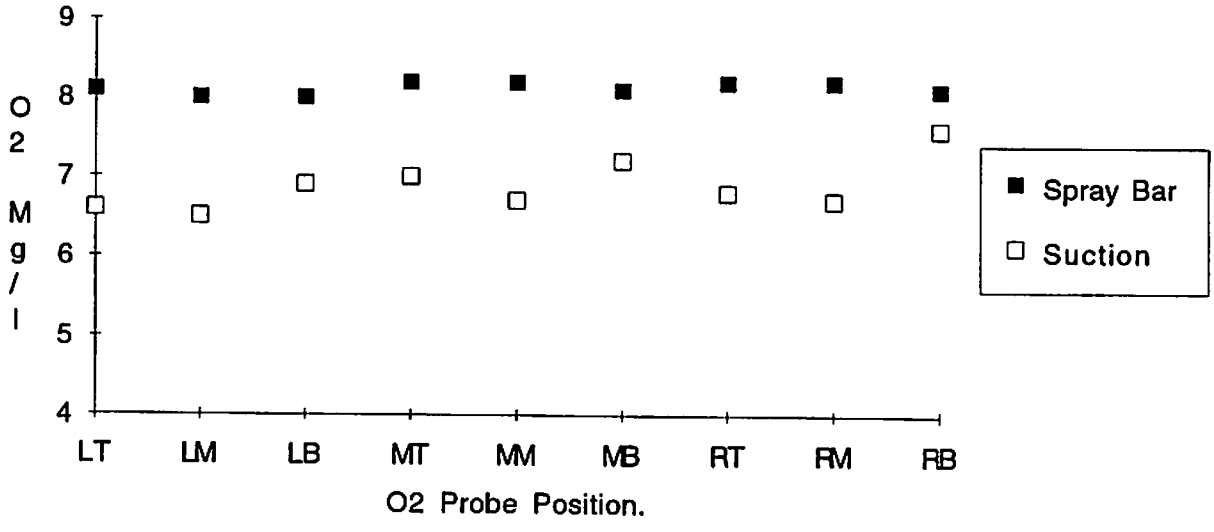
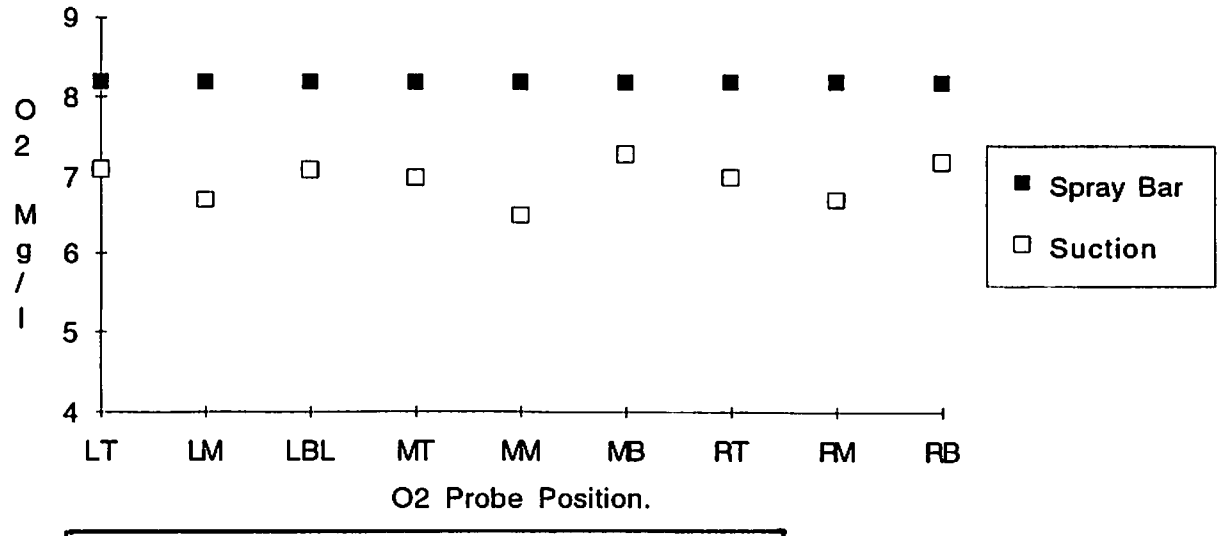


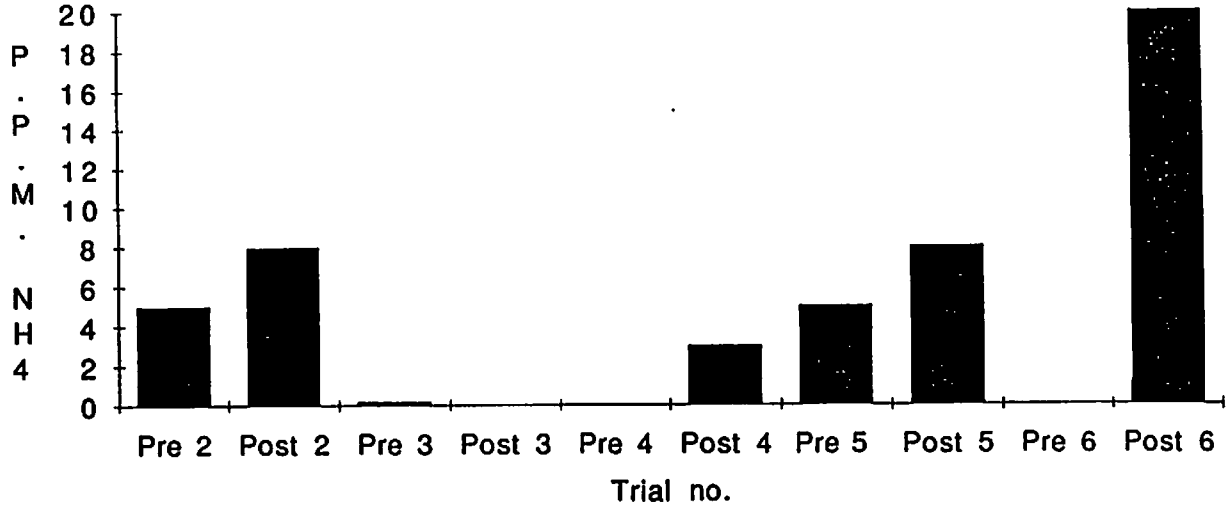
Trial 6



See Section 10.3 for probe position codes

Fig. 9

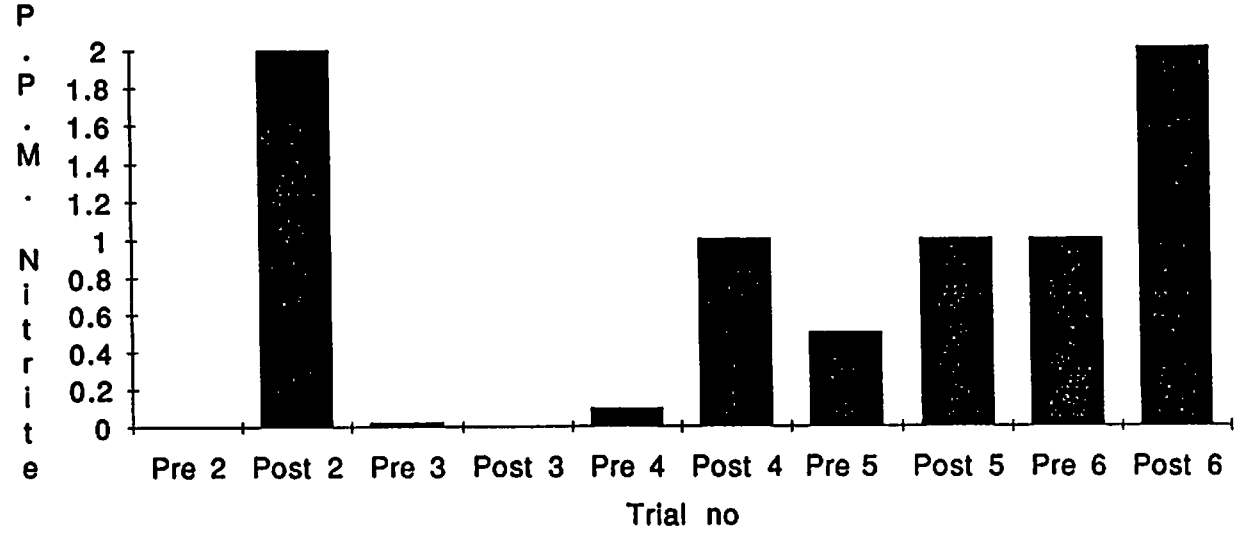
Ammonia Levels at the beginning and end of each trial



To convert ammonium to total ammonia nitrogen multiply by 0.77.

Nitrite Levels at the beginning and end of each trial

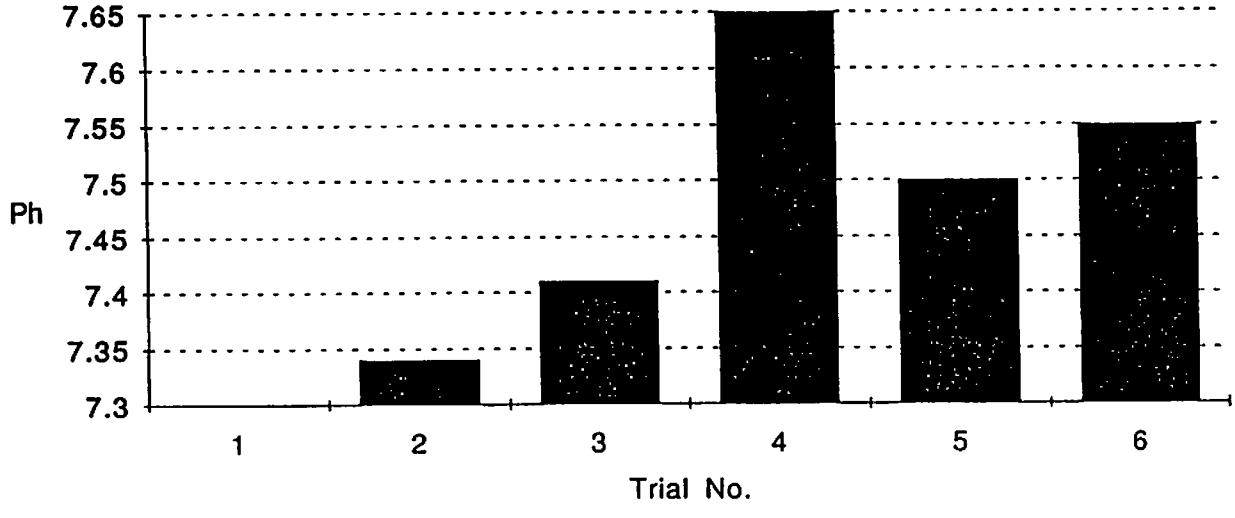
Fig. 10



To convert nitrate to nitrate nitrogen multiply by 0.3.

PH Levels at the end of each trial.

Fig. 11



APPENDIX 1

ARTIFICIAL SEAWATER MIXTURE

Five basic salts as defined in MAFF Laboratory Leaflet No. 39 (Ref. 5) can be mixed in the following proportions to give a salinity of 27 parts per thousand in 1000 litres of water.

Sodium Chloride	NaCl	21.08kg
Magnesium Sulphate	$MgSO_4$	5.18kg
Magnesium Chloride	$MgCl_2$	4.12kg
Flake Calcium Chloride	$CaCl_2$	1.06kg
Potassium Chloride	KCl	0.5kg

Commercial grade salts when obtained from a bulk supplier are in 50kg sacks other than sodium chloride which comes in 25kg sacks.

Costs are given below for sacks bought on individual and per tonne basis:-

	£ per sack (individual)	£ per sack (per tonne basic)
NaCl	5.40	4.20
$MgSO_4$	18.90	13.45
$MgCl_2$	12.00	10.20
$CaCl_2$	19.43	13.90
KCl	50.15	12.35

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

NaCl	3.54
$MgSo_4$	1.39
$MgCl_2$	0.84
$CaCl_2$	0.41
KCl	0.40
	6.58

Source : Ellis and Everard Chemicals - January 1991

SEA FISH INDUSTRY AUTHORITY
Seafish Technology

MUSSEL PURIFICATION, DEVELOPMENT OF A MEDIUM-SCALE DEEP STACK PURIFICATION TANK

Seafish Report No. 387
Project Code No. FT4
MAFF Commission NBC16

March 1990
M. Boulter

This report describes a second, more extensive series of trials of a developed version of the tank which were carried out by Seafish over a three month period in 1989 at the premises of M. Butler. The tank employs high density stacking, a low water mussel ratio and a high water flow rate.

Six purification cycles were made with the tank using artificial seawater to which approximately 4% make-up was added after each use. The trials experimented with high water flow rates and differing stacking densities. Each trial was carefully monitored. For water temperature, pH, dissolved oxygen, salinity, ammonia and nitrite build up. The bacterial quality of the mussels was assessed before and after purification.

It is concluded that the tank operates satisfactorily provided that certain operating criteria are met. A maximum tank loading, minimum water flow, limited operating temperature range and limited water re-use are recommended, in the report. The water exchange rate required is far higher than that previously recommended by the MAFF operating procedures for mussel purification tanks.

The tank has now been granted an operating license by the Department of Health.

The work has been carried out under MAFF Commission NBC16. Seafish would like to acknowledge the help given by the following during the trials :-

M. Butler and D. Coulson, Boston
Environmental Health Department, Boston
Public Health Laboratory, Lincoln
J. Williamson, J J Shellfish, King's Lynn
Eastern Sea Fisheries Joint Committee

SEA FISH INDUSTRY AUTHORITY
Seafish Technology

Seafish Report No. 387 March 1990
Project Code No. FT4 M. Boulter
MAFF Commision NBC16

MUSSEL PURIFICATION, DEVELOPMENT OF A MEDIUM-SCALE DEEP STACK PURIFICATION TANK

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TABLE

No. 1 Bacteriological Results

APPENDIX 1 - Artificial Seawater Mixture

SEA FISH INDUSTRY AUTHORITY
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MUSSEL PURIFICATION, DEVELOPMENT OF A MEDIUM-SCALE DEEP STACK PURIFICIATION TANK

1. INTRODUCTION

The existing criteria upon which existing large-scale mussel purification is based requires mussels to be spread in large shallow tanks with access to a supply of clean seawater. This results in a purification plant that requires a lot of land, is labour intensive and generally remote from the source of mussel supply. To make purification more cost effective, Seafish are developing multi-layer systems with partial re-use of artificial seawater. These plants have a reduced land and labour requirement enabling them to be housed in a building, thereby offering much improved control of environmental and hygiene conditions. It also enables the plants to be sited near to the source of mussel supply, thus reducing transport costs. At an early stage Seafish carried out successful mussel purification trials demonstrating the principle of both multi-layer stacking (Ref. 1 & 2) and the re-use of artificial seawater (Ref. 3 & 4).

