Biological Criteria for the Depuration of the Pacific Oyster (*Crassostrea gigas*) and the Design of a Prototype Small-Scale Depuration Plant

Seafish Report No.433

September 1994

Sea Fish Industry Authority

Seafish Technology

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Summary

The criteria for depuration plant design are discussed; minimum and maximum temperatures are defined and target minimum oxygen levels set. Results of experimental work on oyster density, water flow rates and bacteriological results using model systems and a prototype commercial design are presented. The results obtained in the experimental work were similar in terms of oxygen consumption and depuration to those obtained in the prototype.

The prototype plant fulfilled the design criteria and oysters depurated bacteria successfully within the plant, but further testing and development are required to establish a standard design and its operating criteria.

Contents

1. Introduction

The consumption of bivalve molluscs from sewage contaminated waters is a well established risk to human health. Nowadays the risk is known to be of viral as well as bacteriological origin (7).

Seafish is undertaking a programme of research and development to improve the handling and depuration of bivalve molluscs to provide a safer and better quality product. Initially this work concentrated on live mussels but it is now extending to other species including the Pacific oyster *(Crassostrea gigas).*

The work on depuration is both to improve the performance and reliability of depuration plant and to develop more practical, cost effective plant. To this end Seafish is developing a series of standard plant designs to suit the needs of industry. These are high density plants which utilise artificial seawater and re-use that water. Being compact they can be housed within a building in controlled conditions and by using artificial seawater they can be sited where required rather than close to a supply of natural clean seawater. They are designed to provide controlled and optimal conditions for depuration and in these plants those conditions have been found to be significantly different from the conditions recommended by MAFF (1) for the traditional types of plant.

Seafish have already developed standard designs for large-scale and medium-scale mussel depuration plant (3 and 9) and commenced investigation of optimal conditions for the depuration of Pacific oysters (6). There remains a need to extend that line of work to develop a standard design of small-scale depuration plant for the many businesses handling small quantities of high value bivalve molluscs, primarily Pacific oysters. That plant should ideally be of an open, stacked box design with the water cascading down the stack, rather than the deep tank designs developed for mussels, in order to provide a more useful holding as well as depuration facility but should incorporate the lessons learnt from the earlier development and from further investigation of depuration conditions for Pacific oysters.

(ii) The ratio of oysters to water. This would affect the quantity of oxygen in the water, and the rate at which the metabolites increase in concentration over a period of re-use of water.

(iii) The rate of water circulation. Re-aeration of the water takes place in the `cascade' section of the plant.

Oxygen consumption, uptake of neutral red (Section 3.1) and bacteriological experiments could be used to assess the efficiency of depuration at different densities.

3.4 Rate of Water Flow within Tank Systems

The minimum velocity within a shellfish depuration system is determined by the requirement to supply oxygen to the shellfish; if the velocity of circulation and hence frequency of re-aeration is too low, then insufficient oxygen will reach the shellfish. However, too high a rate of circulation may result in localised turbulence and re-suspension of particulates. The implications of re-suspension of particulate are discussed in reference 6.

The particulate matter is considered to be hazardous because it may contain viral and bacterial particulates which can be reingested by the oysters if re-suspension occurs.

There are two ways of expressing the rate of circulation:

(i) The number of times per hour which the water circulates through the system. This is referred to in this report as the rate of water circulation in numbers of times through the plant per hour (T). If the plant has a cascade system for re-aeration which the water passes through during every cycle then this figure gives an index of the number of times per hour the water is re-aerated.

(ii) The volume of water, in litres per hour pumped by the pump. From a knowledge of the length of the tank (cm) (L) and the number of times the water is circulated per hour (T) an index can be obtained of the velocity of flow (V) in cm/second.

$$V = T*L/3600 \text{ Seconds/Hour} \quad (1)$$

It must be emphasised that this is only an approximate index of velocity for comparison between different systems. Some examples of calculated mean velocities in different systems are given in Table 1 overleaf.

Table 1 - Estimated Mean Water Velocities For Various Depuration Tank Systems

Tank Systems	Water Flow Rate in cm/sec for one circulation per hour using Equation (1)	Normal Operating Water Flow Rate (cm/sec)
Kings Lynn Deep Stack Mussel Tank	0.17	0.17 - 0.20
Boston Deep Stack Mussel Tank	0.11	0.55
Fishtoft, traditional open plan mussel tank	0.17	0.34 approx
Laboratory experimental tanks	0.038	Various see Section 3.2
Prototype commercial oyster tank system as tested in this report	0.2	0.2

4. Experimental Work

4.1 Density of Oysters : Pressure on Oysters

Aims

This experiment was intended to assess whether the oysters were inhibited in their activities by being loaded at high density inside the box. The method used the rate of uptake from the seawater of neutral red which stains the gills of the animals (4). Thus the rate at which neutral red disappears from the water and appears on the gills can be taken as an indication of the activity of the animals.

4.1.1 Apparatus and Method

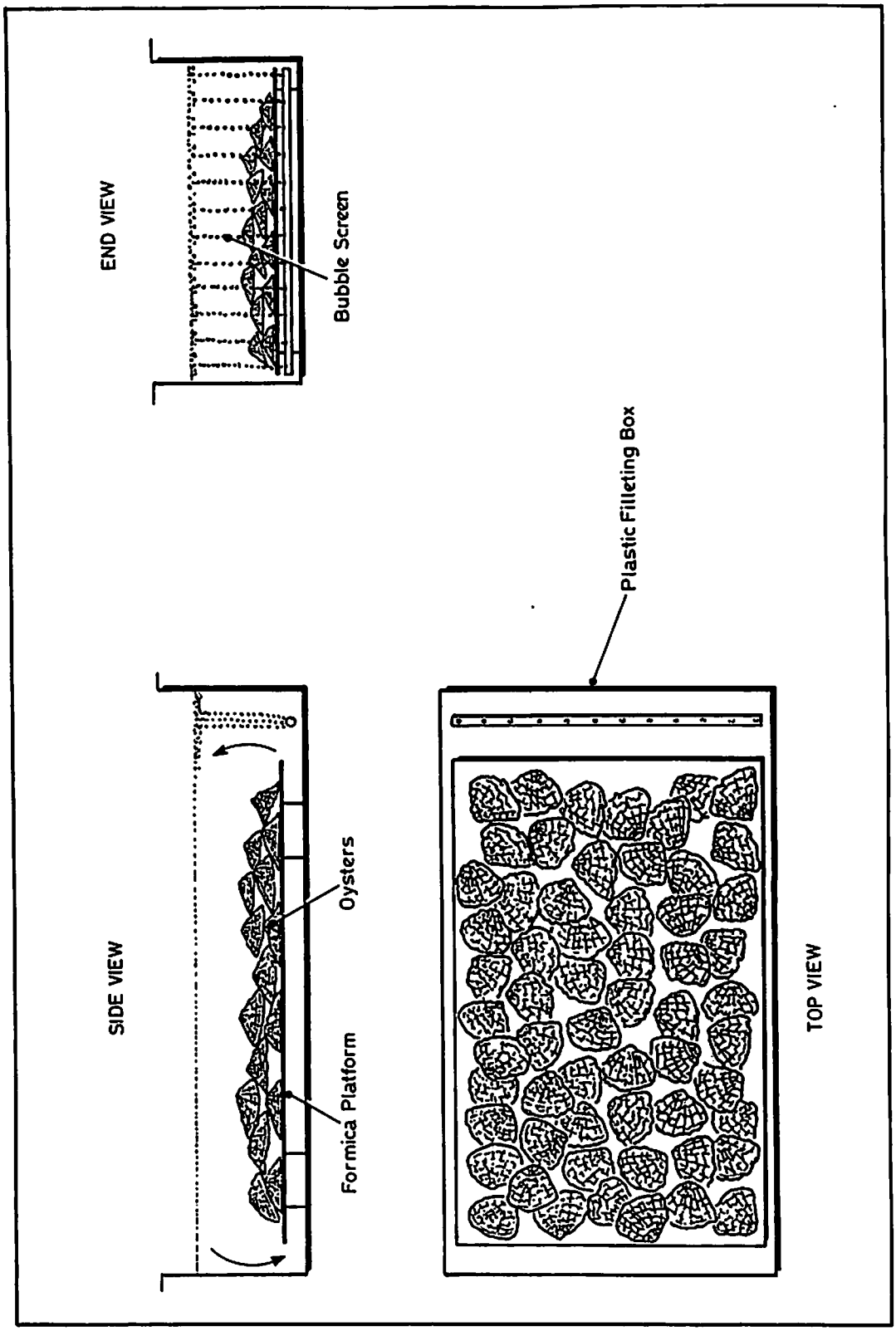
Four different densities of oysters were placed in plastic boxes as shown in Figure 1 overleaf and Table 2. Artificial seawater at 28‰ salinity was used and a temperature of 9-10°C which was maintained throughout the experiment. The aeration system was sufficient to maintain a 5.9mg/litre 0_2 saturation of oxygen. The ratio of seawater to oysters was constant for all four densities (Table 2). Sufficient neutral red solution was added to each tank to make a 0.05% solution. The water in the tanks was sampled at half hourly intervals over the following 3.5 hours and its absorbence read at 838nm (the wavelength of peak absorbence for netural red) in a spectrophotometer. The experiments were continued to visually assess the uptake of neutral red by the oysters according to the scale shown in the Table 3 overleaf.

Table 2
Densities of Oysters

Mean wet weight of individual oysters = 80 g
Mean dry weight of individual oysters = 2.0 g
Number of oysters/litre = 3.3

Number of oysters per m^2 (ft^2)	269 (25)	538 (50)	807 (75)	1076 (100)
Multiple of normal density	0.5	1.0	1.5	2.0
Wet weight of oysters kg per m^2 (ft^2)	21.5 (2.0)	43 (3.4)	64 (6.0)	86 (8.0)
Dry weight of oysters g per m^2 (ft^2)	489 (45.5)	1076 (100)	1646 (153)	2066 (200)
Approximate thickness of the layer : cm (inches)		7.3 (3)	9.7 (4)	12 (5)

Note: The dry weight is the weight of the fleshy parts of the oysters after shucking and then drying in an oven until the weight remains constant.



Apparatus Used to Investigate the Effect of Oyster Density Upon Neutral Red Uptake. Fig.1

Table 3
Scale Used to Visually Assess Staining of Oysters

The ctendium is the gill-like organ which the oysters use to filter water

Stage	Staining
Stage I	No neutral red staining
Stage II	Neutral red in streaks partially covering the ctendium
Stage III	Neutral red covering ctendium but not as dark as IV
Stage IV	Dark neutral colour covering the whole of the ctendium

After four hours an additional quantity of neutral red was added to make a 0.01% solution in each container, in addition to the neutral red which remained. The experiments were then left for 24 hours, drained, and all the oysters opened and assessed on the scale shown in Table 3 above. A note was made of the position of the oyster in each container; top layer, middle layer and lower layer.

4.1.2 Results and Discussion

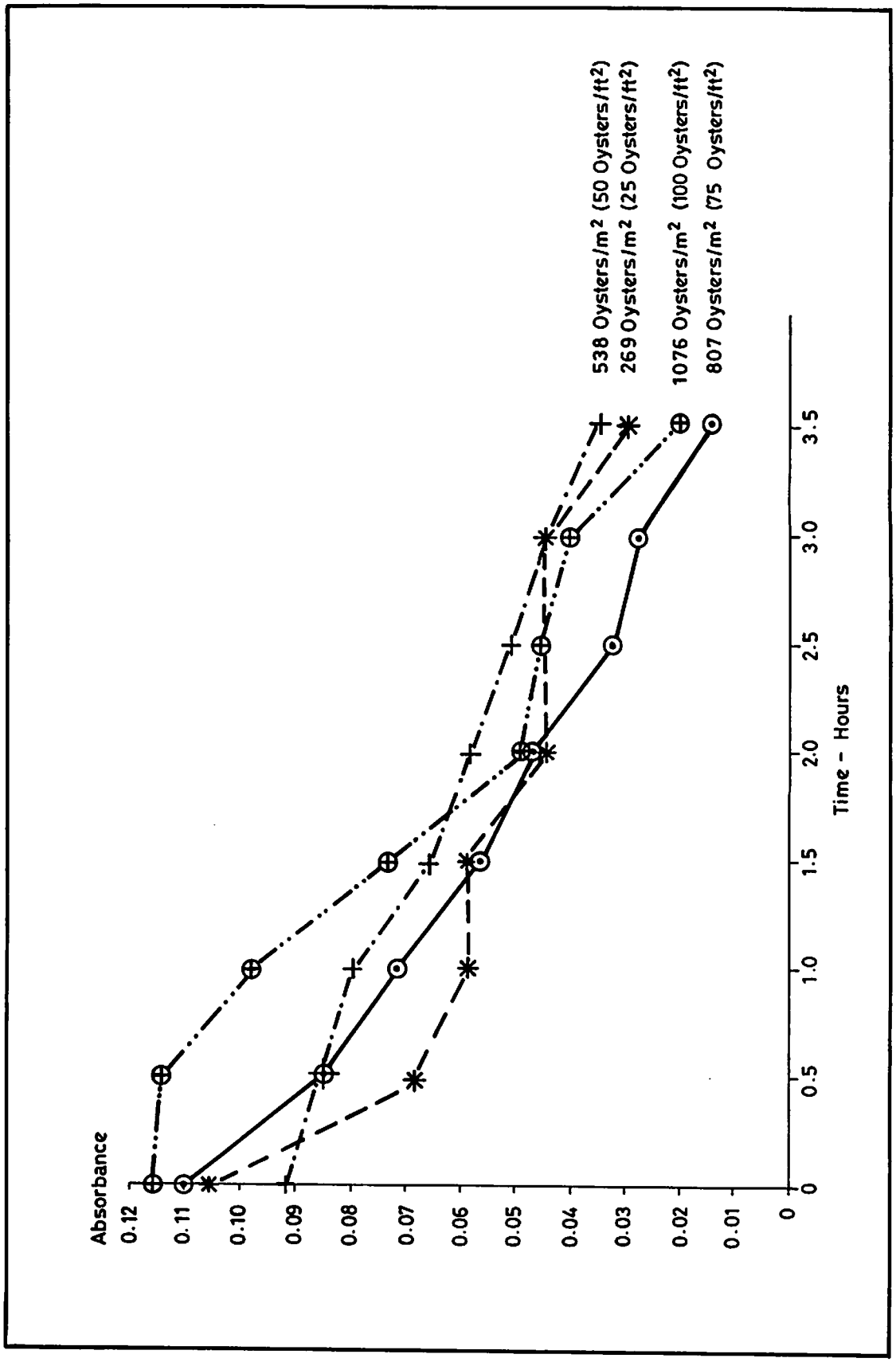
The results are shown in Figures 2 and 3 overleaf. Figure 2 shows that during the initial 3.5 hours the overall rate at which the neutral red solution was taken from the water is similar for all the densities for the oysters although the oysters at the highest density exhibited a slight delay in taking up neutral red and those at the lowest density were initially the most rapid, but all four densities of oysters have reduced the neutral red to similar concentrations at the end of 3.5 hours.

Figure 3 shows the degree of redness of the oysters after 30 hours using the stages defined in Table 3. It seems probable that the degree of redness and hence the stage which the animals were allocated to would be dependent upon :-

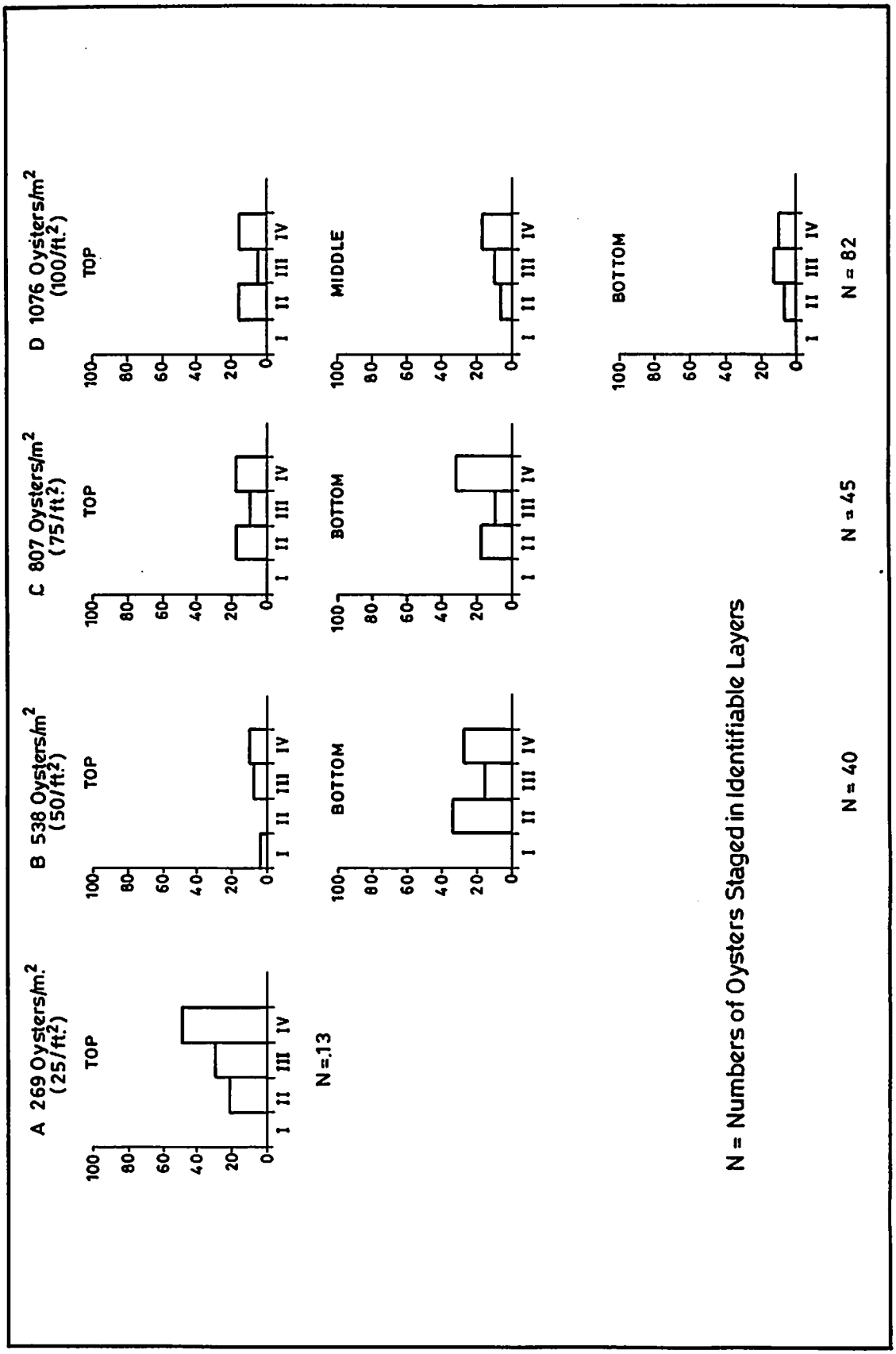
(i) The length of time the animals opened and pumped.

(ii) The length of time the animals spend closed before they opened and filtered. Animals which opened later in the period would have encountered neutral red at a lower concentration and hence the degree of staining would be reduced.

There is considerable variance in the results but considered overall there was a greater absorption of neutral red in the lowest density of oysters than in the higher densities, particularly the highest density, and there is a slight indication in the higest density of lower absorption in the bottom oysters. However, this may be a result of point (ii) above.



Absorbance of Neutral Red / Time for Oysters at Different Density Fig.2



Percentage of Oysters in Each Stage of Neutral Red Uptake (see Table 3 for stages) After 30 Hours. Fig.3

4.2 Density of Oysters : Depuration Experiments

The experiement in the previous section (Section 4.1) established that oysters at densities of up to 1076 per m^2 (100 per ft^2) did not stop the animals ability to open and filter. Therefore it was considered that a depuration experiment at these densities would be worthwhile.

4.2.1 Apparatus and Method

Two identical model depuration systems were set up as shown in Figures 4 and 5 overleaf. Oysters were contaminated in the natural environment and transported by road (approximately 6 hours) before depuration commenced. The oysters were then divided into two groups and placed in the two model depuration systems at different densities:-

(i) Control density at 538 oysters per m^2 (50 per ft^2)

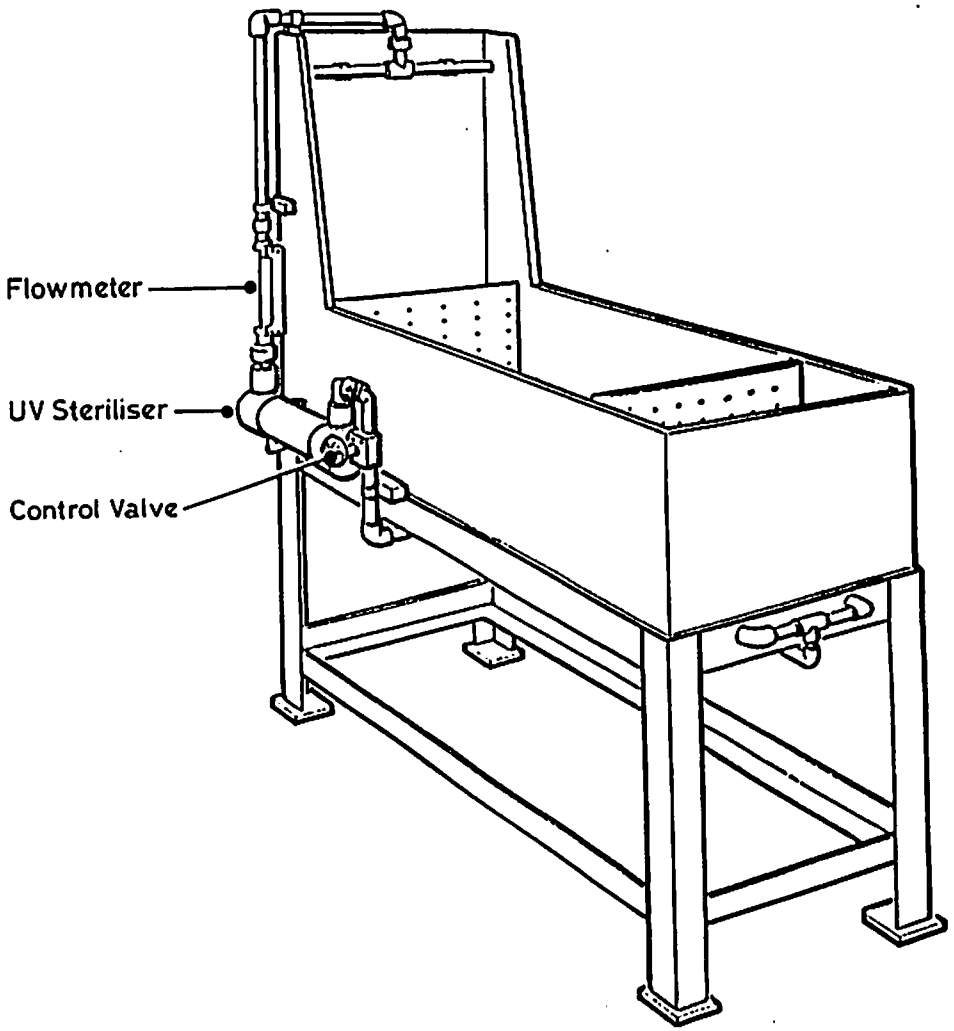
(ii) Experimental density at 1076 oyster per m^2 (100 per ft^2)

Other parameters were kept identical and are shown in Table 4. Both experimental and control tanks were depurated at 15°C and at a salinity of 28‰ for 42 hours, oxygen differences being monitored at intervals.