Seafish is now developing a series of standard plant designs to cater for the varying needs of industry.

Trials on the large-scale high density mussel purification tank at Kings Lynn successfully demonstrated the application of these principles of multi-layer stacking of mussels and partial re-use of artificial seawater in a commercial plant. The capacity of the tank at 1500kg was considered, however, to be too large for some operators. A scaled down version of the Kings Lynn tank was considered but a smaller multi-layer tank of 750kg nominal capacity, already under development at Boston, was worthy of further investigation. Following the limited success of initial trials in March 1989 of this medium-scale deep stacking purification tank (Ref. 13) which had been developed over three years by M. Butler and D. Coulson. Seafish and M. Butler have developed an improved version of this medium-scale deep stacked purificiation tank. The most significant of the improvements was a substantial increase in the pump capacity and hence flow rates.

The trials of this tank incorporated three changes to the standard MAFF purification criteria: multi-layer stacking up to five boxes deep, a maximum water flow based on up to five tank changes per hour, and boxes filled up to 150mm deep. The new tank differs from the large-scale tanks at Kings Lynn as it relies on a more rapid water circulation and a lower water/mussel ratio. However, the effective water flow rate is not controlled by the number of water changes alone but relates also to the tank length, and the Boston tank is relatively short. A series of monitored purification trials have been carried out by Seafish with this more developed tank to assess its effectiveness.

A small single layer Control Tank used in previous trials and operating to existing MAFF criteria was run alongside the experimental tank throughout the trials to obtain comparative data.

2. OBJECTIVES

To develop and prove a standard design of medium-scale deep stacked mussel purification tank, and in particular:-

2.1 To investigate the effect on purification of increased water flow and reduced ratio of water to mussels.

2.2 To investigate the effectiveness of purification with increased mussel density in the boxes.

3. OUTLINE OF TRIALS SEQUENCE

The sequence consisted of six consecutive purification trials in the deep stack tank over a period of 10 weeks in December 1989 - February 1990, re-using the artificial seawater. Comprehensive bacteriological, physical and chemical monitoring was carried out.

The first trial was restricted to initial investigation of various water flow rates and subsequent levels of dissolved oxygen, and no bacteriological analysis was made.

In the second trial the flow rate was set to its maximum and the quantity of mussels in each of the boxes was increased significantly to increase the total loading of the tank. In the third trial the high level of loading remained and the flow rate was decreased but had to be returned to its maximum level during the trial when dissolved oxygen levels dropped as tank temperature rose. Thereafter the flow rate was left at the maximum.

For the fourth trial the number of boxes in the tank was reduced but their individual loading was further increased to investigate the effects of a deeper layer of mussels. High water temperatures were becoming a problem and an alternative water input system was experimented with.

Up to this point the water had been re-used with a small percentage make up after each trial. However, in trial 4 there appeared to be problems caused by deteriorating water quality and so the old water was discarded and a fresh batch of artificial seawater was made up for trial 5 and re-used for trial 6.

In trial 5 the number of boxes was returned to the original number and the loading per box was increased slightly from that in trials 2 and 3. In an attempt to overcome the problem of low *E. coli* counts in the mussels prior to purification a method of artificial contamination was investigated.

In trial 6 the tank loading was returned to that of trials 2 and 3 but in view of the problems in trial 4 the loading of the control tank was increased to further investigate depuration in a deeper layer of mussels.

Following the trials the Department of Health granted an operating license for the tank conditional on certain operation criteria.

4. TRIALS SITE

The trials were conducted within the premises of M. Butler at the Riverside Industrial Estate, Boston.

5. TRIALS EQUIPMENT

5.1 Purification Boxes

50 Allibert type 11037 stack nest boxes of 37 litres capacity and external dimensions of 600mm x 400mm x 236mm were used. This is a significantly deeper box than those usually used for purification but forms part of an integrated handling system from fishing ground through to purification.

5.2. High Density Purification Tank

The tank is of wooden construction with an external wooden frame and lined with G.R.P. It has internal dimensions of 2.44m x 1.2m x 1.2m. It was painted with a 2 pot epoxy paint. General views of the tank are shown in Figure 1.

Pipework and fittings are in 1.5 inch ABS and UPVC plastics. P.V.C. sheet is used inside the tank for flow control screens.

Diagramatic views of the tank and water flow system are shown in Figure 2. The tank has its own circulation pump and U.V. sterilizer (2 x 30 watt tubes). Flow rate can be monitored by a flowmeter and can be adjusted by means of a valve. Water is delivered across the width of the tank at one end via a spray bar mounted above the water surface (see Figure 3). Two types of flow control screen have been used at the input end of the tank. One is a solid screen with a 150mm gap underneath. The other is a perforated screen with 140-10mm holes in rows of 10 holes. The water suction consists of a pair of ABS tee's joined in the centre of the tank (see figure 4). The tank is filled and emptied through two, 3 - way valves into a reservoir tank.

The tank is designed to hold 50 boxes stacked 5 deep and to be loaded and unloaded manually. At the maximum 75mm depth of mussels that MAFF has required for purification each box holds approximately 11.5kg of mussels and in this condition the capacity of the tank is 575kg. However, the tank is designed with high water flow rates in the hope of achieving a 100mm depth

of mussels to give a nominal 15kg per box and capacity of 750kg. At this nominal capacity there is approximately a 100mm gap above each layer of mussels which permits water circulation and prevents embyssment of the top layer of mussels on the box above.

At the nominal capacity the tank holds approximately 2.6m^3 of water and this corresponds to a water/mussel ratio of 3.5 litres/kg which compares with 6.1 litres/kg for the large-scale tanks at King's Lynn and 6.6 litres/kg for the control tank. The maximum output of the pump is 13.5m^3/hr which corresponds to an exchange rate of 5.1 times per hour at nominal capacity which compares to the MAFF previously recommended range of 1-2 times per hour and one change per hour in both the large-scale and control tanks. At maximum pump output and nominal capacity the water flow rate through the tank is approximately 12.5m/hr which compares with 6m/hr for the large-scale tank and 0.180m/hr for the control tank.

The bottom boxes sit on ribs moulded on the base of the tank which raise the boxes approximately 50mm from the base. The base slopes toward a drain for flushing out. When the purification water is pumped to the reservoir approximately 4% remains in the bottom purification tank with the detritus (mud, shell, faeces, etc) and is then washed away to prevent contamination of the next purification cycle. This 4% of lost water then has to be made up with new artificial seawater for the next purification cycle.

Provision was made in the design of the tank for a water chiller unit but it was not used during these trials as it was desired to investigate any water temperature problems without resorting to refrigeration.

5.3. Artificial Seawater Reservoir Tank

A G.R.P. lined tank of similar construction to the purification tank is used both as a reservoir and for the mixing of artificial seawater.

5.4. Control Mussel Purification Tank

Seafish installed on site a small Control Tank (Ref. 3 and 4), capable of holding a single tray of mussels (see Figure 5). The tank has its own U.V. sterilizer, pump, control and aeration and is designed to operate within the existing MAFF criteria for mussel purification.

5.5. Environmental Chamber used for Storage Trials

Manufactured by Cee-Tel Thermal Equipment Limited, the test chamber installed at IDU premises, Hull, has a 1000mm x 1000mm x 1000mm chamber and any required temperature profile between -30°C to 100°C can be maintained. The chamber was used for holding live mussels at 15°C. The accelerated spoilage so induced can be related to storage at lower temperatures and other comparable trials data.

6. INSTRUMENTATION

6.1. Temperature

Comark 9001 digital thermometer with probe.

6.2. pH

Jenway portable PH meter with temperature compensation.

6.3. Oxygen

Oxyguard Handy portable oxygen meter with temperature compensation.

6.4. Salinity

Dryden portable salinity meter.

6.5. Ammonia

Merck Aquaquant colourmetric test kit. Ammonia measured in the range 0.2 to 8.0 mg/l but can be extended to 80 mg/l.

6.6. Nitrite

Merck Aquamerck colourmaster test kit. Nitrite measured in the range 0.02 to 20 mg/l.

7. ARTIFICIAL SEAWATER

The artificial seawater (ASW) was made up using the five basic salts, as defined by MAFF Laboratory Leaflet No. 39, (Reference 5). Details are given in Appendix 1.

The salt content or salinity of seawater is usually expressed as number of parts by weight of salt in one thousand parts of weight of water. The unit 'parts per thousand' is indicated by the symbol °/oo. Salinities of 22°/oo, 27°/oo and 30°/oo are specified, all using five basic salts. The salinity of 27°/oo was chosen for re-use even though a lower salinity of 22°/oo is satisfactory. This was to allow a working margin for error (in a commercial environment) and avoid the danger that if salinity fell too low the mussels would not purify.

8. MUSSEL SUPPLY

D. Coulson supplied the mussels for trials 1,2,3,5 and 6.
An alternative source at Kings Lynn supplied the mussels for the fourth trial. All came from The Wash.

9. BACTERIOLOGICAL ANALYSIS

Several quantitative methods exist in the United Kingdom for the examination of bivalve molluscs for sewage contamination. These include roll tubes, pour plates and most probable number (MPN) techniques but there is apparently no national standard method. For the purposes of this trial an MPN method specified by P.A. West and M. R. Coleman was used (see Ref. 6). The Lincoln Public Health Laboratory were contracted to take samples throughout the trials. At the start of each trial three or four mussel samples were taken and at the end a further five with a water sample as well. Mussel and water samples were examined for Escherichia coli (E. coli) and Group D faecal streptococci. Faecal streptococci were to be used as an indication that purification had occurred in the absence of sufficient numbers of E. coil.

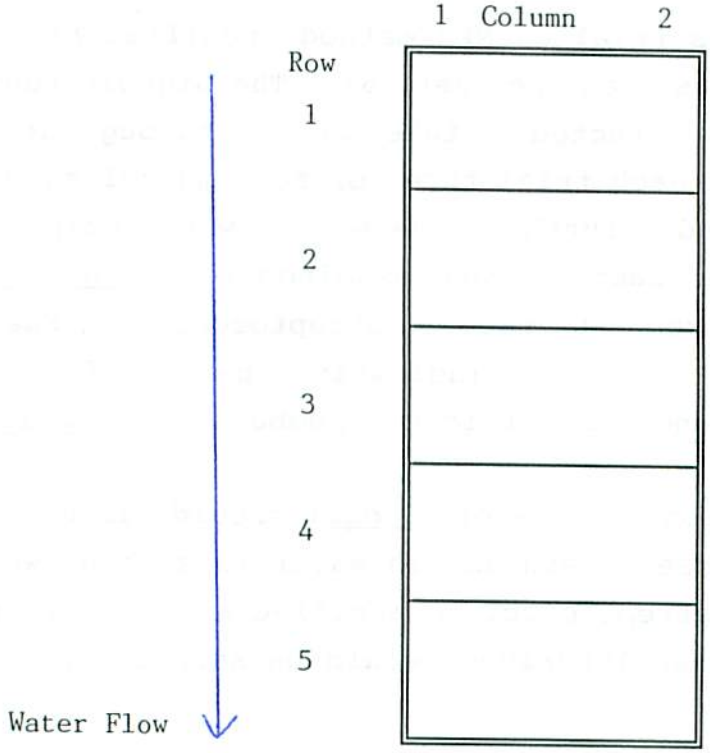
At end end of purification, counts of E. coli should all be less than 230 E.coli/100g mussel flesh and in water less than two E. coli/100ml. Counts of streptococci in purified mussels are not well defined but more than 1000/100gm would be suspicious.

9.1 Mussel Sampling

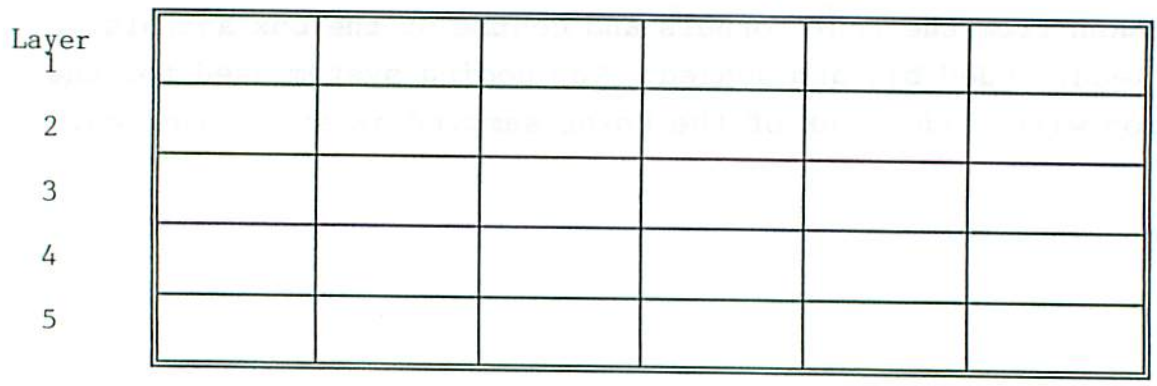
Each sample for bacteriological analysis contained a sufficient number of mussels to provide 50gm of shelled mussel meat (usually 12 to 15 mussels) and was taken from an individual box. Mussels were taken from the four corners and centre of the box and placed in a clean, coded bag and sealed. The coding system used for the location within the tank of the boxes sampled is shown overleaf.