At the end of the experiment both tanks were drained down and samples of oysters removed from positions in the tanks specified in Figure 6 overleaf.

Table 4
Environmental Parameters and Oyster Densities in the Experimental and Control Tanks

	No of Oysters per m^2 (ft^2)	Dry weight Oysters per m^2 (ft^2)	Volume of water litres	Oyster Water Ratio dry wt g/litre	no./litre	Water circulation Rate time/hour (t)	Pumping rate litres/hour
Experiment	1076 (100)	1732 (161)	103	5.3	3.3	4.4	450
Control	538 (50)	866 (80.6)	51.5	5.3	3.3	4.4	225



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

PIPEWORK/VALVES - 1/2" A.B.S.

PUMP - Nikkiso Magpon CPO8. Centrifugal pump with magnetic drive. Maximum capacity 6 1/min at 1 metre head. Mounted under tank.

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

FLOWMETER - Paton PG1 - 10 1/min.

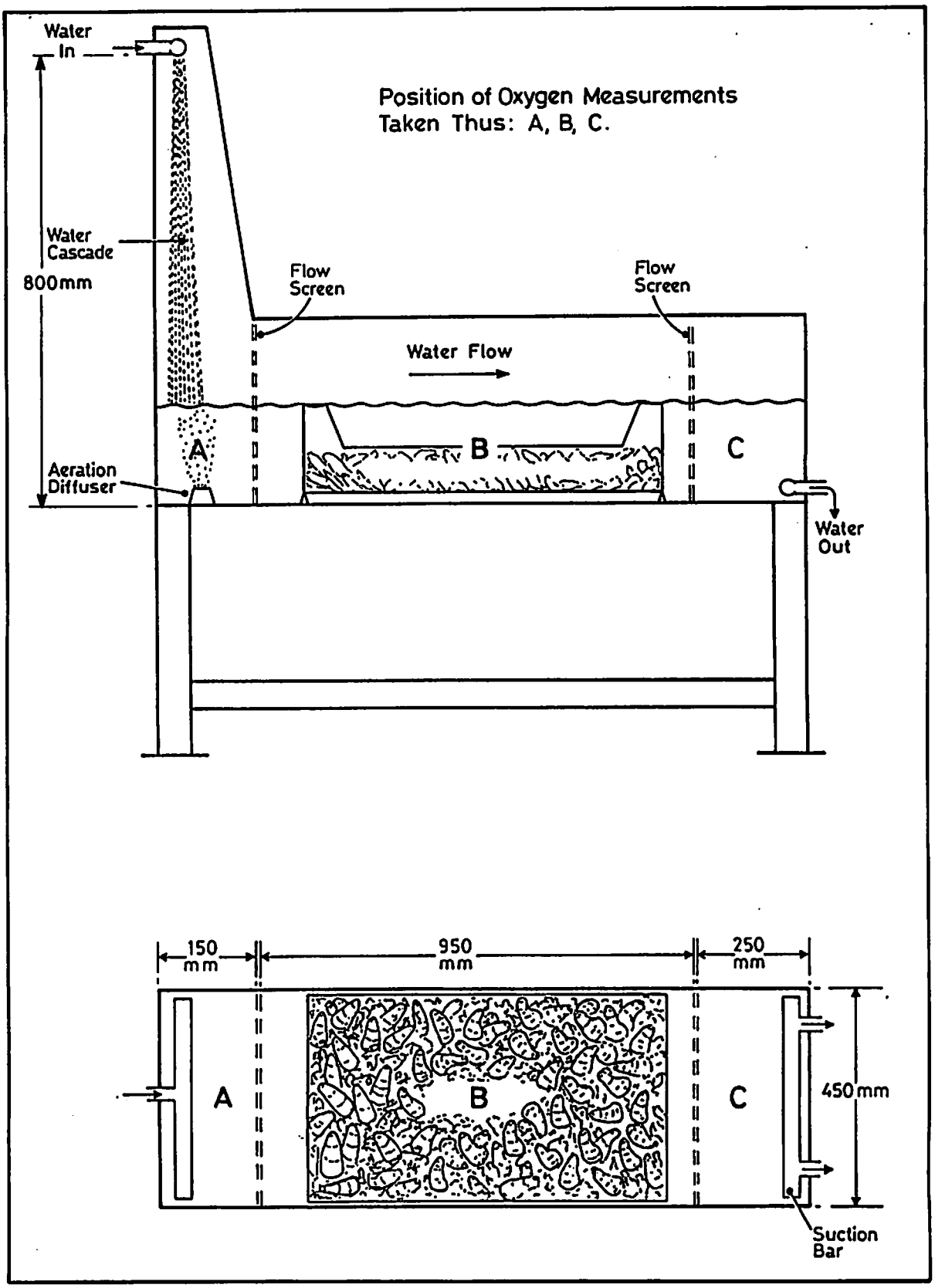
AERATION - Atlantis B800 air pump mounted on back of tank feeding two 300mm diffuser blocks on tank base.

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

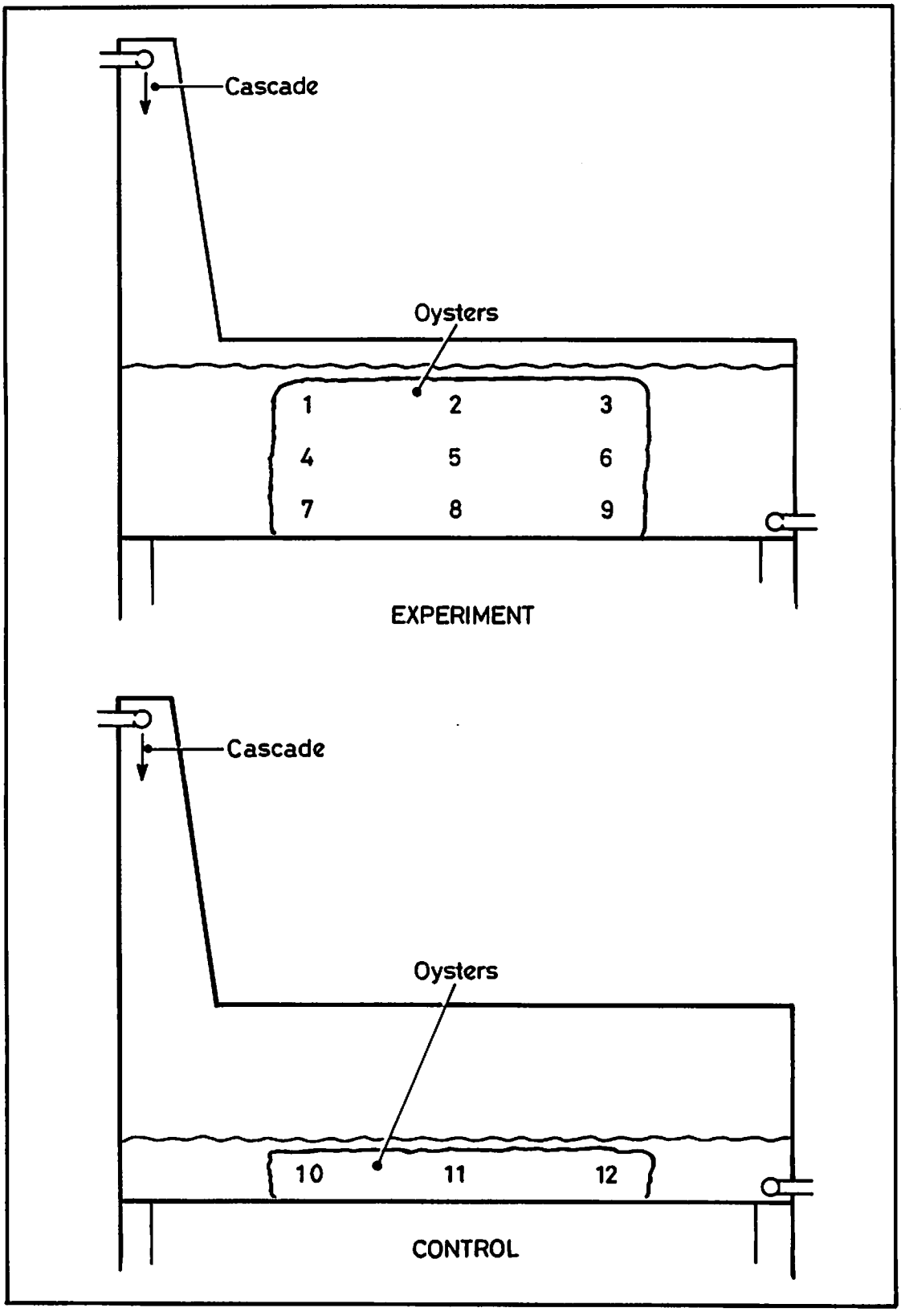
TRAY - Up to two Allibert Type 41042 (752 x 448 x 167mm).

HEATING - 300 watt aquarium heater used with Digistat temperature controller

Experimental Oyster Depuration Plant - Equipment Specification Fig.4



Experimental Oyster Depuration Plant - Schematic Layout Fig.5



Cross Section Through Model Depuration Plant Showing Positions of Oyster Sampling Stations. Results Obtained Are Shown in Table 7. Fig.6

4.2.2 Results and Discussion

4.2.2.1 Oxygen Concentrations

Dissolved oxygen levels were measured at the 3 locations (A,B & C) shown in Figure 5. The minimum oxygen concentrations and mean differences in oxygen concentration across the tanks (from input to output ends) for experiment (2 x normal density) and control (1 x normal density) are shown in Table 5 below.

Table 5

Minimum oxygen concentration (0_2) and mean oxygen differences, (see equation 2 below) for experimental and control tanks. Other parameters; water/oyster ratios, rate of circulation of water and temperatures were identical and given in Table 4.

	Minimum 0_2 mg/litre	Mean 0_2 Difference mg/litre	Standard deviation of difference
Experiment	6.6	0.6	0.05
Control	6.5	0.7	0.1

Oxygen difference across tank $\Delta\ 0_2 = (0_2)\ A - (0_2)C$

Where (0_2) A = Oxygen concentration at position A (figure 5)

(0_2) C = Oxygen concentration at position C (figure 5)

There is a small but statistically significant difference ($p>0.05$) in the mean oxygen concentration difference across the tanks, the control tank having a very slightly greater oxygen difference than the experiment. This may be accounted for by differences in the behaviour of the oysters, the experimental oysters consuming slightly less oxygen per oyster. However, it may also be due to differences between the two tanks aeration capacities, and although the difference is statistically significant it may not be important. If it is a true difference and not an artificat, then it suggests that the higher density inhibits oyster activity.

4.2.2.2 Bacteriological Results

Samples of oysters were taken for bacteriological analysis before and after purification. The bacteriological methodology is given in Appendix I.

The initial levels of bacteria in three replicate samples of the oyster flesh are shown in Table 6 overleaf. Final, post-purification levels of bacteria in oyster samples taken from stations within the oyster mass for both the control and experiment are shown in Table 7 overleaf.

Table 6
Initial Concentrations of Bacteria in Oysters: Replicate Samples

Replicate	Total Coliforms		*E.coli*		Faecal Streptococci
	Counts/100g flesh	95% Confidence Interval	Counts/ 100g flesh	95% Confidence Interval	100g Flesh
1	2200	570-7000	800	100-1900	100
2	1700	430-4900	800	100-1900	100
3	2100	440-5000	400	50-1100	100

Table 7
Final concentrations. Bacteria/100g wet weight oyster flesh in experimental and control samples taken from the positions within the oyster mass shown in Figure 6

Station		Total Coliforms at 37°C		Faecal Streptococci 100g flesh	*E.coli* at 44°C	
		Counts/ 100g flesh	95% Confidence Interval		Counts/100g flesh	95% Confidence Interval
Experiment	1	230	70-700	<100	<20	-
	2	20	4-34	<100	20	5-70
	3	70	20-210	<100	<20	-
	4	170	43-490	<100	20	5-70
	5	500	50-1300	<100	40	5-110
	6	130	35-300	<100	20	5-70
	7	130	35-300	<100	<20	-
	8	170	43-490	<100	<20	-
	9	80	10-190	<100	20	5-70
Control	10	80	20-190	100	20	5-70
	11	40	5-150	100	40	5-110
	12	130	5-70	100	20	5.70

These results show that oysters depurated bacteria successfully in both the experiment and control from moderate levels of contamination. However, the highest coliform and *E.coli* results were found in the middle of the higher density experimental tank and in general the total coliform counts were higher in the experimental tank. There would be a need for further experiments to investigate this should the higher density be considered for commercial practice.

4.3 Oxygen Concentrations/Rate of Water Circulation

In recirculating systems most re-aeration of the water occurs when the water is cascaded. The rate at which the oxygen concentration falls as the water passes through the tank is related to three factors.

(i) The activity of the oysters in the tank.
(ii) The rate at which the water in the tank is being returned to the re-aeration cascade (See Section 1.3)
(iii) The ratio of oysters to water in the tank.

In addition the solubility of oxygen (oxygen carrying capacity) of seawater decreases with increasing temperature. In these experiments the ratio of oysters to water was kept constant while the water temperature and rate of water circulation was varied.

4.3.1 Method

Two identical tanks of the type shown in Figures 4 and 5 were used. A total of 170 oysters were loaded into each of the tanks at a density of 538 oysters/m^2 (50/ft^2), this being the normal commercial density specified by Ayres (1). The oyster/water ratio was 3.3 oysters/litre and the dry weight of oysters was estimated as 6.45 g per litre. This was the same as the control in the density experiments (Section 3.2).

The experiments were carried out as follows:-

The tanks were placed in a chill room set at a constant 15°C. In the experimental tank, a thermostatically controlled heater was placed, which was set to the temperature required. The control was kept at the constant 15°C. The rate of water circulation was kept constant in the control and varied in the experimental tank. The oysters were removed from the water overnight, between experiments to ensure that they built up an oxygen deficiency before the experiments. The water flow rate and the temperature were set before the oysters were placed in the tanks. Oxygen concentrations were monitored using an oxygen meter at intervals during the subsequent 5-6 hours. Three positions (A, B and C) were monitored in each tank as shown in Figure 5.

(i) At the cascade end of the tank (A)
(ii) In the middle of the mass of oysters (B)
(iii) At the downstream end of the tank adjacent to the suction point (C)

4.3.2 Results and Discussion

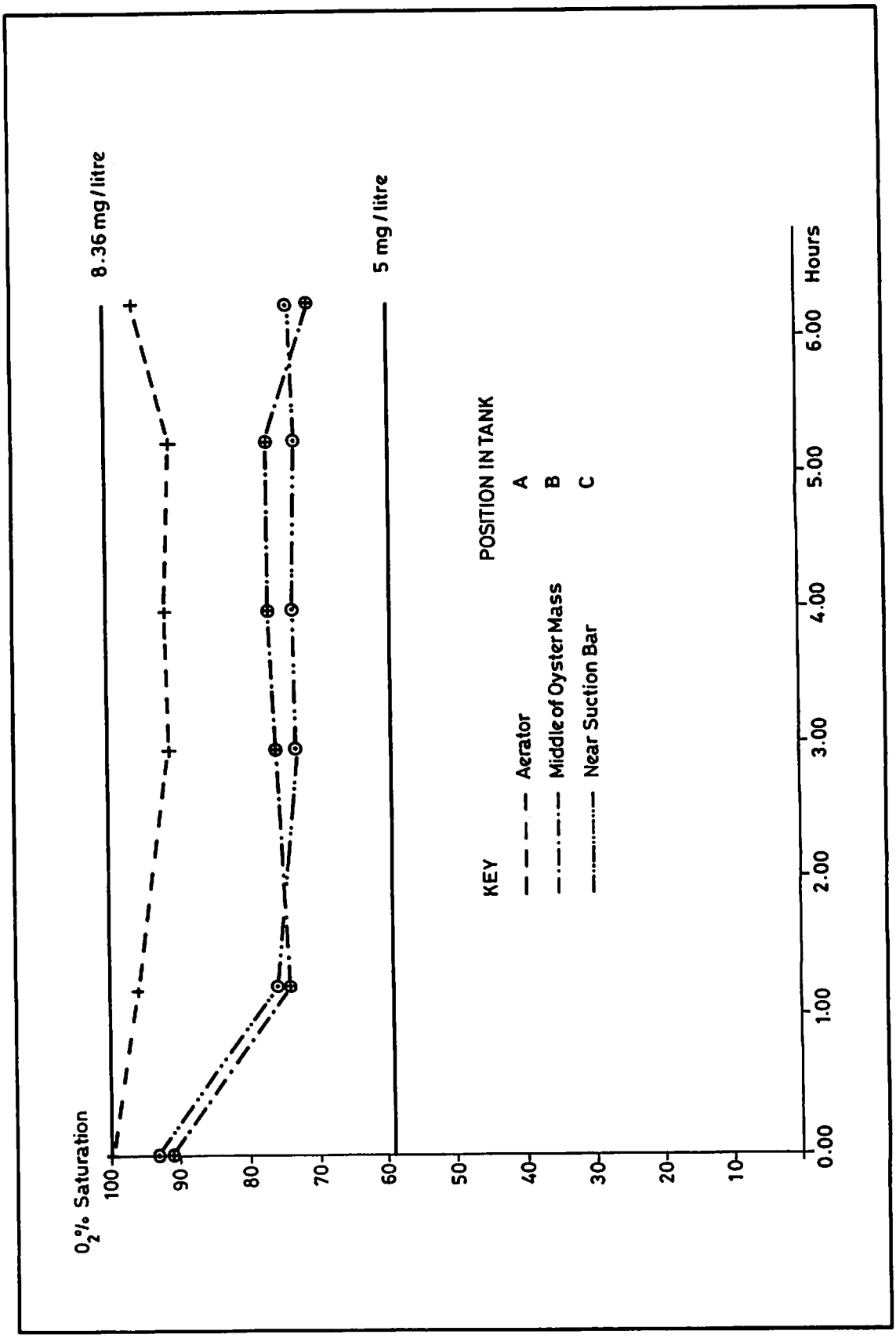
Table 8 and Figures 7-12 overleaf show the experimental permutations and the percentage oxygen saturation against temperature for the 5 different conditions of temperature and flow rate examined.

These results show that higher temperatures result in reduced oxygen levels in the plant as a consequence of increased oxygen demand by the oysters and the reduced solubility of oxygen in the water. Taking 21°C as the maximum operating temperature and $5mg0_2$/litre as the minimum level of oxygen which can be tolerated (Section 1.2) the situation in Figure 12 would appear to be the optimum. Table 8 shows that at this oyster/water ratio, the conditions shown in Figure 12 correspond to 2 litres of water/oyster/hour. This could, therefore, be used as a guide to the flow required through the trays in a similar commercial system with flow along a length of one box. However, this assumes that there is adequate re-aeration at the cascade end of the plant or adequate re-aeration between trays in a stack system.

Table 8
Rates of Flow in Litres/Hour/Oyster for Figures 7-12

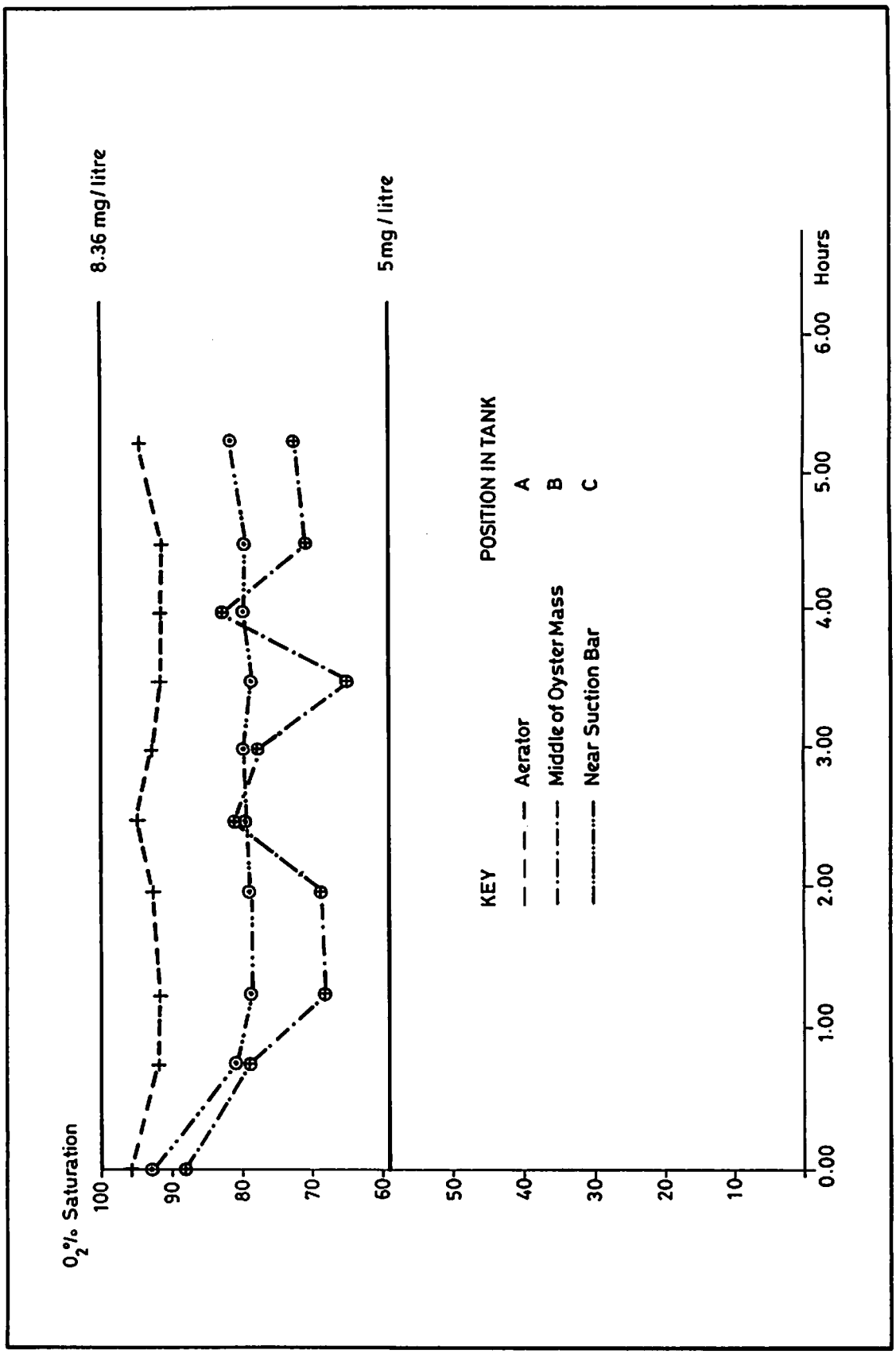
Figure No	Rate of Flow Litres/Hour	Rate of Flow		Temp °C
		Litres/Hour/ Oyster	Litres/Hour/g Dry Wt	
7	114	0.7	0.41	15
8	225	1.3	0.81	15
9	114	0.7	0.41	18
10	225	1.3	0.81	21
11	225	1.3	0.81	18
12	336	2.0	1.21	21