TRIALS 1,2,3,5 AND 6

Plan View of Tank

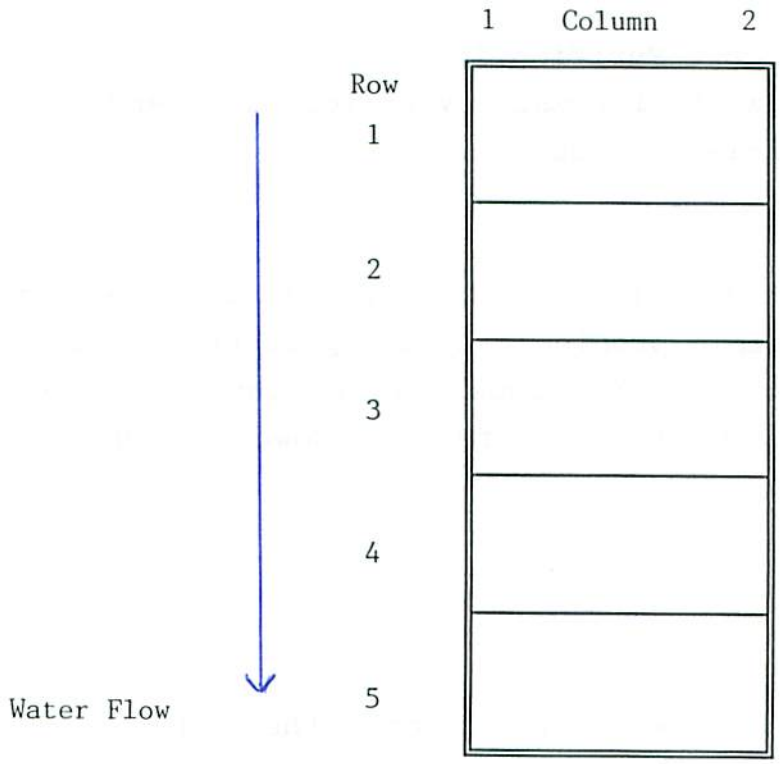


Side Elevation of Tank

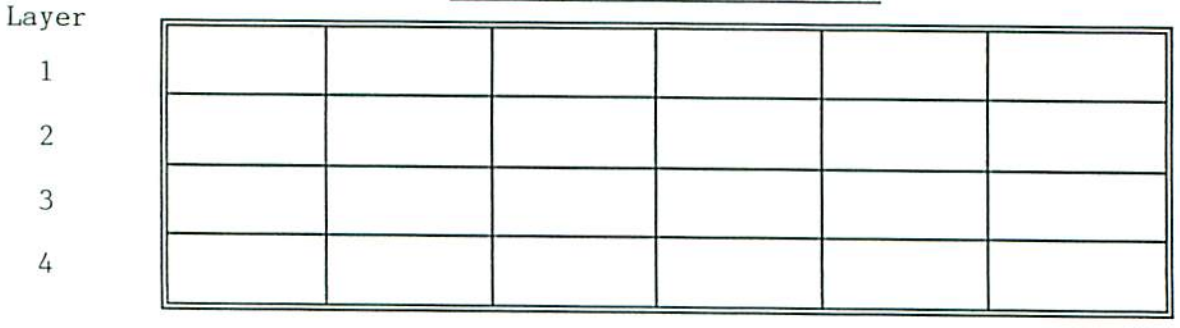


TRIAL 4

Plan View of Tank



Side Elevation of Tank



9.2 Mussels Samples - Pre-purification

Samples were taken from a variety of locations throughout the tank for each trial. Details of the locations are given in Table 1.

9.3 Mussels Samples - Post Purification

Samples were taken from a similar variety of locations and from the control tank as detailed in Table 1.

The bottom of the tank at the suction end is considered the most likely area for any possible problems as it represents the area where we might expect to see the lowest oxygen levels coupled with the maximum effect of detritus falling down through the tank.

9.4 Water Samples

A single water sample was taken just before the end of each purification cycle at the suction end of the tank.

10. TRIALS PROCEDURE

All trials involved the operation by Seafish staff of both multi-layer and control tanks with each purification cycle being monitored.

Each trial/purification cycle consisted of loading the boxes of mussels into the tank, filling the tank with water then circulating the water through the tank and UV for a minimum of 42 hours. The water was then returned to the reservoir, the boxes of mussels removed and the tank flushed out.

10.1. Summary of Tank Loading

Trial Number	No. of Boxes	Mussels per Box	Total Loading
1	50	11.5 kg	575 kg
2	50	14.3 kg	715 kg
3	50	14.3 kg	715 kg
4	40*	16.7 kg	668 kg
5	50	15.7 kg	785 kg
6	50	14.3 kg	715 kg

* This would correspond to 835 kg if 50 boxes were used

The control tank was filled with 12-14 kg of mussels in a 75mm layer in single box except for trial 6.

10.2. Filling Purification Tank with Artificial Seawater

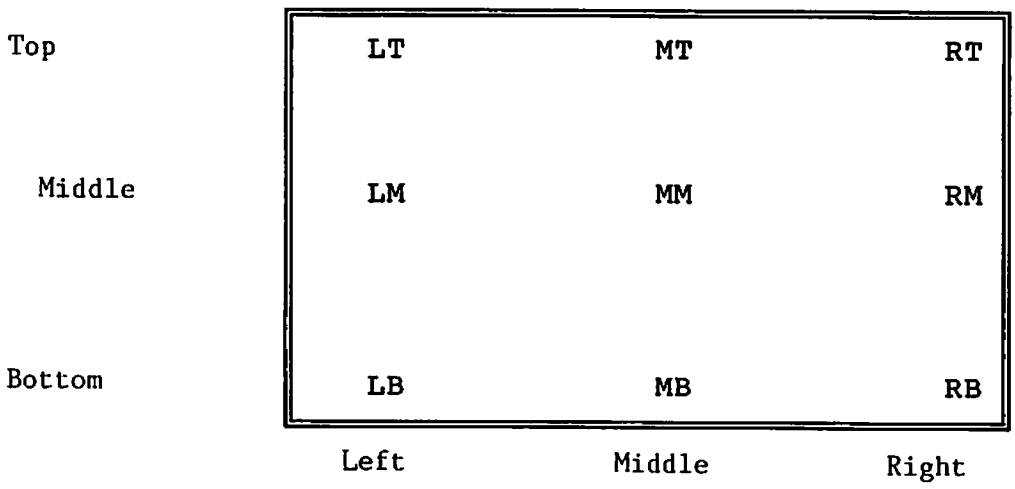
For the first trial 100% freshly made artificial seawater was transferred from the reservoir into the purification tank through the U.V. until the top layer of mussel boxes were covered. For trials 2,3, and 4 the tank was filled with 96% from the previous trial and 4% freshly made. The water was then replaced totally with fresh ASW on run 5 as there were problems with mussel activity on run 4 probably caused by water quality. The control tank was always filled with freshly made ASW.

10.3. Monitoring Trials

pH, ammonia and nitrite levels were measured at the end of each trial, with salinity checked at the start. Water temperature and dissolved oxygen levels were monitored throughout each trial. Dissolved oxygen levels were measured between the boxes and flow screens at either end of the tank, as this was the only accessible place for the DO_2 probe. See diagram below for the DO_2 measurement locations across the cross section of the tank.

OXYGEN PROBE LOCATIONS

Flow Screen



10.4 Emptying Purification Tank

Each trial operated on a 48 hour cycle which included filling and emptying the tanks. The minimum immersion time of the mussels in water was 42 hours.

In the high density tank, having first taken the water sample, the artificial seawater was pumped back to the reservoir.

After the tank was drained, the mussel samples for bacteriological assessment and holding trials were taken out. The remaining boxes were then mannually removed and re-bagged for disposal as unpurified mussels, bait or dumped on a tip.

10.5 Holding Trials

After trials 2 and 4, samples of mussels were taken from the deep stack tank and from the control tank. These were taken back to the Seafish laboratory in Hull. Here they were put into shallow trays and held at 15°C, whilst kept moist, in the Environmental Chamber. Mortality in each sample was then recorded (Ref 7).

10.6. Details of Individual Trials

10.6.1. Trial 1

This initial trial was to investigate the flow rates at which the tank could operate. The fifty boxes were filled with an average of 11.5kg (at the MAFF specified depth of 75mm) to give a total mussel mass of 575kg. This gave a water/mussel ratio of 4.6:1. Water flows of 13.5m^3/hr, 7.5m^3/hr, 8.2m^3/hr and 5.5m^3/hr were tried during the trial. The solid front flow screen was tried at 9inch and 6 inch clearance off the tank floor to assess any differences in flow patterns. Monitoring concentrated on measurement of dissolved oxygen levels throughout the tank to assess the adequacy and uniformity of flow. Bacteriological analysis was not carried out.

10.6.2. Trial 2

In this trial the fifty boxes were filled with an average of 14.3kg per box (nearly to the nominal capacity) to give a total mussel mass of 715kg. This gave a water/mussel ratio of 3.7:1. During this trial the flow was left at 13.5m^3/hr. The spray bar was modified during the trial to a "T" configuration from its original end feed to get more even water distribution. The spray bar holes were also enlarged from 10mm to 13mm.

10.6.3. Trial 3

In this trial the boxes were filled as in Trial 2. The water flow initially was only 8.5m^3/hr. However, by the second day this was increased to 13.5m^3/hr because oxygen levels were falling alarmingly low as water temperature increased.

10.6.4. Trial 4

In this trial only forty boxes were used. They were filled to an average weight of 16.6kg giving a total mass of 666kg in only 2.2m^3 of water. This gave a water mussel ratio of 3.33:1. Some of the boxes were filled to a depth of 150mm.

The spray bar on this trial was raised by 4 feet in an attempt to lower the water temperature. It was thought that the high water temperatures found in the tank would drop to the ambient air temperature with a larger water drop allowing the water droplets to cool in the air. The maximum water flow rate possible at this spray bar height was 12.5m^3/hr, this maximum rate was used henceforward during the trials.

There were problems during this trial as the mussels showed little sign of activity. Mussels taken out of the tank after the purification cycle and then put into fresh A.S.W. started to respond rapidly. This lead to the conclusion that the re-used water quality was suspect and therefore it was dumped.

10.6.5. Trial 5

The number of boxes was returned to 50. They were filled to an average weight of 15.7 kg giving a total mass of 785kg. The water mussel ratio was 3.38:1.

Due to low E.coli counts pre-purification in the previous trials, it was decided on this trial to try artificially dosing mussels to in E. coli rich water prior to purification. A fresh batch of A.S.W. was made up into which we poured an E. coli culture of nominally 2.5×10^7 E. coli. This would dilute down to 1000/m.p.n. per 100ml of A.S.W. This water was then pumped into the purification tank holding the mussels and circulated for two hours without the U.V. lights on. It was hoped this would be adequate to boost the E. coli levels.

The contaminated water was drained down and dumped and mussel samples taken out for bacteriological analysis. The tank was then refilled with fresh A.S.W. and the purification process started.

During the trial the solid flow screen at the front of the tank was replaced by a perforated flow screen to compare oxygenation patterns between the two types.

The boats from Boston were not fishing during this period and so an alternative source from King's Lynn was employed. It turned out that these mussels had extremely high levels of contamination prior to artificially dosing.

10.6.6. Trial 6

In this trial the original source of mussels at Boston was employed and the boxes were filled the same as in Trial 2, approximately 715kg. This gave a water/mussel ratio of 3.7:1.

For further investigation of purificaton in deep layers the box was removed from the control tank and the entire tank base between the flow screens was filled with 41 kg of mussels laid out in a sloping layer increasing in depth from 3 inch at the input end of the tank to 6 inch at the suction end.

11. RESULTS AND DISCUSSION

11.1 Bacteriological Results

The bacteriological results are shown in Table 1 overleaf.

In order to show that satisfactory purification has occurred it is necessary to have high initial counts in the mussels before purification and final counts of less than 230 E. coli per 100gms. Although in general initial results were low, 7 out of the 17 pre-purified samples were over 230 E. coli per 100gms and of these four were over 1000 E. coli per 100 gms.

Satisfactory results were obtained from 17 of the 19 post purification counts. The two poor results are both from Trial 5 where levels pre-purification were very high at 16,000 E. coli per 100gms. It is assumed that one of these results must have been from a dead mussel as it gave a reading of 5400 E. coli per 100gms. The other result was just above the permitted level at 270 E. coli per 100gms. Counts of faecal streptococci can be used as an indicator of purification when E. coli counts are low. The faecal streptococci showed higher initial counts with 12 out of 17 pre-purified samples giving readings into the thousands. The reduction required to indicate purification is a factor of 10. This is apparent in all cases. In 17 out of the 19 post-purification samples the levels dropped to zero.

Considered overall, the bacteriological results indicate that purification was occurring.

The artificial dosing of the mussels in trial 5 did not lead to any significant increase in the levels of E. coli. Further reading indicates that the dosing level should have been 10 times greater and for a period of six hours (Ref 11 and 12).

Table 1 — Bacteriological Results

Trial and Sample	Pre E.coli	Post E.coli	Pre F.strep	Post F.strep
T2 1-1-1	60	<20	600	0
T2 3-3-2	110	<20	200	0
T2 5-5-1	50	<20	400	0
T2 5-1-1		<20		0
T2 Control		<20		0
Mussel Mass	715KG			
T3 1-1-1	170	<20	5700	0
T3 1-2-2	170	<20	400	0
T3 3-2-1	130	<20	3100	0
T3 5-5-1	490	<20	2000	0
T3 Water		<1		
T3 Control		<20		0
Mussel Mass	715KG			
T4 1-1-1	20	<20	3000	0
T4 3-3-1	60	20	5800	350
T4 4-2-2	70	20	1400	0
T4 5-4-1	80	20	200	0
T4 Water		<1		
T4 Control	70	20	1400	0
Mussel Mass	666KG			
T5 1-1-1	9200/16000	150	3100/3300	0
T5 3-3-1	16000/16000	5400	11000/6200	0
T5 3-2-2		270		50
T5 5-5-1	16000	220	9100	0
T5 Water		<1		
T5 Control	16000	<20	3100	0
Mussel Mass	784 KG			
T6 1-1-1	1100	<20	1300	0
T6 3-3-1	490	40	1400	0
T6 5-5-1	330	<20	1600	0
T6 Water		0		
IControl top		<20		0
Control Middle		<20		0
Control Bottom		<20		0

Sample position code | Row - layer - Column

11.2 Mortality

Trials by Seafish have shown a relationship between mussel storage temperature and mortality. Storage for one day at 15°C approximates to three days storage at 5°C and three and half days storage at 0°C. Samples of mussels from both control and multi-layer tanks were taken after trials 2 and 4 and held at 15°C in the environmental chamber at Hull. The incidence of mortality is shown in Figure 6. The results from trial 2 show a small difference in the mortality of mussels from the deep stack and control tanks. Mortality results typically show variations from trial to trial depending upon the handling and condition of each individual sample of mussels (the control sample came from a single bag of mussels) and given the weight of evidence already accumulated on deep stock purification this particular result is not considered significant. Trial 4's results show that there was a large and significant adverse effect upon the mussels in the deep stack tank during that trial, probably caused by poor water quality although testing for salinity, pH, ammonia and nitrates did not reveal exceptional levels. It is assumed that some biological action had occurred after trial 3 resulting from the high water temperature at the end of that trial, 16.2°C, and the water being left still for 5 days in the reservoir tank.

11.3 Water Temperature

Purification relies upon the shellfish filtering naturally. Mussel filtration activity is effected by water temperature and will reduce significantly once temperatures fall below 5°C (Reference 7). MAFF recommend a minimum water temperature of 5°C (Reference 8). It was observed during the trials that mussel activity appeared to increase at water temperatures of 10°C or more.

Unfortunately as water temperature rises, its ability to hold oxygen decreases and consequently a maximum water temperature

needs to be specified. As discussed in 11.4 below, a maximum water temperature of 10-15°C is recommended.

The temperatures in the deep stacked tank, control tank and ambient air for each trial are shown in Figure 7. The straight lines are between plotted points and do not represent a constant rate of temperature change between readings.

High water temperatures in the deep-stack tank during the six trials proved a problem with the temperature rising to a maximum of 16.2°C. The temperature rise during was due to the high flow rates and an inefficient type of pump transferring heat into the water. The insulative property of the G.R.P. lined wooden tank prevented the temperature from dropping back to ambient. Increasing the height of the input cascade had only a minor effect. Fitting a more efficient pump with a less direct heat path from the motor to the impellor would improve the situation. Using a refrigeration system to chill the water would no doubt be effective but at additional cost and complexity.

11.4 Oxygen

Dissolved O_2 levels (mg/l) in the multi-layer purification tank are shown in Figure 8. The points represent average oxygen levels during each trial at the particular location stated.

Monitoring of oxygen levels is a good indicator of the effectiveness of tank design in producing an even flow rate throughout the mass of mussels of water sufficiently oxygenated to permit natural filtration activity. Low levels of oxygen in the water will inhibit filtration activity, a level of 5mg/l or above is recommended at tank water temperatures of 10°C.

This tank had a major design difference in respect to how it achieved an adequate oxygen flow through the tank, compared to the large scale purification tank system designed by Seafish (Reference 2). In this tank the means of maintaining adequate

dissolved oxygen levels and even flow is to pass the water through the input cascade and tank at a high rate without using an aeration system in the tank.

Figure 8 shows that there is little difference in oxygen levels throughout the cross-section of the tank. This is an indication that an even flow is present and that stratification is not occurring. This is essential if the tank is to purify mussels successfully. Both the solid flow control screen with gap at base and the perforated flow control screen were effective in this respect.

In Trial 1 there was a large variation in oxygen levels as the water flow rate was altered from 5.5m^3/hr to 13.5m^3/hr. Below 7.5m^3/hr the water flow rate was unable to sustain oxygen levels above 5.0mg/l.

In Trial 2 the oxygen level in the water was high at a sustained 13.5m^3/hr flow rate despite the increased loading of the tank, the levels of dissolved oxygen reducing along the tank on average to 7.5mg/l at an average temperature of 10.9°C.

In Trial 3 the oxygen levels in the water were again lower at a reduced flow rate of 8.5m^3/hr and higher temperature, the levels of dissolved oxygen reducing on average to 5.0mg/l at an average temperature of 11.9°C.

In Trials 4,5 and 6 the oxygen levels were high even though the temperature rose up to 16.2°C. The water flow rates were at a maximum of 12.5-13.0m^3/hr. Trial 4 had levels of dissolved oxygen reducing on average to 7.2mg/l with an average temperature of 13.3°C (although in this trial the mussels were not very active because of the water quality). Trial 5 had levels of dissolved oxygen reducing on average to 6.5mg/l with an average temperature of 10.9°C. Trial 6 had levels of dissolved oxygen reducing on average to 6.5mg/l with an average temperature of 11.8°C.

It is known that mussel activity increases with water temperature, with the mussels becoming particularly active at temperatures greater than 10°C (Reference 2). If dissolved oxygen levels above 5mg/l are to be maintained to ensure optimum purification, then a maximum water temperature must be specified. An increase in mussel mass will also increase oxygen consumption thus a maximum mussel density must also be specified.

To make it unlikely that oxygen levels will drop below 5mg/l and thus possibly affect mussel purification, it is recommended that a maximum temperature of 15°C is allowed. This should be in conjunction with a maximum mussel mass of 750kg. The tank must have a water flow of at least 8.5m^3/hr at temperatures of 5 to 10°C and a water flow of at least 12.5m^3/hr at temperatures of 10-15°C if adequate oxygenation is to be maintained. It is important to stress that the system will not work at MAFF specified flow levels of one change per hour. Specification of flow in changes per hour is not necessarily relevant to all types of system, particularly when the water/mussel ratio is low.

11.5 Water Analysis

11.5.1. Ammonia

Figure 9 shows the ammonia levels at the start and finish of each purification cycle, expressed in terms of ammonium concentration in parts per million (PPM). During the trials the ammonia level rose, then reduced before the following trial one week later. The rises in the deep stack tank were up to 20 ppm dropping to 0.2 ppm before the next run.

The water/mussel ratios used in this tank are significantly less than in the large-scale tank at King's Lynn and this leads to a more rapid ammonia increase in the water after two or three re-uses. After a third re-use in the large-scale tank ammonia levels rose to 5 ppm compared to up to 20 ppm in the Boston tank after two re-uses.

If the water was re-used on a longer term basis the ammonia level would rise until it reached a stabilised level. This occurs when any additional ammonia is being broken down by bacteria into nitrites and subsequently nitrates in the water. Laboratory trials have shown some evidence of higher than 40ppm of ammonia starting to effect mussel mortality (Reference 10). This would be unacceptable in a commercial situation.

A greater build up of ammonia might be seen with a more prolonged use under normal operating practice where the tank could be used up to three times per week. A maximum re-use of three times is recommended for this tank.

11.5.2 Nitrite

Figure 10 shows the nitrite levels at the beginning and end of each purification expressed in terms of total nitrite in ppm. The level is seen to rise during the trials and then fall before the next trial.

The build-up of nitrates is due to the effect of nitrifying bacteria attached to the boxes and surfaces of the tank, breaking down the ammonia. Any subsequent drop in nitrites is due to other nitrifying bacteria breaking the nitrite into nitrates. However nitrates were unable to be measured with the test kits available. Nitrate is toxic to mussels but at what level is unclear.

11.5.3. pH

Figure 11 shows the pH levels in the tank. They are all well within the expected region for such a system.

The rise in pH up to Trial 4 (when the water was dumped) and again from Trial 5 to Trial 6 is probably due to the aeration of the tank. The aeration removes CO^2 from the water, which is naturally present in tap water, and thus the pH level rises.

11.6. Deep Box Stacking

Operation at the nominal capacity, at a mussel depth of 100mm, appeared to present no problems.

In Trial 4 some of the boxes were filled deeper but unfortunately the mussels were very clean prior to purification and, in addition, the mussels showed very little signs of activity due to a water quality problem. Thus it is not possible to conclude anything from that trial.

In trial 6 when one end of the control tank had been filled to a depth of 150mm, after 24 hours the mussels had all moved so that there was even layer of 110mm depth across the tank. These mussels purified, dropping from 1100, 490 and 330 m.p.n. *E. coli* per 100gms to <20 m.p.n. *E. coli* in three samples. The faecal streptococci results dropped from 1300, 1400, 1600 to 0.

12. CONCLUSIONS AND RECOMMENDATIONS

1. The purification of mussels in this design of purificiation plant, of high density, low water/mussel ratio, and high flow rate was successful provided that certain operating criteria are satisfied.

2. A nominal tank capacity of 750kg is recommended, this gives a water/mussel ratio of 3.5 litres/kg and a 100mm depth of mussels in each box without apparent harmful effect.

3. A tank operating temperatures range of 5 to 15° C is recommended. To avoid overheating in warm weather, refrigeration of the water or operating environment may be required.

4. At an operating temperature of 10°C a minimum water flow rate of 8.5m^3/hr is recommended to provide adequate oxygen to the mussels but at 15°C this must be raised to at least 12.5m^3/hr. Flow rates as high as 13.5m^3/hr, which corresponds to 5.1 water changes per hour and a flow rate through the tank of 12.5m^3/hr have been tested without apparent harmful effect.

5. It is recommended that for this high water/mussel ratio design the artificial seawater should be completely replaced after three purification cycles.

6. Despite exceeding the previously recommended MAFF general operating criteria, particularly in flow rate, on the basis of the above this particular design of plant has been approved by MAFF and granted an operating license by the Department of Health.

7. It is further recommended, that the water circulation through the U V is continued to inhibit biological action in the water. This is based on the problems of maintaining

the water quality when the plant is left idle at high ambient temperatures and particularly for extended periods. Similar difficulties were encountered at Kings Lynn.

13. FURTHER WORK

1. A trial carried out over a week's commercial operation should be carried out to monitor the ammonia and nitrate build-up which occurs.

2. A trial should be carried out on the large-scale tanks at King's Lynn to investigate replacing the direct aeration by increased flow rates.

3. Further work on the permissable depth of mussel layers in purification plants should be carried out as no technical limitation has been found in the trials to date.

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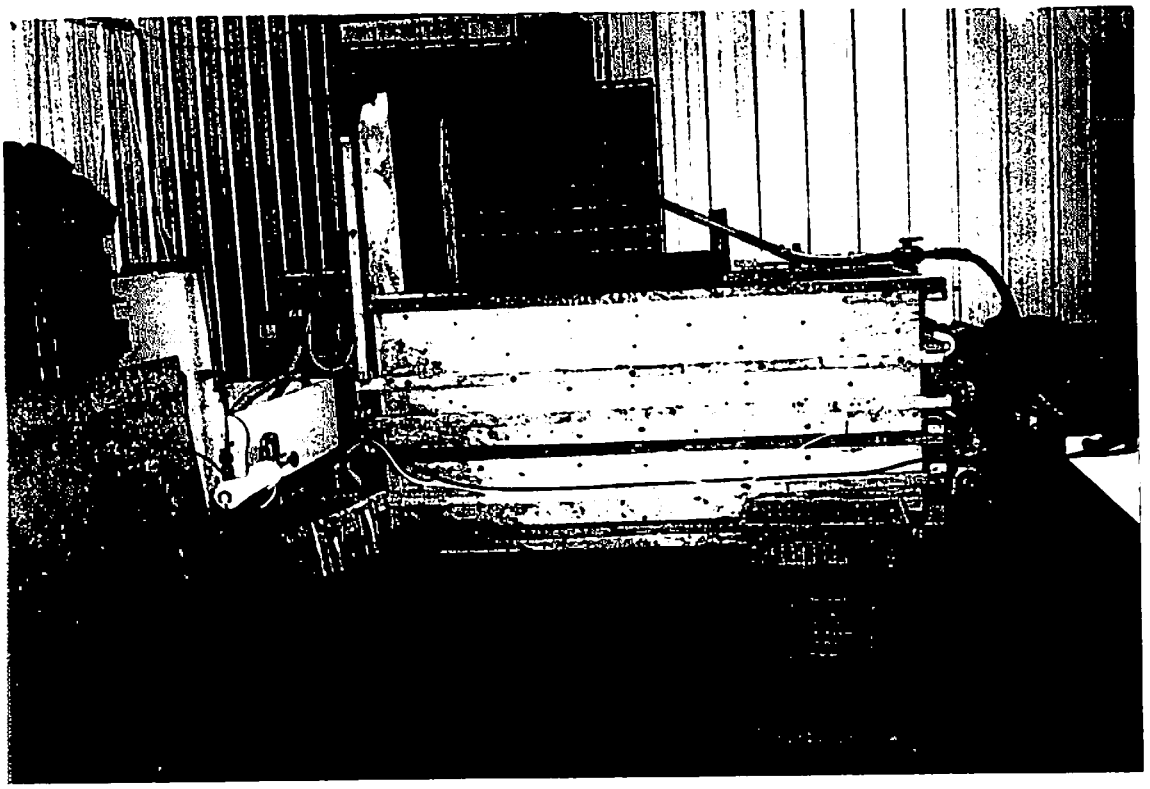
View of the Boston Tank with type 11027 boxes Fig 1