Number of oysters = 170
Dry Weight = 227g
Oyster: Water Ratio = 3.3 oysters/litre
6.45g dry wt oysters/litre



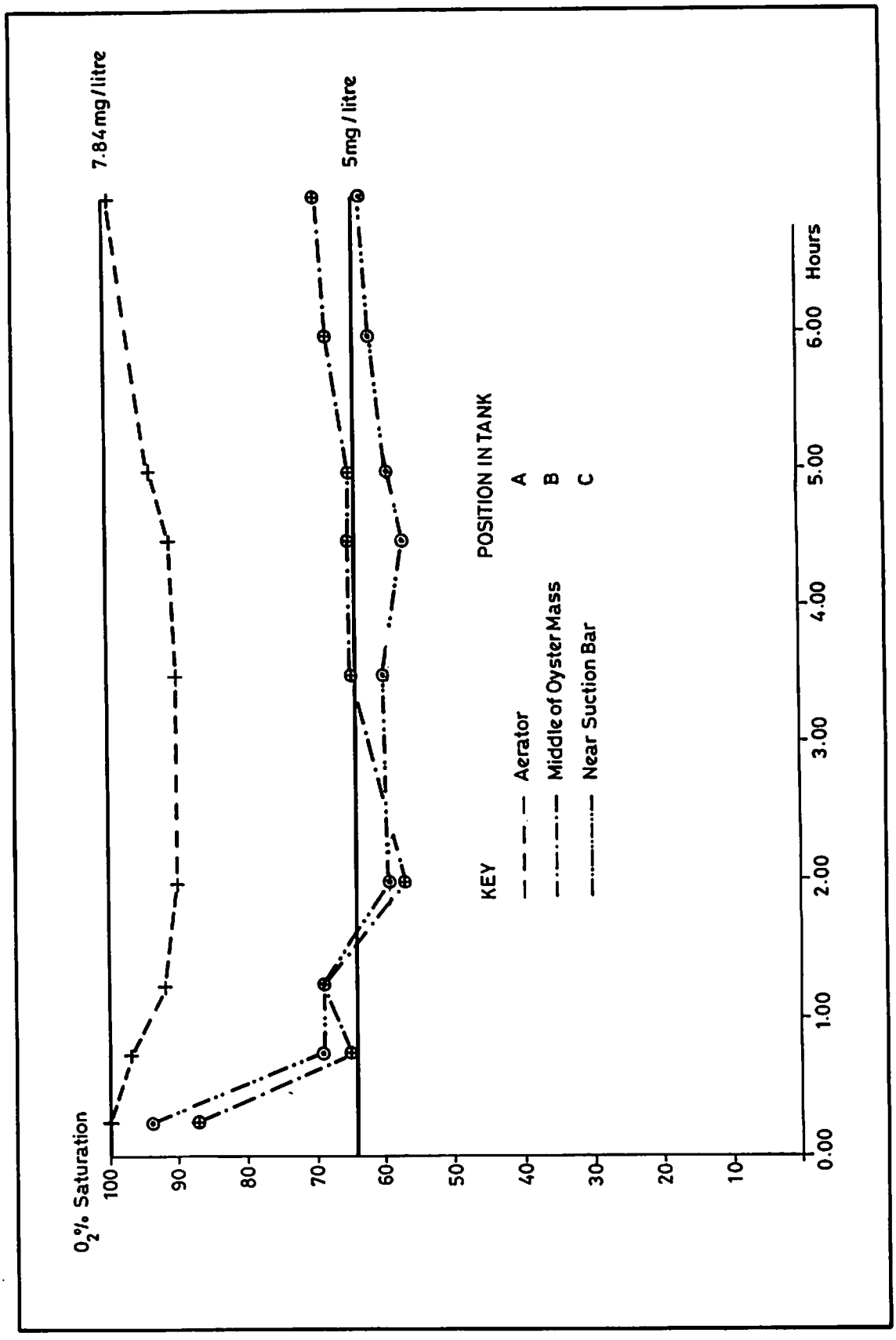
Oxygen Saturation Against Time 15°C
2·2 Circulations /Hour (114 litres/hour)

Fig. 7



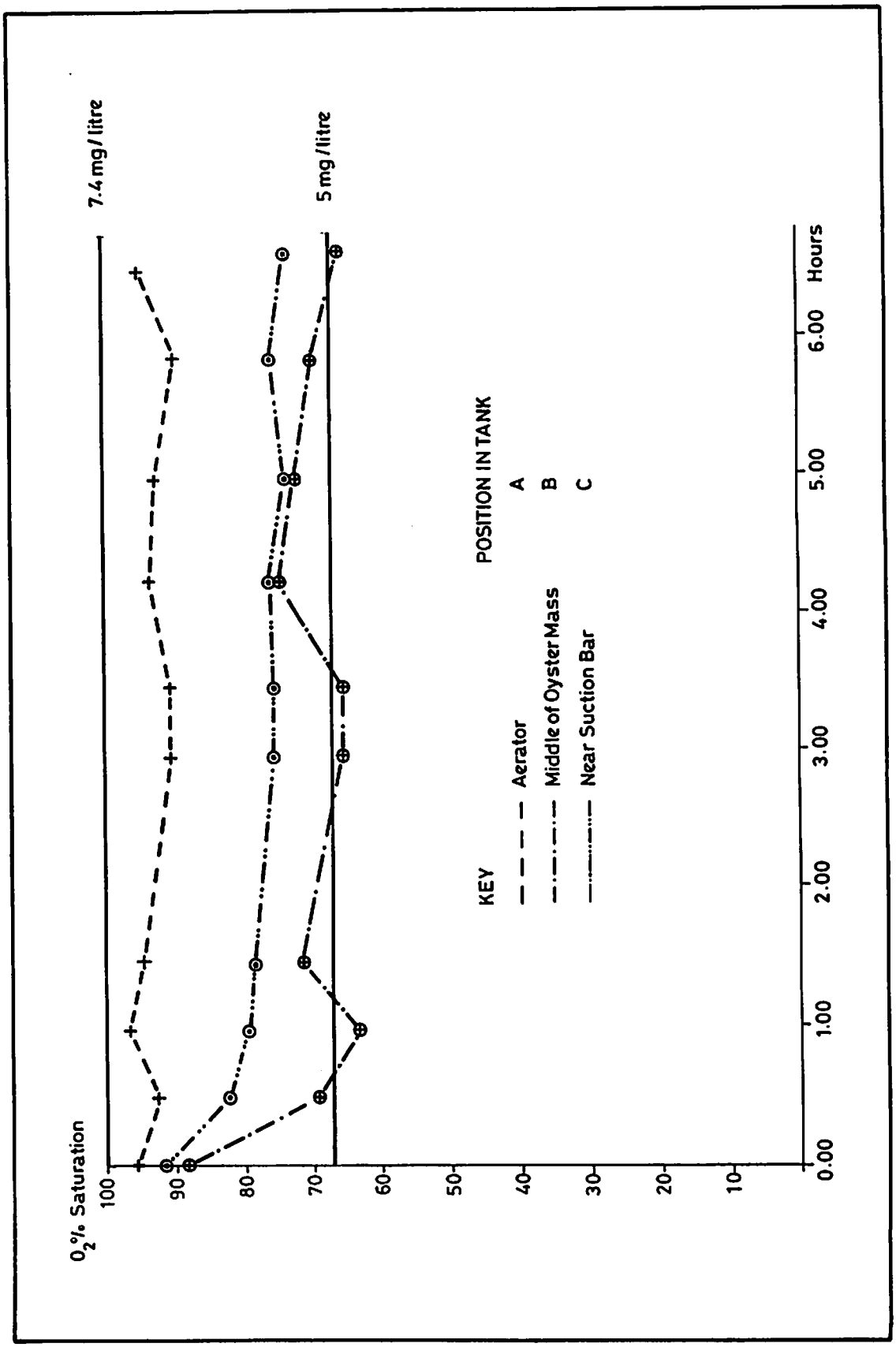
Oxygen Saturation Against Time at 15°C
4·4 Circulations/Hour (225 litres/hour)

Fig. 8



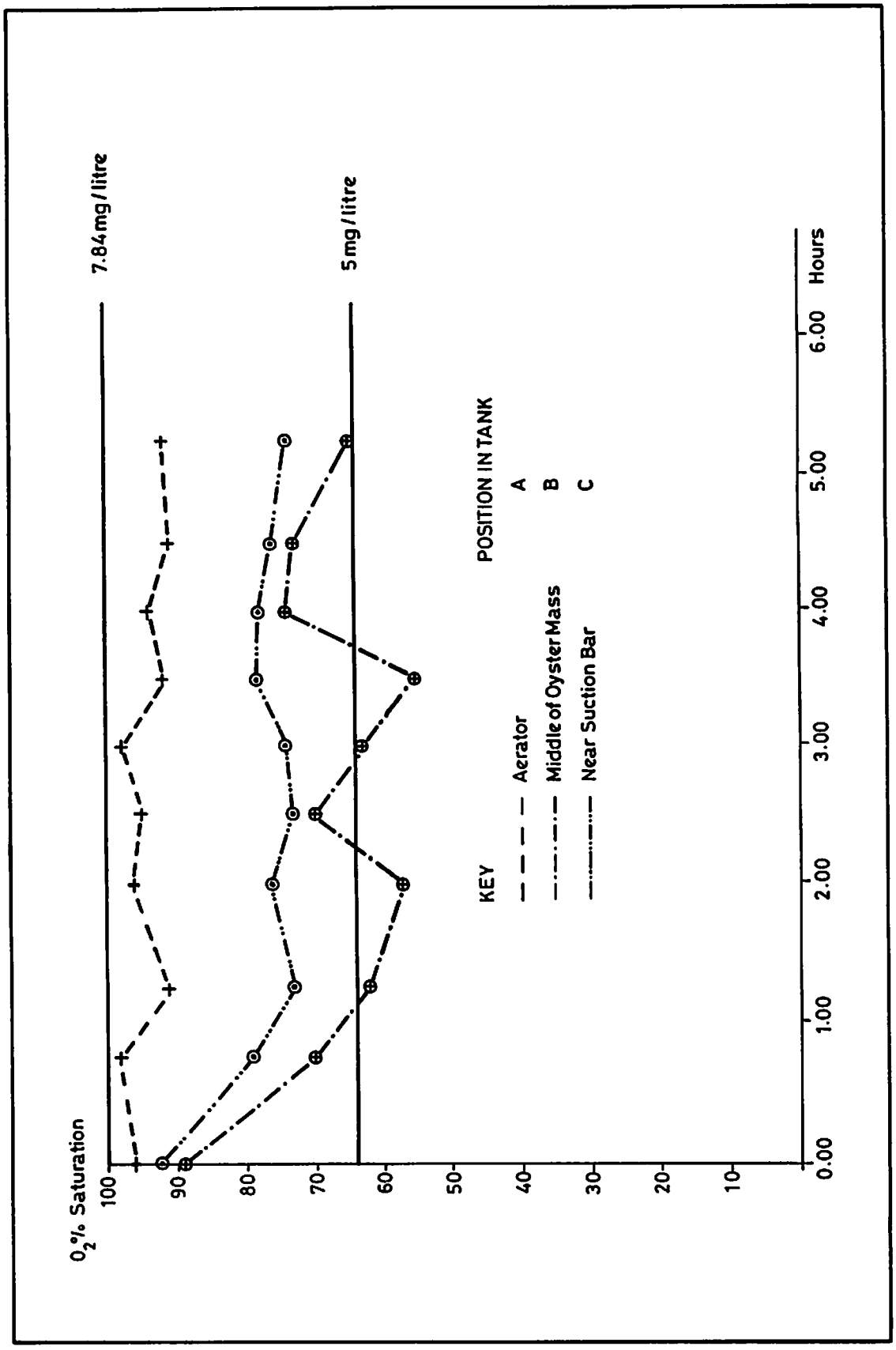
Oxygen Saturation Against Time at 18° C
2·2 Circulations / Hour (114 litres/hour)

Fig. 9



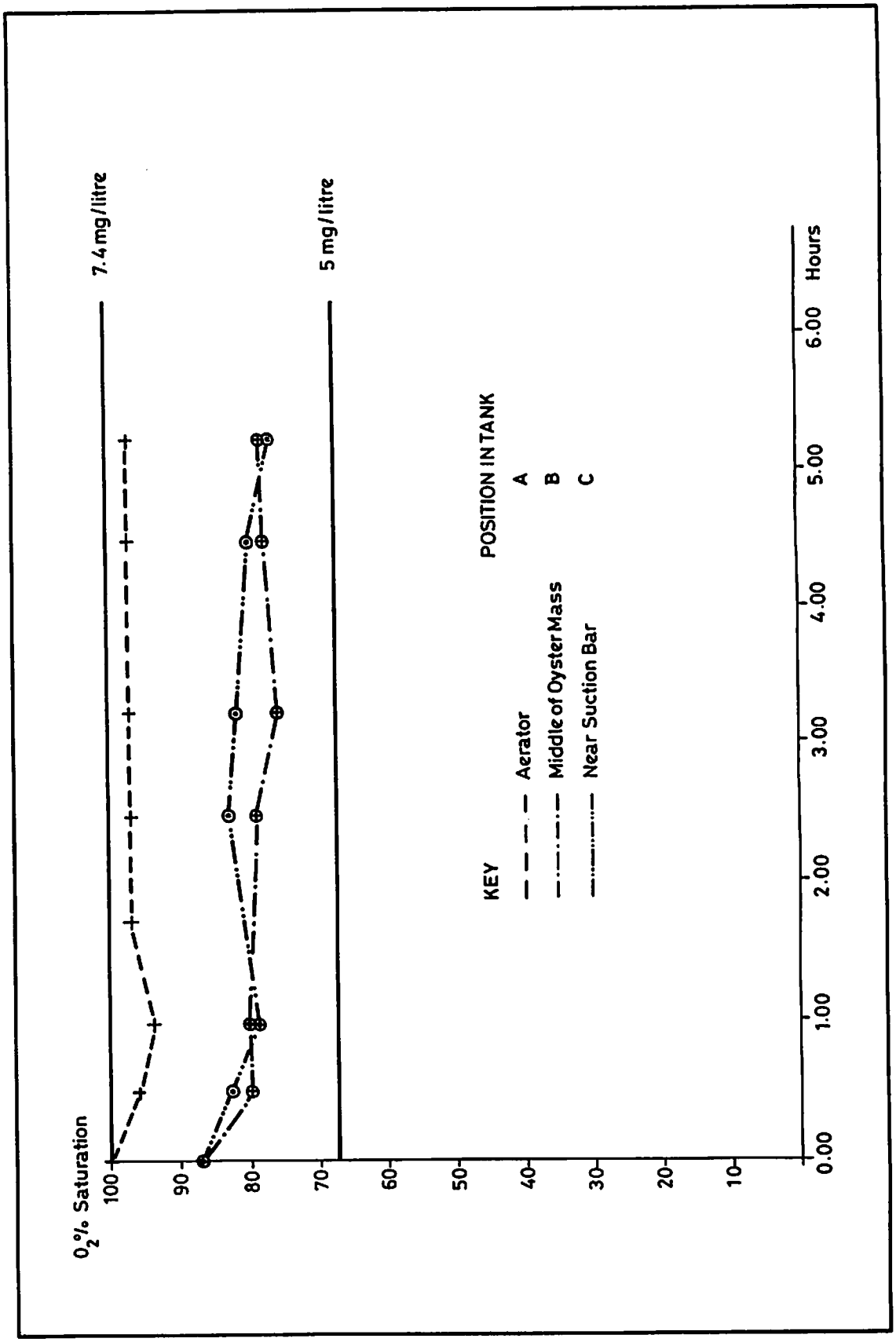
Oxygen Saturation Against Time at 21°C
4·4 Circulations/Hour (225 litres/hour)

Fig.10



Oxygen Saturation Against Time at 18°C
4.4 Circulations/Hour (225 litres/hour)

Fig. 11



Oxygen Saturation Against Time at 21°C
6·6 Circulations /Hour (336 litres/hour)

Fig.12

5. Experimental - Prototype Commercial Depuration System

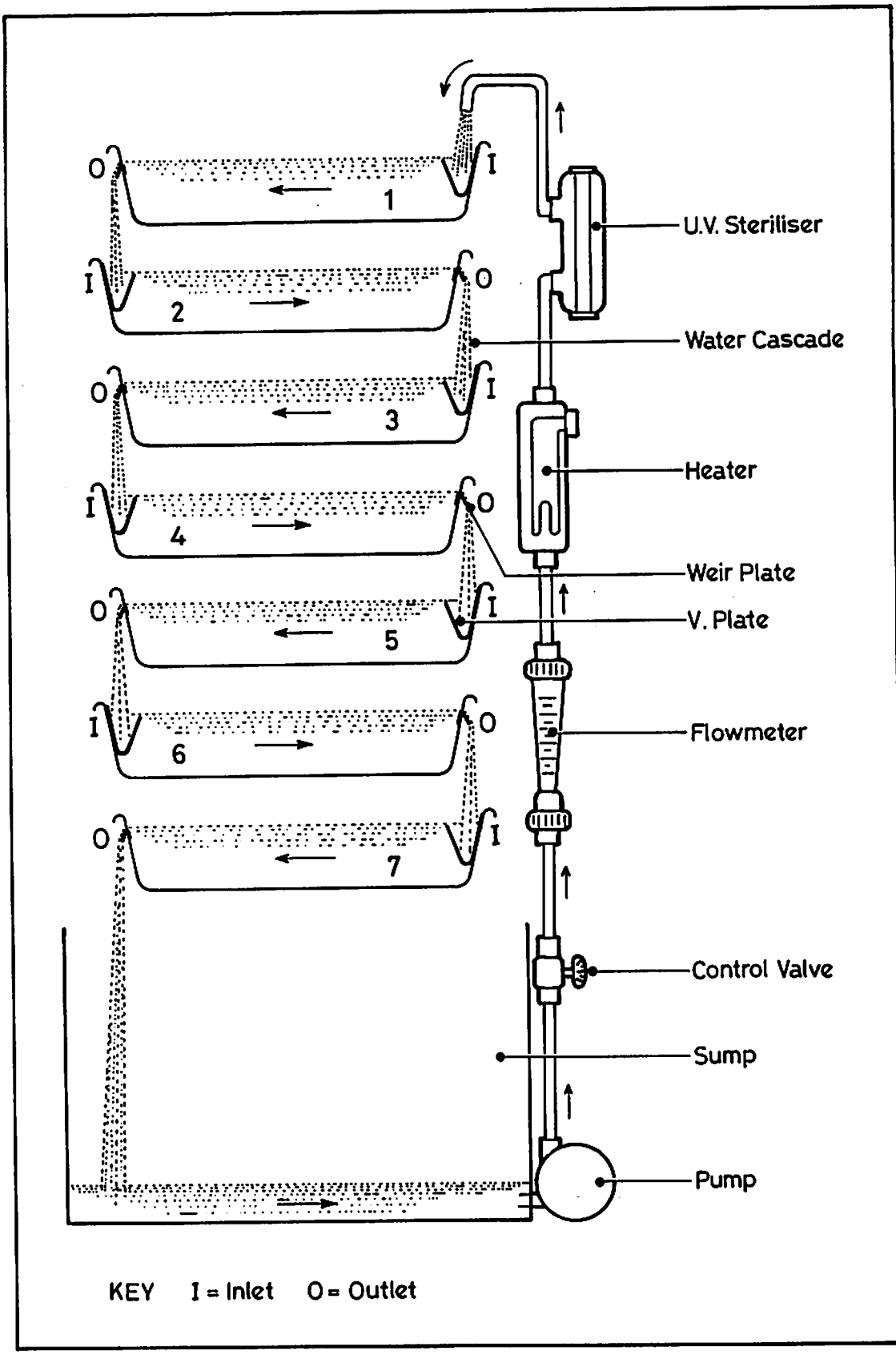
5.1 Apparatus - General Description

The apparatus used for these experiments was a prototype for a commercial plant, full details of which are given in Appendix II. The plant consisted of a stack of seven fish boxes (Allibert 12030) on a metal frame, shown in Figures 13 and 14 overleaf. The seawater was pumped from the sump tank, through the heater and UV systems to fall into the tank at the top. The water then flowed from box to box, cascading between the boxes. The numbering code identifying the boxes is shown in Figure 13. The plant had a number of special features which are considered improvements upon previous designs.

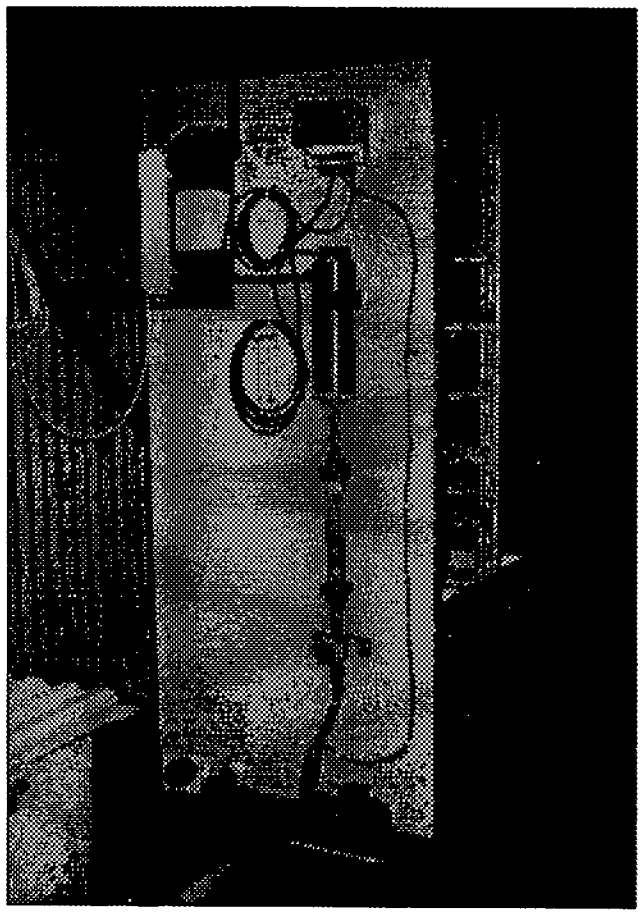
(i) The heater and electronic thermostat. This enabled control of water temperatures to be obtained. The heating system chosen (see Appendix II) enabled a temperature of 18°C to be obtained in the plant. However, the chilling of the water as it cascaded down the stack resulted in a reduction of 1-3°C in temperature dependent upon the ambient conditions. When there was a difference between the top and bottom of the stack the mean temperature was recorded in the experiment results.