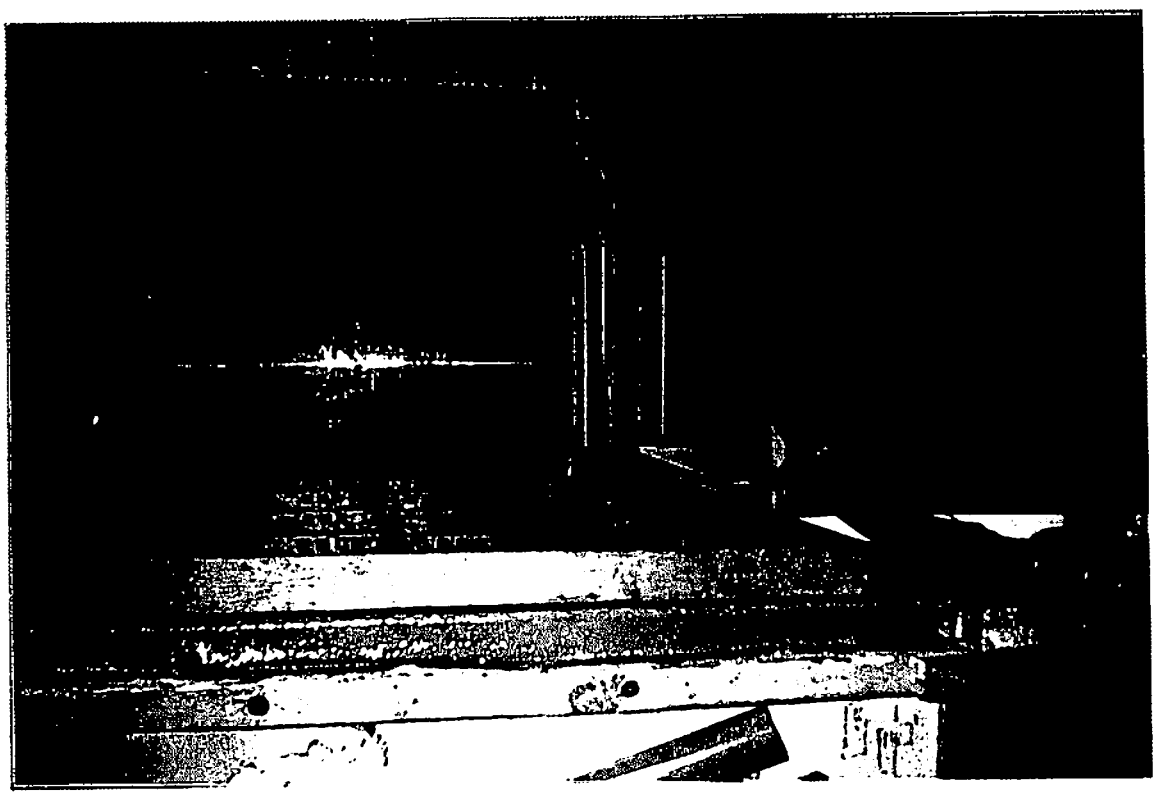
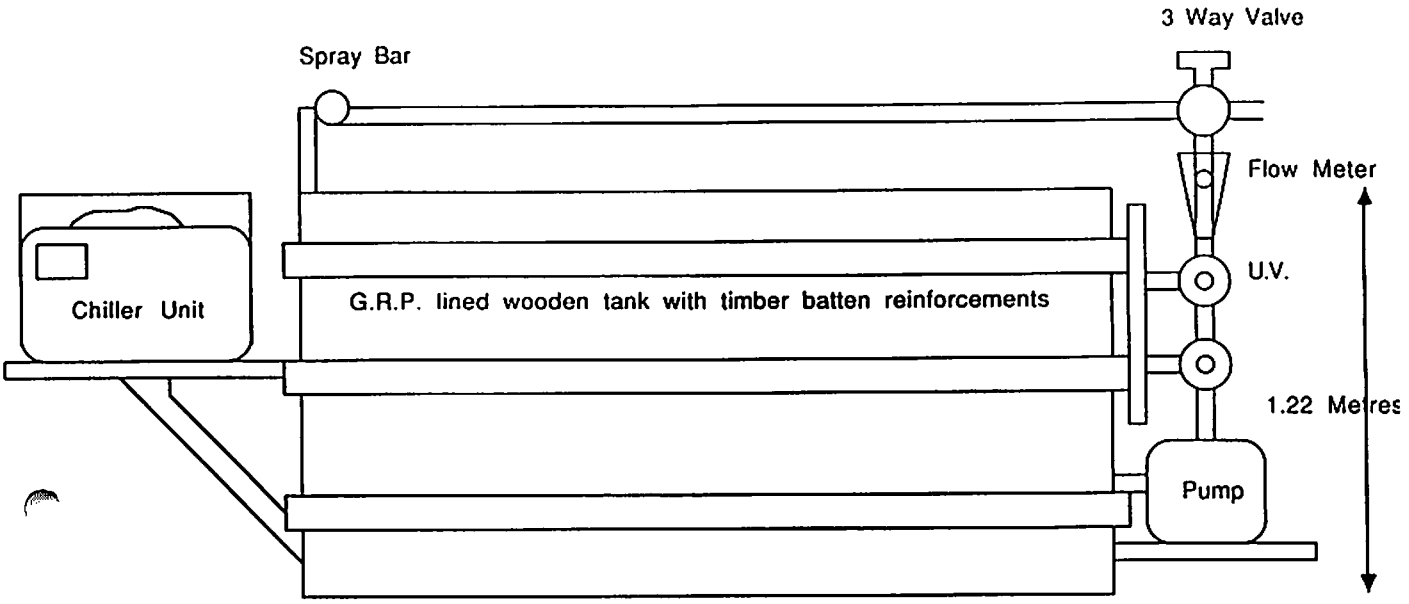
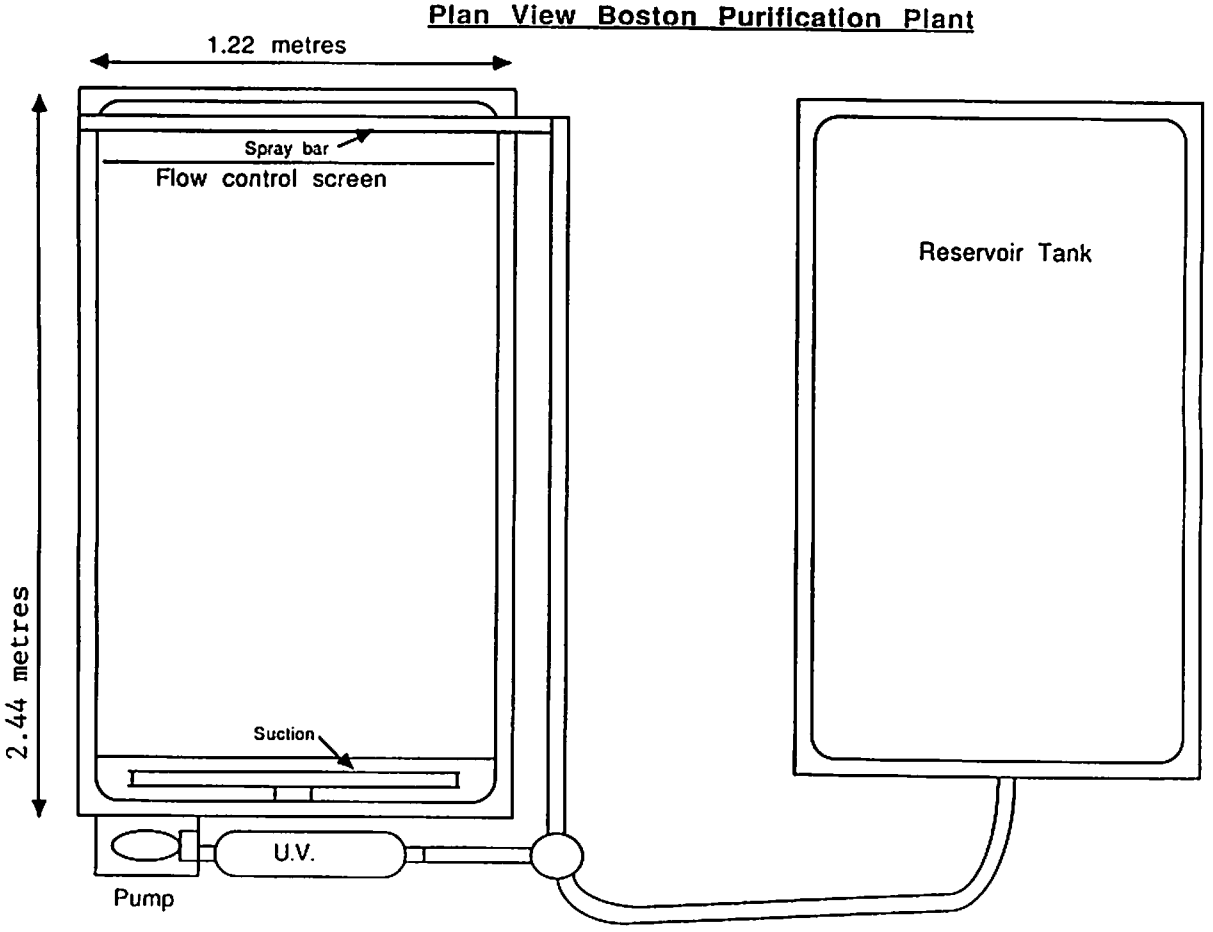


Fig. 2

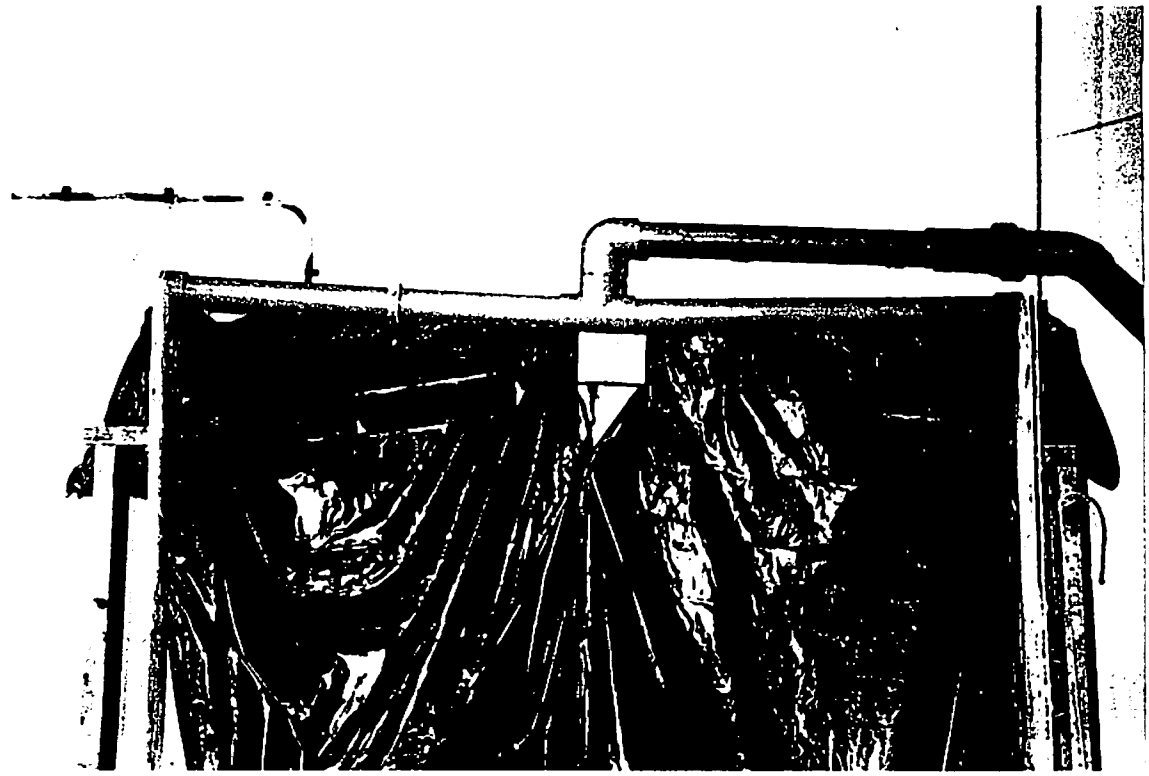
Side Elevation of Boston Purification Plant