(ii) The cascade system was designed with shaped stainless steel plates attached to the boxes to create a through-box flow with a controlled cascade at one end to prevent the cascading water from disturbing the oysters in the box below or the particulate matter surrounding them (see Appendix II).

(iii) Plastic trays were arranged within the boxes to contain the oysters. These have the effect of lifting the oysters off the bottom of the tray and away from accumulations of particulate matter. When the plant is drained down the oysters are above the residual water containing particulate matter in the bottom of the tanks. The oysters are removed in their trays. The small amount of remaining below the trays is then discarded with the detritus. The trays also improve oyster handling: after the plant has been drained down the oysters can be easily removed from the plant without the need for tipping them out of the boxes. The oysters which are to be depurated during the next depuration cycle can be prepared and placed in a spare set of trays ready to be placed in the plant for the next depuration.

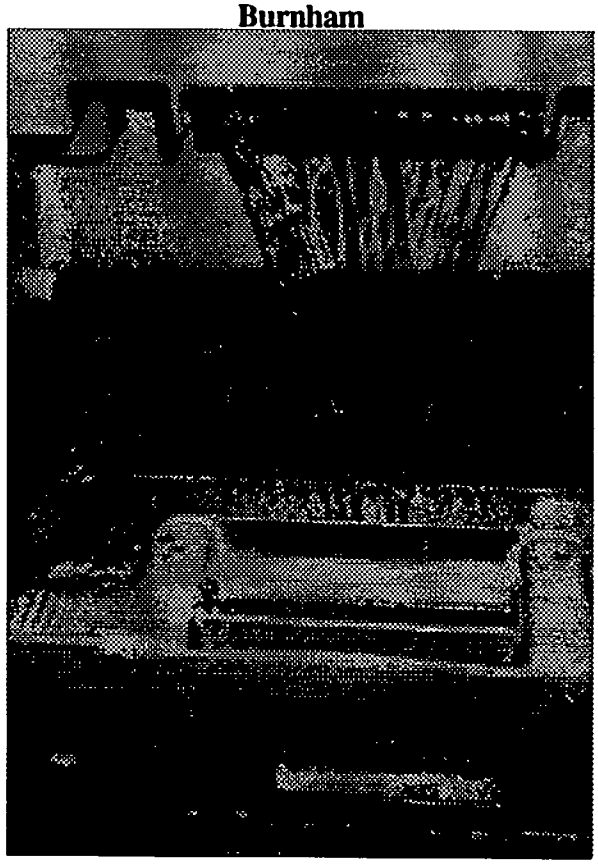


Water Flow Through Purification Unit Fig.13



Prototype Purification Unit Installed at Burnham

Figure 14



Water Cascade

5.2 Method

Experiments were carried out using this system which was set up on different occasions in the conditions described in Table 9 overleaf. These experiments were carried out in order to:-

(i) To determine that the rate of circulation is adequate to maintain adequate oxygen levels in the plant at up to 18°C. The plant was run at normal density of 110 oysters per box and at the higher density of 140 oysters per box, and oxygen measurements were taken over a period of at least four hours. The distance between the trays was also reduced, keeping the other parameters of 110 oysters/tray and 18°C constant. (Experiments 3 and 4).

(ii) Oysters were then depurated in the plant at normal densities of 110 oysters per box at 10°C, 14°C and 18°C with bacteriological analysis carried out before and after depuration to test the effectiveness of depuration. (Experiments 1,2 and 5).

(iii) To investigate the bacterial hazards associated with re-suspension of the particulate matter surrounding the oysters. After purification the plant was drained down and the oysters removed in their trays. All the particulate matter in the bottom of the trays was then poured into the sump. The oysters were then replaced in the plant and the plant run for 3 hours with the UV lamp switched off. The particulate matter was mixed to keep it in suspension in the sump tank and water samples were taken at appropriate intervals. Oyster samples were taken before and after this procedure was carried out (Experiment 1).

Table 9
Conditions for Experiments in Prototype Oyster Depuration Plant

Experiment Number	1	2	3	4	5
Mean temperature of Seawater °C	9.6	14	18	18	18
Salinity of Seawater ‰	25	26	24.5	24.5	25
Density of Oysters per unit area					
No.of oysters per m^2 (ft^2)	592 (55)	592(55)	595(55)	625(70)	592(55)
No of oysters per box	110	110	110	140	110
Mean dry weight of oysters g/m^2 (g/ft^2)	568(86)	568(106)	443(83)	487(54)	443(93)
Density of oysters per unit volume					
No.of oysters per litre					
In whole plant	2.56	2.56	2.56	3.26	2.56
In individual trays	4.4	4.4	4.4	6.22	4.4
Dry weight of oysters (g/litre)					
In whole plant	2.46	2.46	2.0	2.58	2.0
In individual trays	4.22	4.22	3.4	4.85	3.4
Individual oysters	0.83	0.83	0.83	0.69	0.83
Water volumes					
Total volume of water in the plant (litres)	310	310	310	310	300
Water volume in each tray					
With oysters approx. (litres)	25	25	25	22.5	25
Without oysters approx. (litres)	35	35	35	35	35
Circulation Rates					
Rate of pumping (litres/hour)	300	300	300	300	600
No. of times the water is recirculated per hour					
In whole plant	1.0	1.0	1.0	1.0	2.0
In each tray with oysters in place	12	12	12	13.3	24
Approx. mean velocity of water through the plant with oysters in place (cm/sec)	0.21	0.21	0.21	0.21	0.442

5.3 Results and Discussion

5.3.1 Oxygen Levels

Figures 15 and 16 overleaf show the percentage saturation of oxygen at the inlets and outlets of each tray for oysters in trays at normal loading of 110 oysters/tray and for increased loadings of 140 oysters/ tray. These figures show the maximum oxygen deficit obtained during the first 16 hours of operation. Figure 17 overleaf shows the aggregate oxygen deficit across all the boxes in mg/litre plotted against time. The aggregate is defined as follows:-

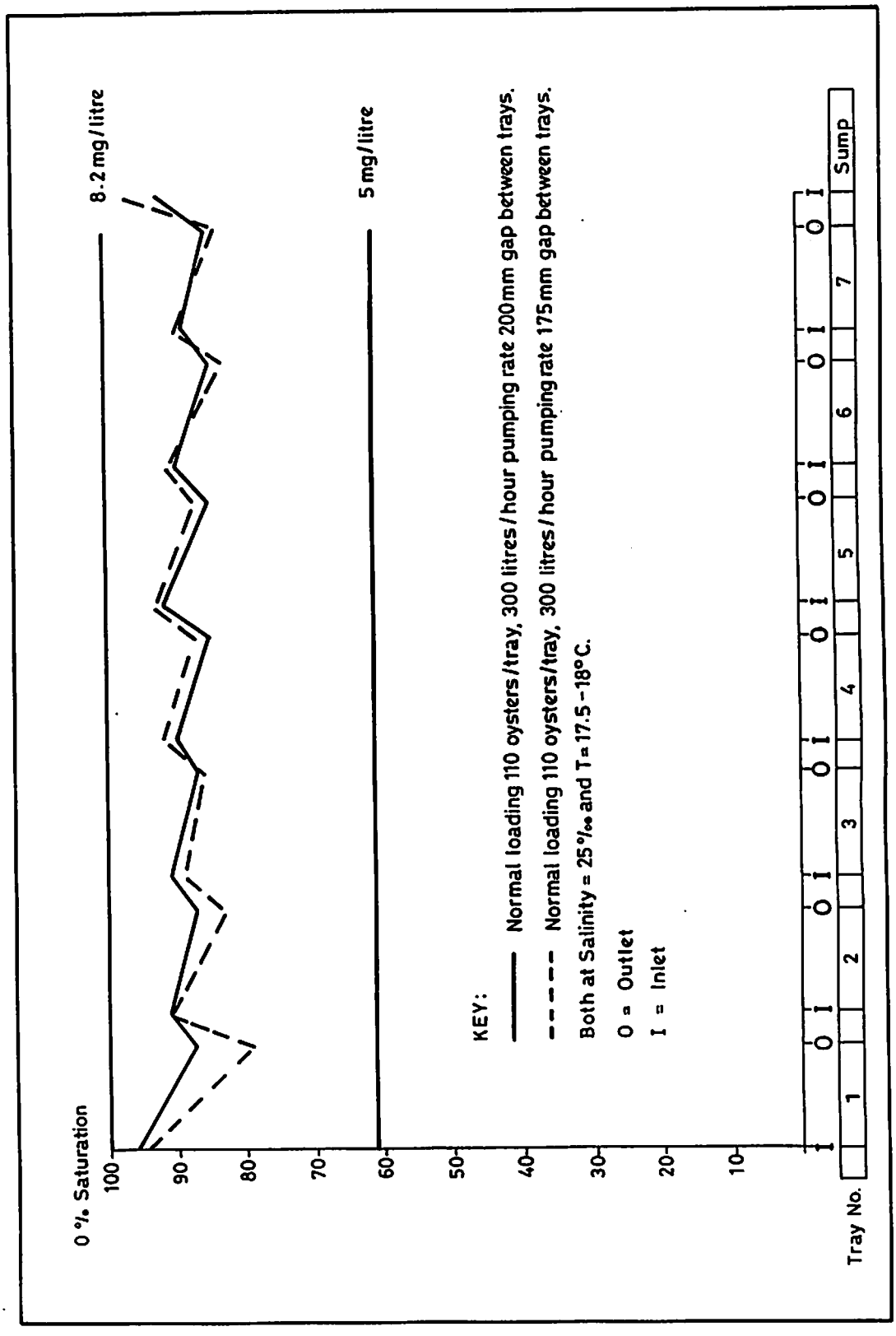
$$\Sigma \Delta \ 0_2 = \sum_{t=1}^{t=7} (0_2)\, i - (O_2)\, o$$

where $(O_2)\, i$ = Concentration of 0_2 at the inlet of trays 1 to 7
$(O_2)o$ = Concentration of 0_2 at the outlet of trays 1 to 7