Plan View Boston Purification Plant



Tank Spray Bar



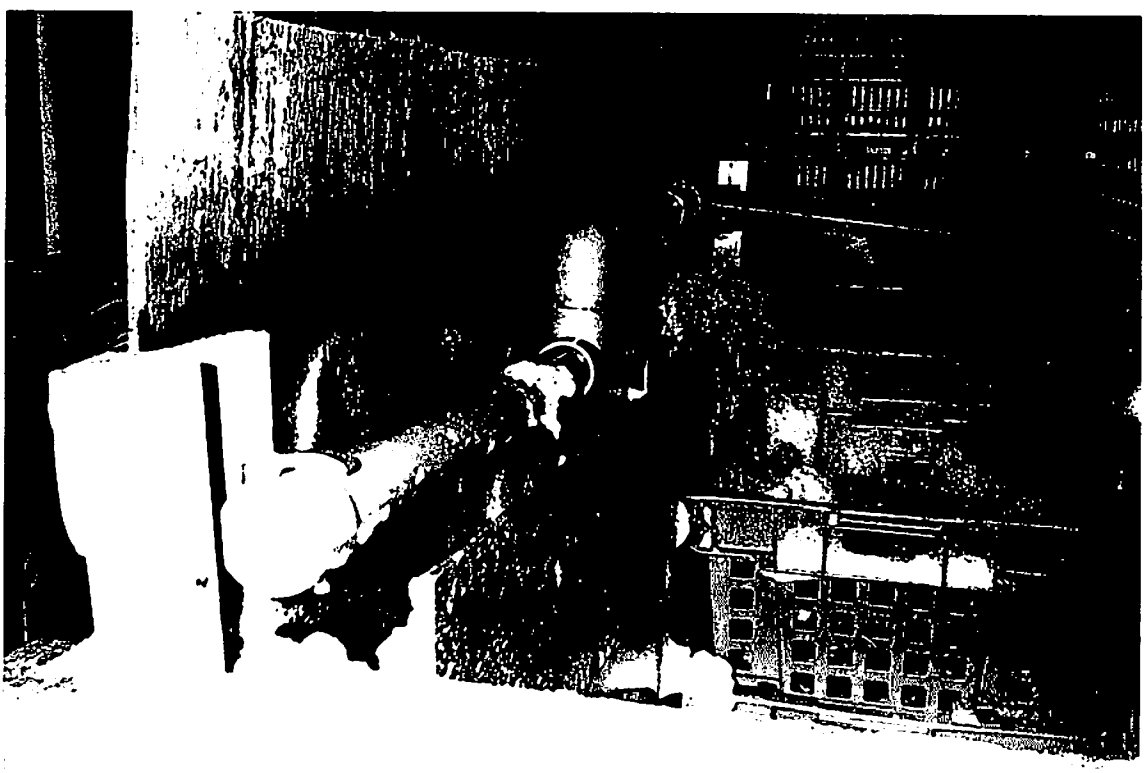
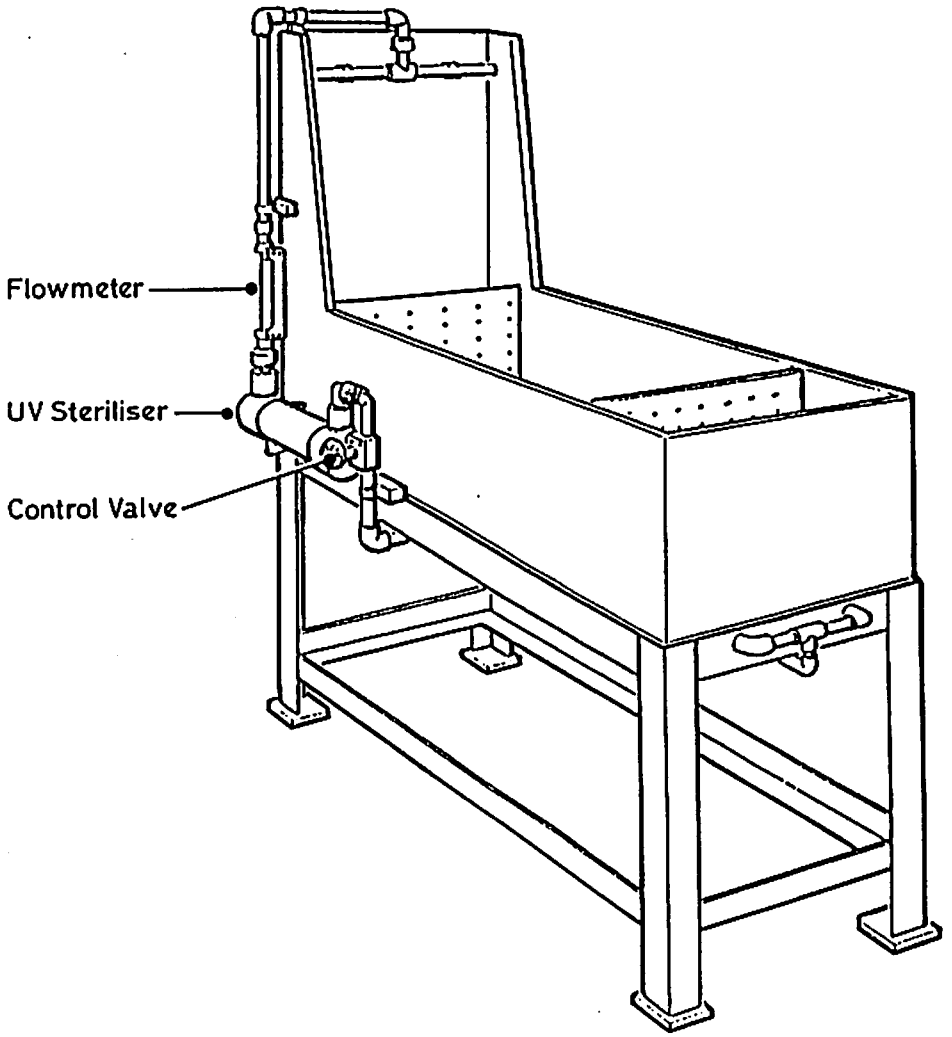


Figure 5 Control Purification – Equipment Specification



TANK - Constructed in marine ply with light blue epoxy resin surface finish. Mild steel stand.

PIPEWORK/VALVES - ½" A.B.S.

PUMP - Nikkiso Magpan CPR8. Centrifugal pump with magnetic drive. Maximum capacity 6 l/min at 1 metre head. Mounted under tank.

ULTRA VIOLET STERILIZER - UVAQ 15/3P with 15 watt tube.

FLOWMETER - Paton PG 1 - 10 l/min.

AERATION - Atlantis B800 air pump mounted on back of tank feeding two 300mm diffuser blocks on tank base.

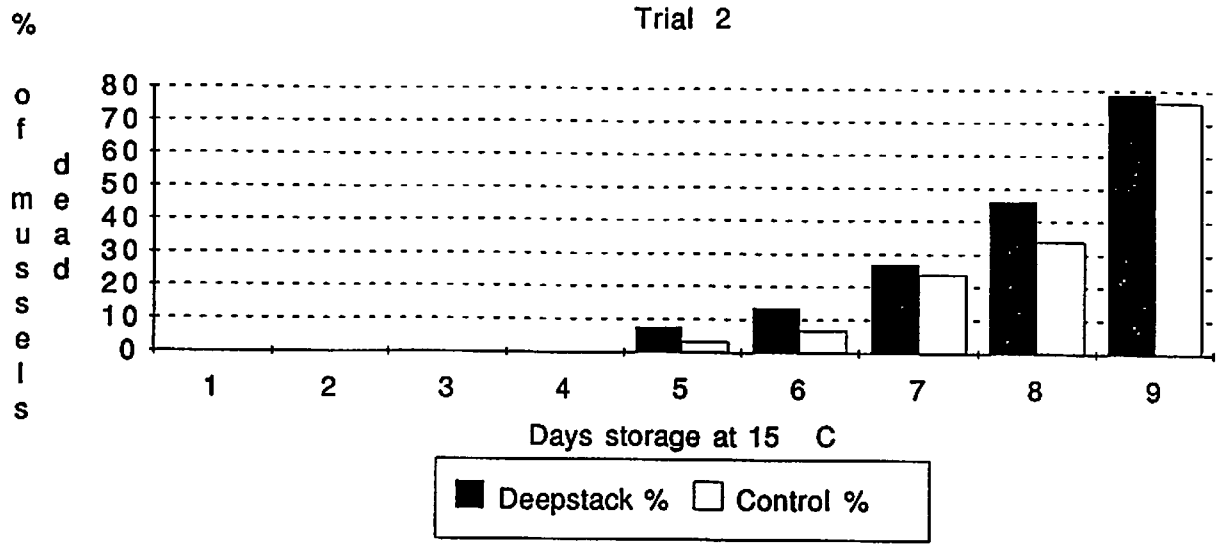
WATER CAPACITY - At working depth of 180mm, 110 litres (24 gallons) and 340mm, 206 litres

TRAY - Up to two Allibert Type 41042 (752 x 448 x 167 mm)

HEATING - 300 watt aquarium heater used with Digistat temperature controller.

Fig 6

Comparison of mortality rate between deep stack and control tank



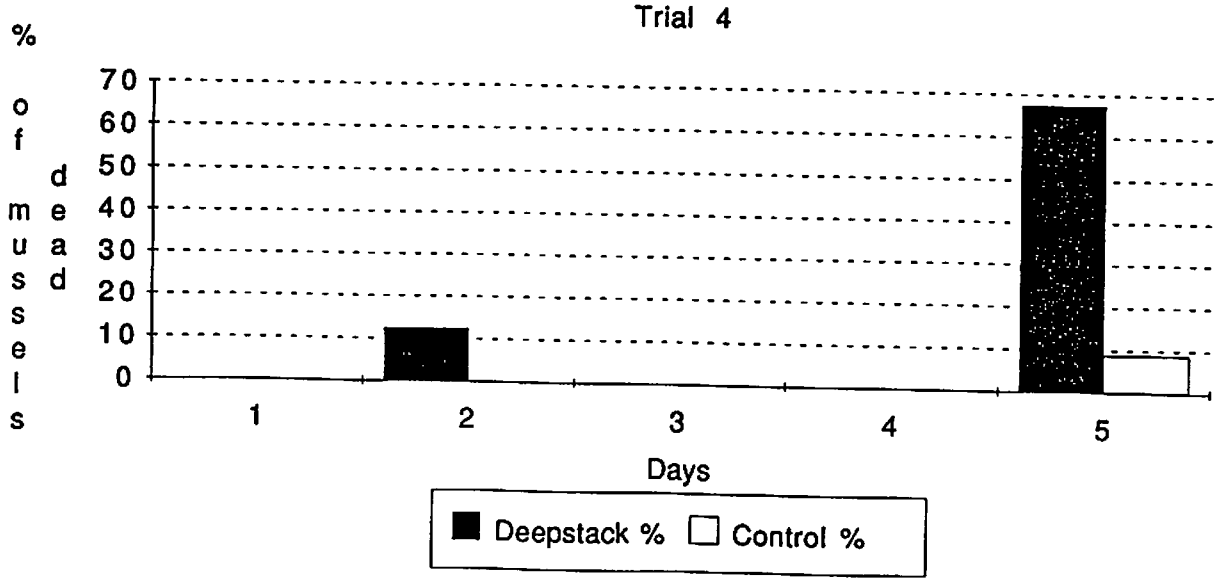
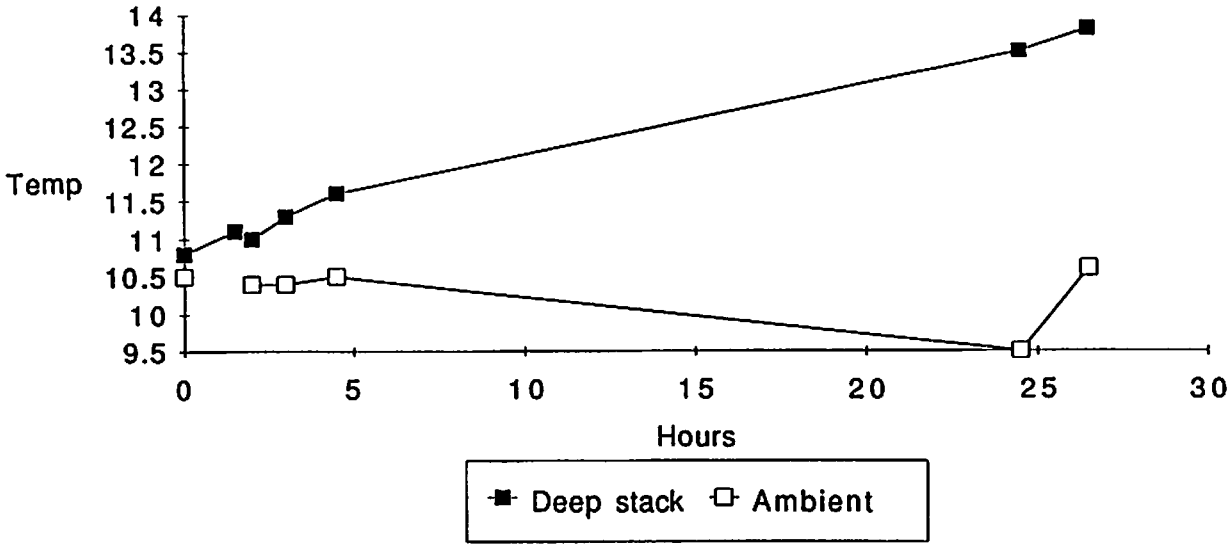


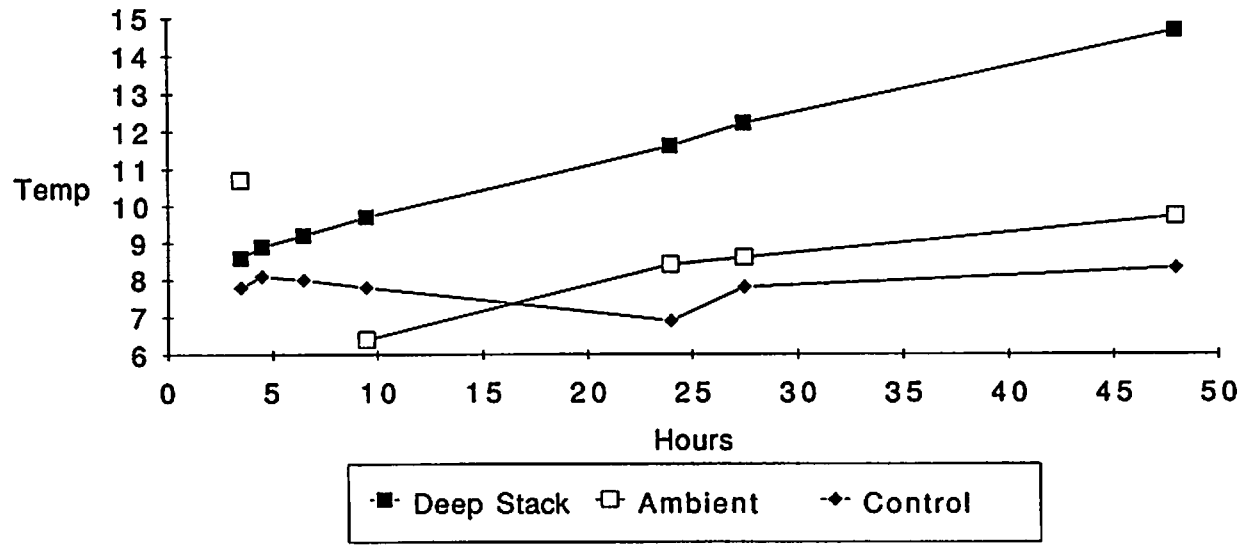
Fig. 7

Temperature changes during purification

Trial 1



Trial 2



Trial 3

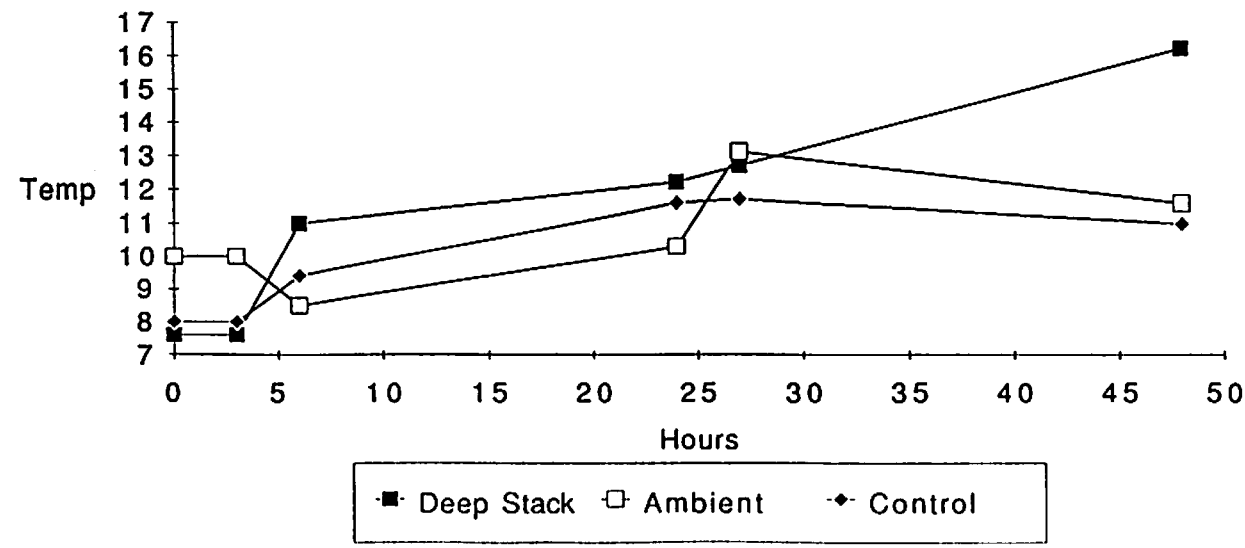
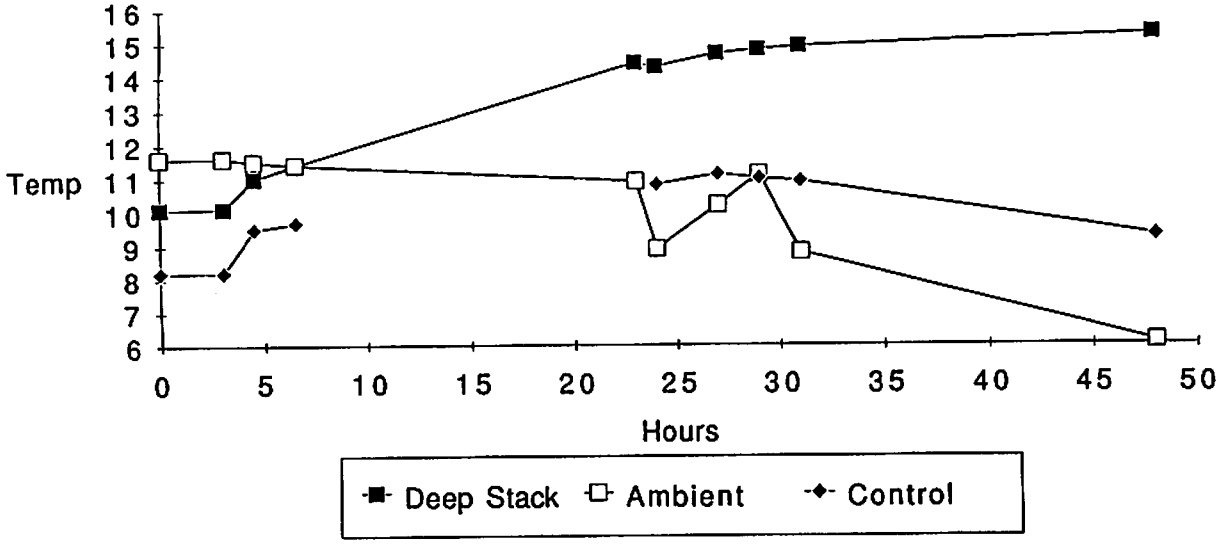
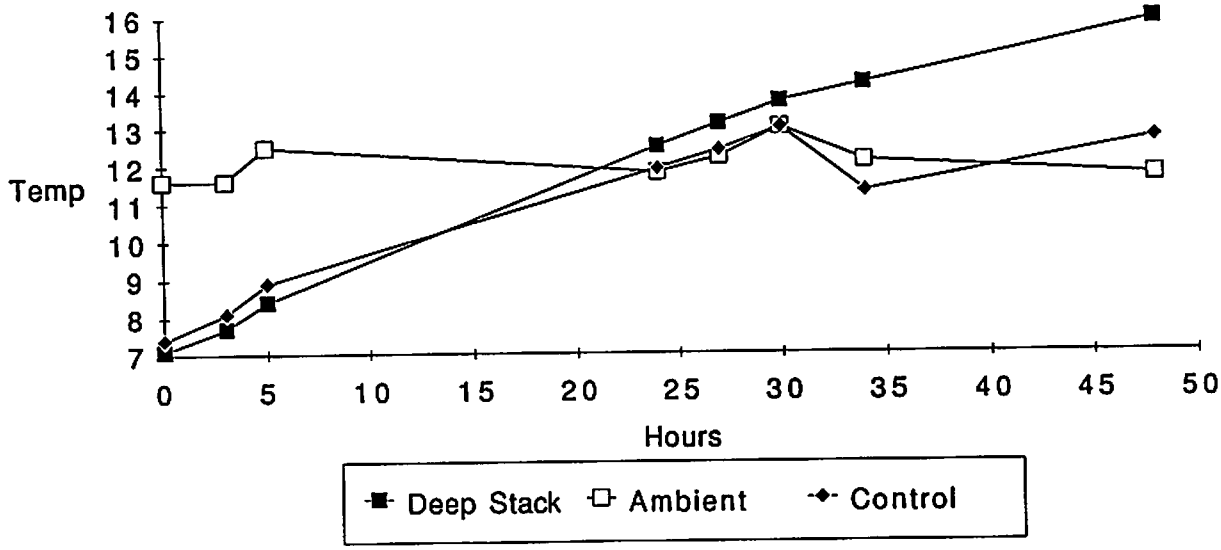


Fig. 7
(cont.)

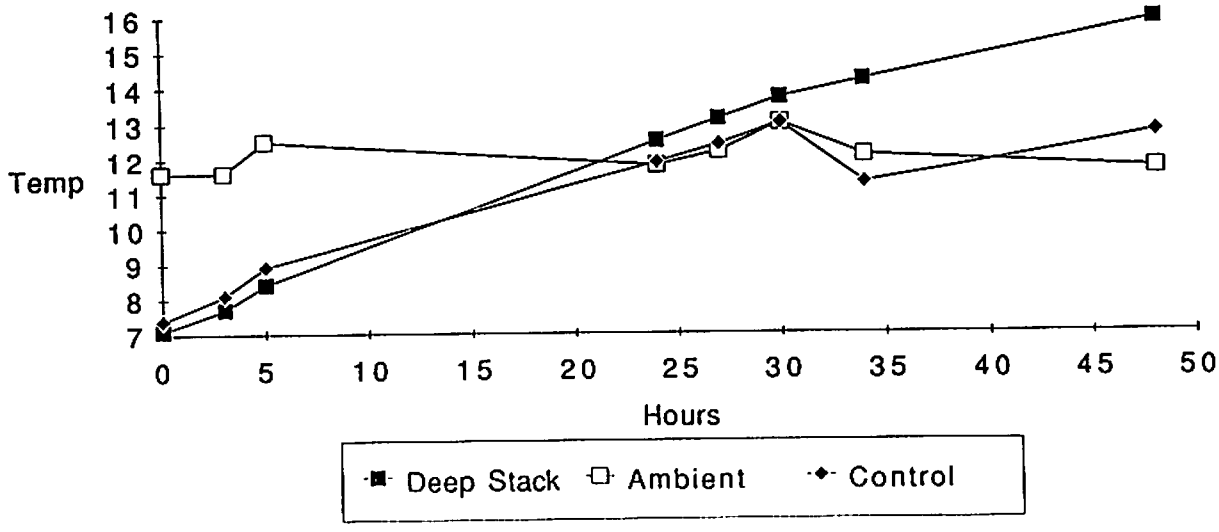
Trial 4



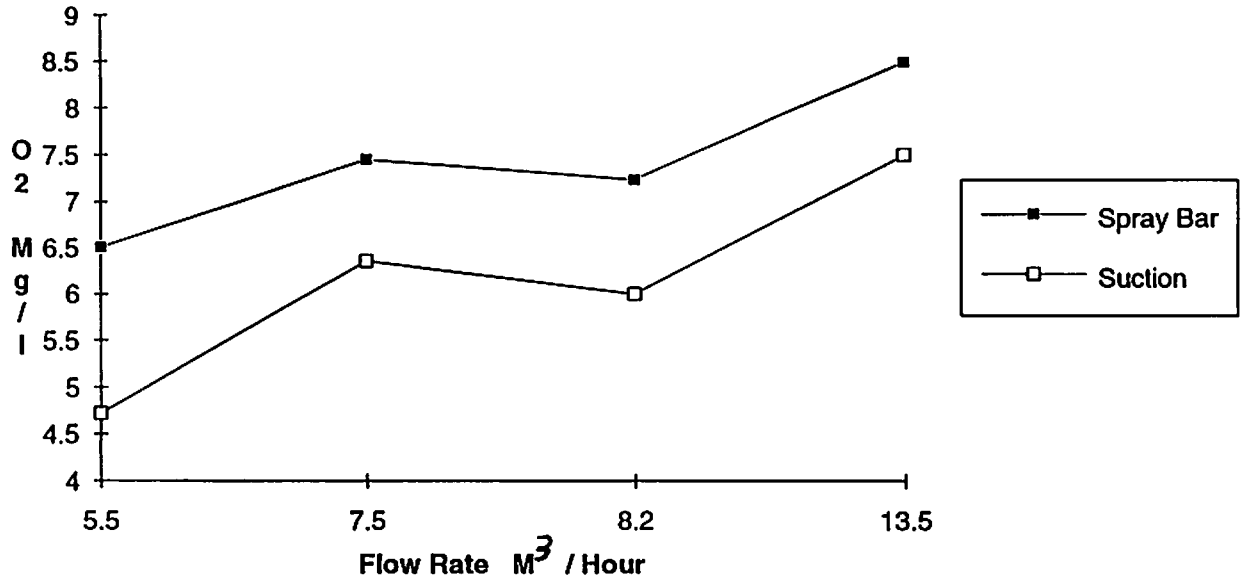
Trial 5



Trial 6



Trial 1 Mean Oxygen Levels During Purification



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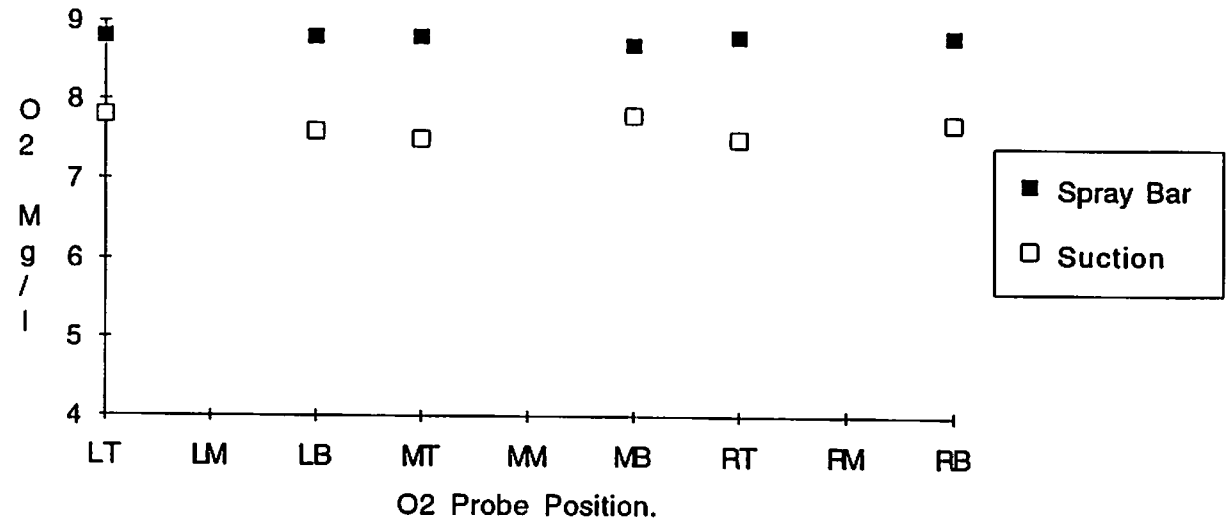

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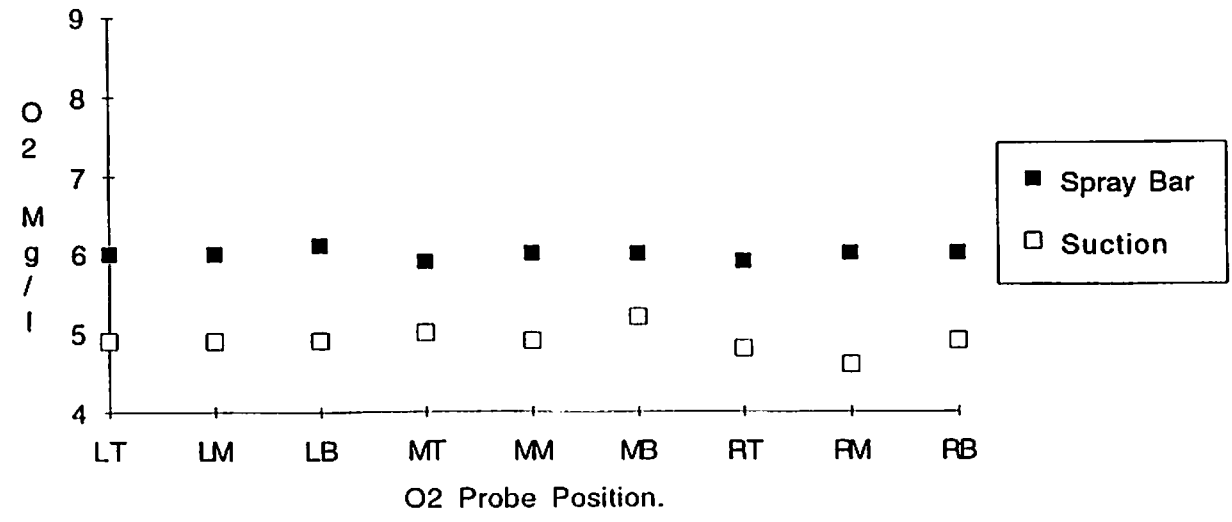
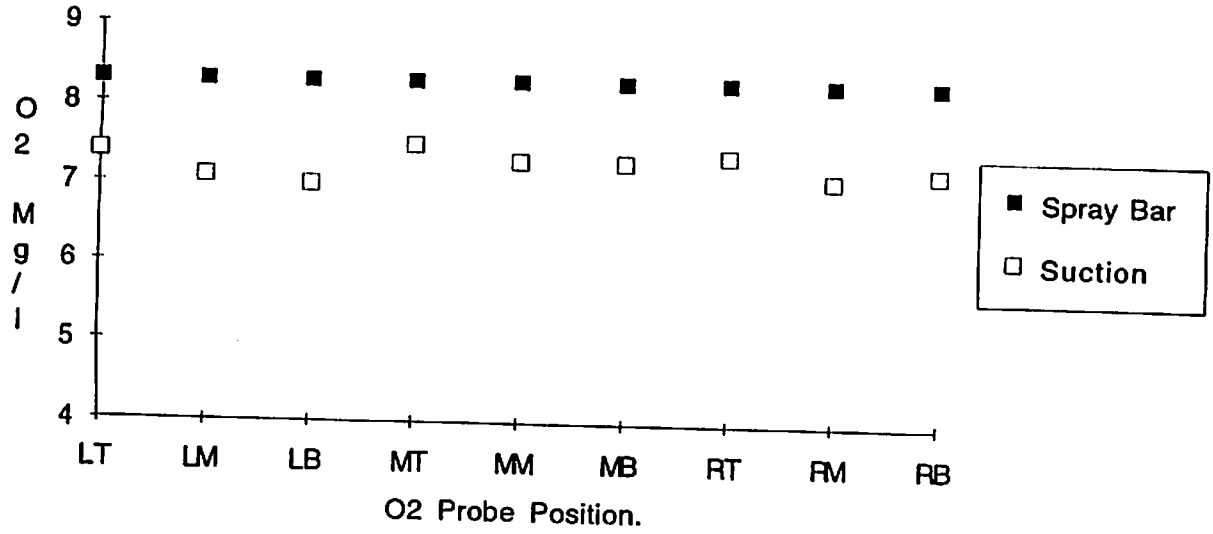


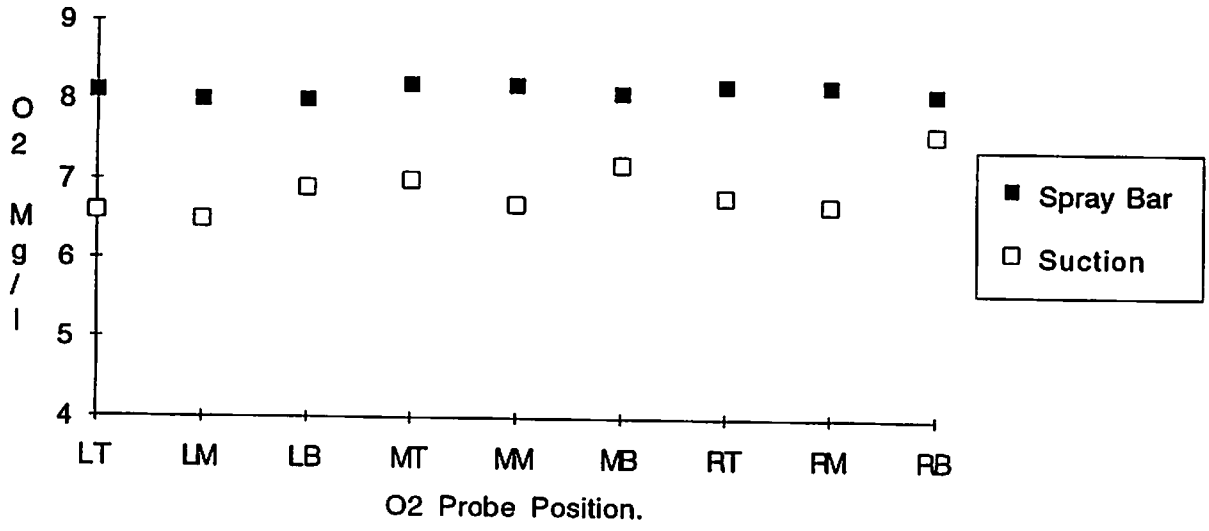
See Section 10.3 for probe position codes

Fig. 8
(cont.)

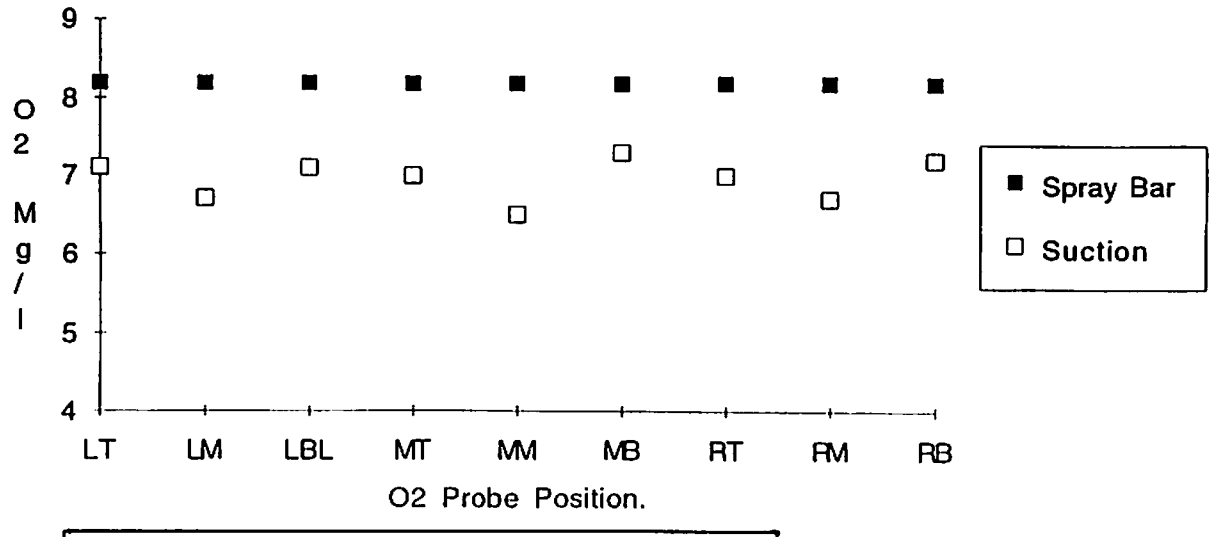
Trial 4



Trial 5



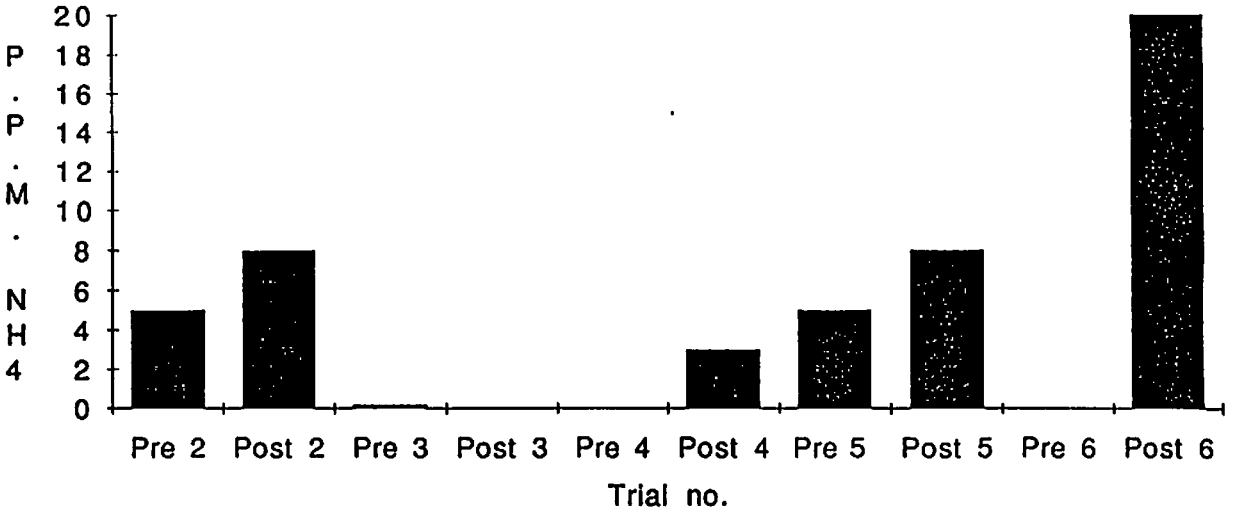
Trial 6



See Section 10.3 for probe position codes

Fig. 9

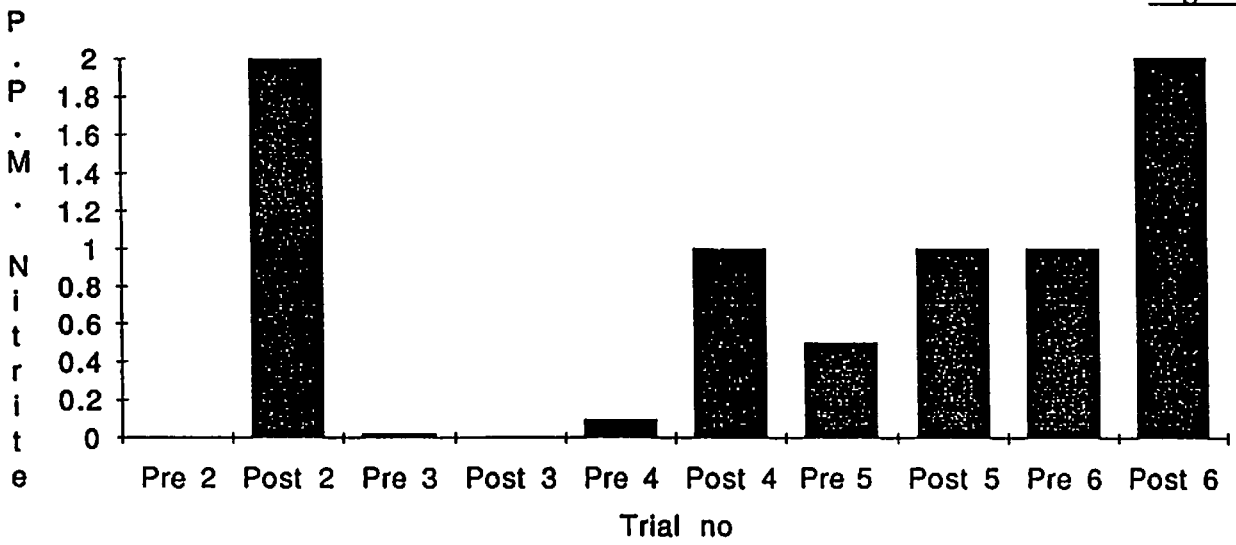
Ammonia Levels at the beginning and end of each trial



To convert ammonium to total ammonia nitrogen multiply by 0.77.

Nitrite Levels at the beginning and end of each trial

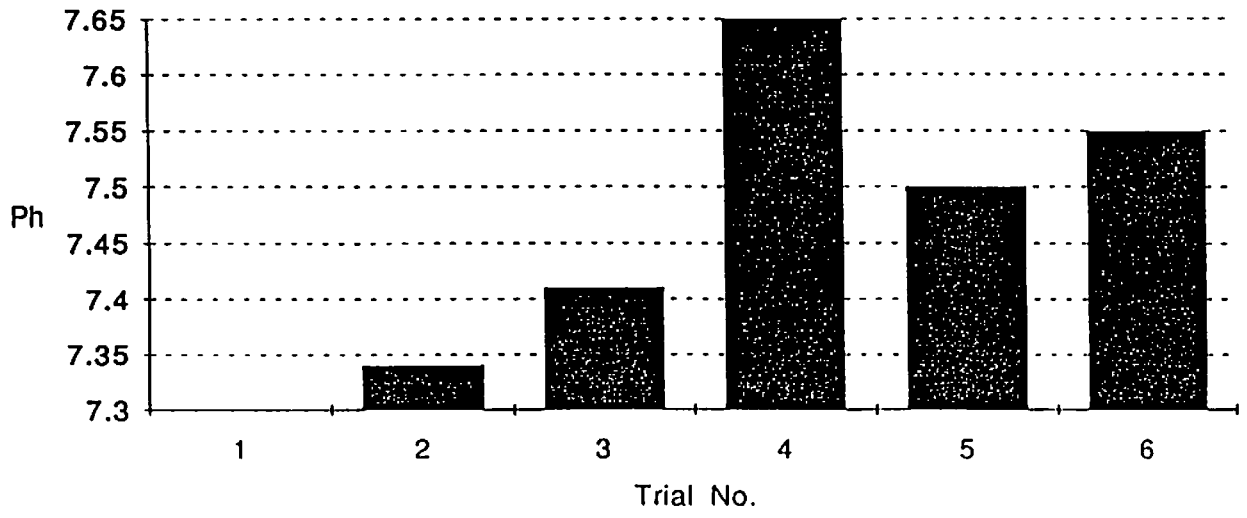
Fig. 10



To convert nitrate to nitrate nitrogen multiply by 0.3.

PH Levels at the end of each trial.

Fig. 11



APPENDIX 1

ARTIFICIAL SEAWATER MIXTURE

Five basic salts as defined in MAFF Laboratory Leaflet No. 39 (Ref. 5) can be mixed in the following proportions to give a salinity of 27 parts per thousand in 1000 litres of water.

Sodium Chloride	NaCl	21.08kg
Magnesium Sulphate	$MgSO_4$	5.18kg
Magnesium Chloride	$MgCl_2$	4.12kg
Flake Calcium Chloride	$CaCl_2$	1.06kg
Potassium Chloride	KCl	0.5kg

Commercial grade salts when obtained from a bulk supplier are in 50kg sacks other than sodium chloride which comes in 25kg sacks.

Costs are given below for sacks bought on individual and per tonne basis:-

	£ per sack (individual)	£ per sack (per tonne basic)
NaCl	5.40	4.20
$MgSO_4$	18.90	13.45
$MgCl_2$	12.00	10.20
$CaCl_2$	19.43	13.90
KCl	50.15	12.35

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

NaCl	3.54
$MgSo_4$	1.39
$MgCl_2$	0.84
$CaCl_2$	0.41
KCl	0.40
	6.58

Source : Ellis and Everard Chemicals - January 1991