t = tray numbers 1-7

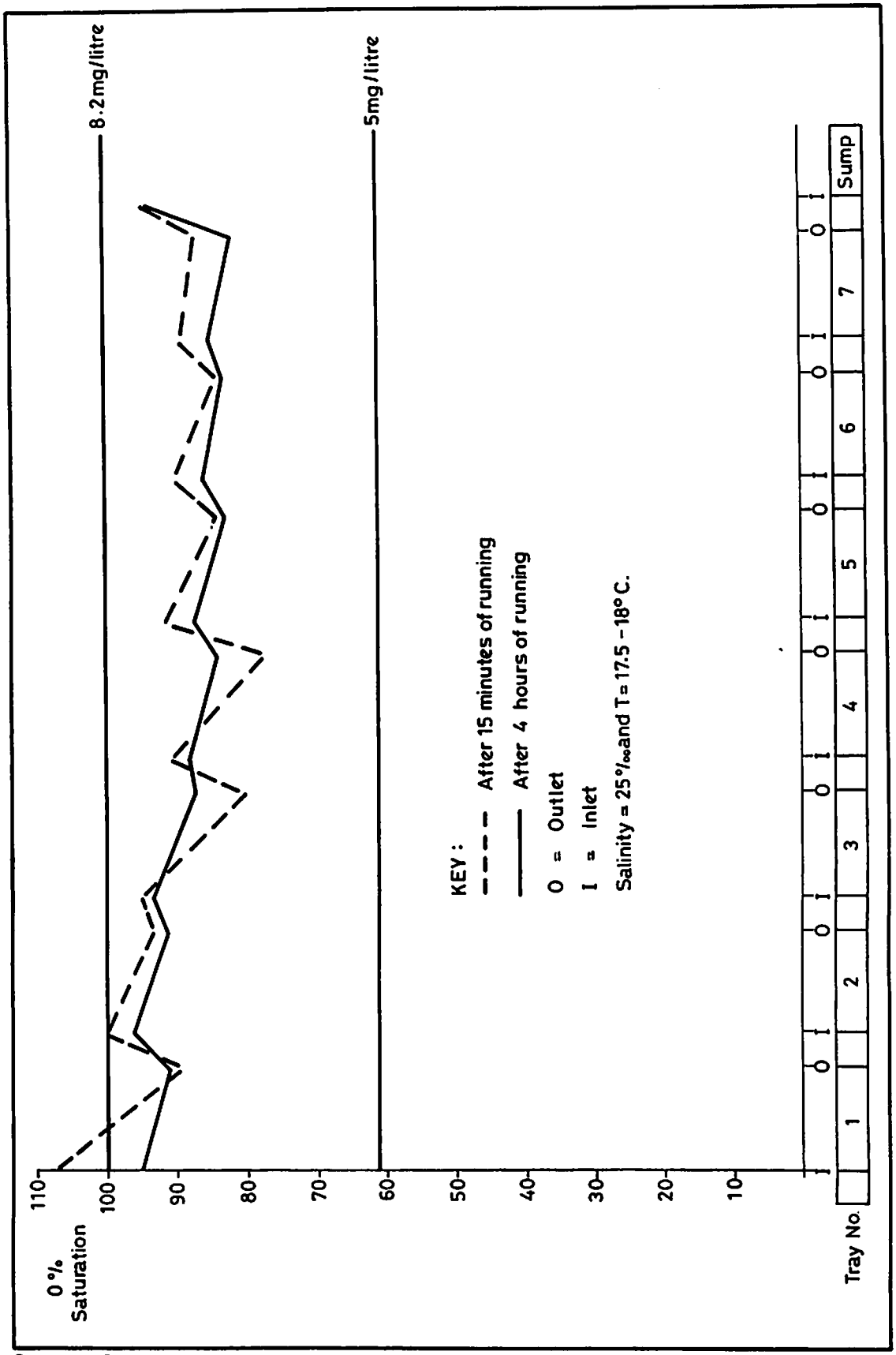
This shows that the net activity of all the oysters is fairly constant throughout the depuration cycle.

Both Figures 15 and 16 show the oxygen consumed by the oysters during the water's passage through the boxes is replenished when the water falls into the box below. Reducing the gap between the boxes from 200mm to 175mm does not appear to seriously affect the plants capacity to re-aerate the water between boxes (Figure 15). There is further replenishment when the water falls into the sump. This suggests that there is no limit to the number of boxes which could be placed in a stack, provided loadings are kept to 110 oysters/box. However, it is important to have an effective cascade at the base or an aerator, to ensure that oxygen is fully replenished before recirculating.



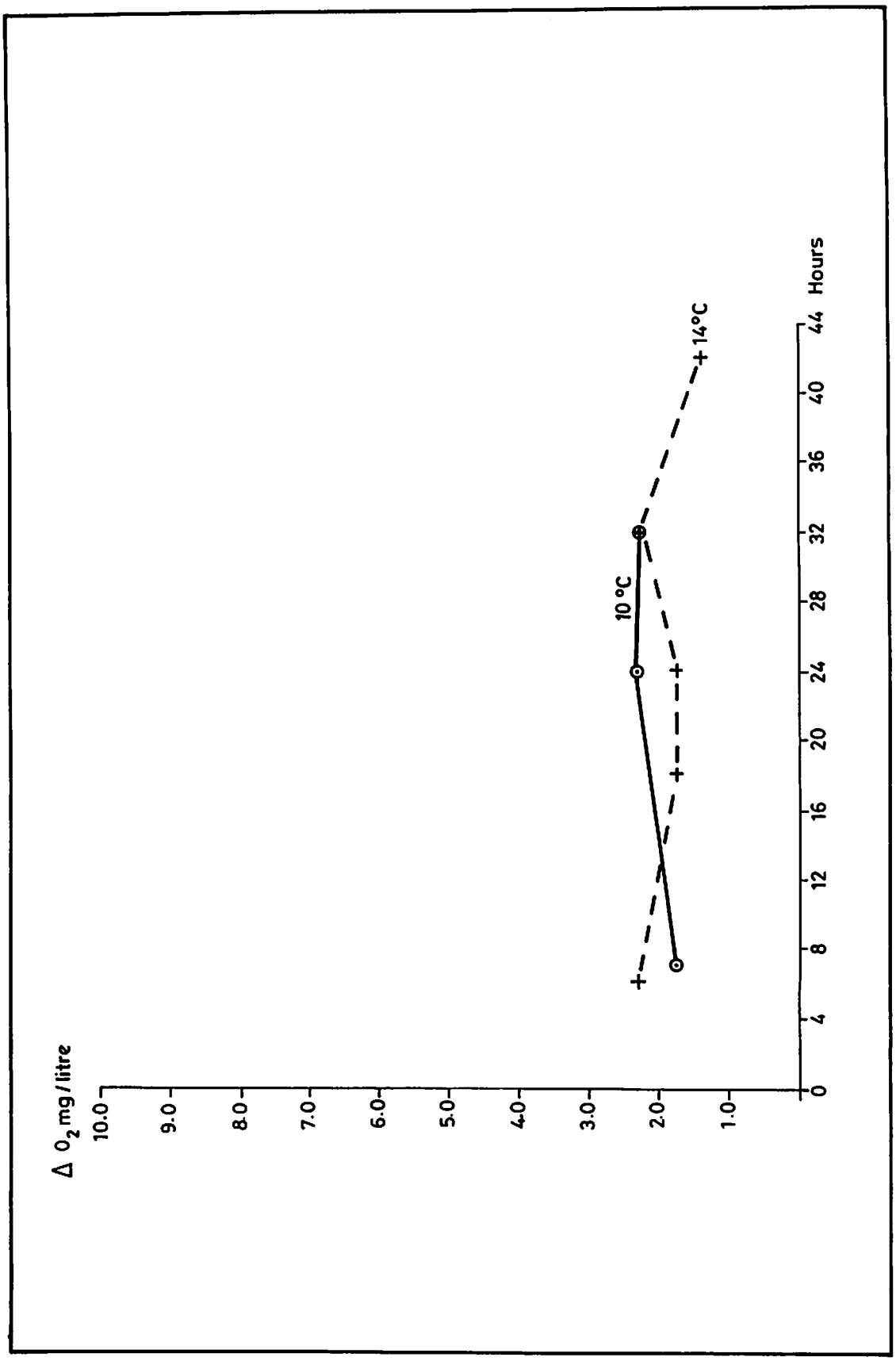
Oxygen Saturation at the Inlets and Outlets of Trays in Prototype Commercial Depuration Plant

Fig.15



Oxygen Saturation at the Inlets and Outlets of Trays in Prototype Commercial Depuration Plant. The Plant was run at an Increased Loading of 140 Oysters / Tray and 300 litres / hour Pumping Rate, with a 200 mm Gap Between the Trays.

Fig.16



Aggregate Oxygen Deficit / Time for Prototype Commercial Depuration Plant. Details of Oyster / Water Ratios Given in Table 9 for Experiments 1 and 2.

Fig.17

Considering individual boxes a flow of 300 litres/hour at 140 oysters/tray corresponds closely to the requirement for 2 litres of water/oyster/hour set in Section 3.3.2. At normal densities of 110 oysters per tray this guideline is considerably exceeded. However, the oyster/water ratio is slightly higher in numbers terms but lower in dry weight terms, in the boxes of the commercial prototype plant than in the experimental depuration systems (compare Tables 8 and 9). Also the effective velocity of water in the boxes is similar to other commercial systems (Table 1) so there seems no justification for reducing the flow.

5.3.2 Depuration

Three depurations were carried out at temperatures of 10°C, 14°C and 18°C. In each experiment three replicate samples of oysters were taken initially pre-depuration. Intermediate samples were taken from boxes 2,3,5 and 6 at 21 hours and final samples post depuration were taken from boxes 1,4 and 7. The bacteriological procedure is detailed in Appendix I. The results together with water samples are shown in Tables 10 below and 11 and 12 overleaf.

Table 10
Oyster Depuration at 10°C
Results from Experiment 1

Time	Oysters					
	Sample	Bacteria/100g flesh				
	Tray Number or pooled sample	Total Coliform		E.coli		Faecal Streptococci
		MPN	±95%	MPN	±95%	530
Pre-purification	Mean of three replicates	2800	370-10,000	1300	350-3,000	530
After 21 hours	Tray 2 & 3 Tray 5 & 6	20 80	4-34 5-150	20 80	4-34 5-150	<20 <20
After 42 hours Post-depuration	Tray 1 Tray 4 Tray 7	20 20 80	4-34 4-34 5-150	<20 <20 40	 150	<20 <20 <20
Water						
Sample	Bacteria/100 ml					
	Tray	Total Coliform		E.coli		
		MPN	±95%	MPN	±95%	
At the start of depuration	I=1 O=7	7 79	1-170 25-190	7 33	1-170 11-93	
After 21 hours	I=1 O=7	<2 2	0.57	<2 <2		
After 42 hours of depuration	I=1 O=7	<2 <2		<2 <2		

Key I=Inlet of Tray O = Outlet of Tray

Table 11
Oyster Depuration at 14°C
Results from Experiment 2

Time	Oysters					
	Sample	Bacteria/100g flesh				
	Tray Number or pooled sample	Total Coliform		E.coli		Faecal Streptococci
		MPN	±95%	MPN	±95%	
Pre-purification	Mean of three replicates	23,500	170-14,000	7000	170-49,000	<20
After 21 hours	Tray 2 & 3 Tray 4 & 5	490 110	170-1,300 21-150	490 110	170-1,300 21-150	<20 <20
After 42 hours Post-depuration	Tray 1 Tray 7	80 80	4-150 5-150	50 80	5-130 5-150	<20 <20

Sample	Water				
	Bacteria/100 ml				
	Tray	Total Coliform		E.coli	
		MPN	+95%	MPN	+95%
At the start of depuration	I=1 0=7	<2 110	2-250	<2 70	1-170
After 21 hours	I=1 0=7	<2 <2		<2 <2	
After 42 hours of depuration	0=7	<2		<2	

Key I=Inlet of Tray 0 = Outlet of Tray

Table 12
Oyster Depuration at 18°C
Results from Experiment 5

Time	Oysters					
	Sample	Bacteria/100g flesh				
	Tray Number or pooled sample	Total Coliform		E.coli		Faecal Streptococci
		MPN	±95%	MPN	±95%	530
Pre-purification	Mean of three replicates	>18,000	-	>18,000	-	530
After 21 hours	Tray 3 & 4 Tray 5 & 6	>18,000 >18,000	- -	16,000 >18,000	6400- 58,000-	<20 <20
After 42 hours Post-depuration	Tray 1 Tray 4 Tray 7	330 5400 460	110-930 1800-14,000 160-1,200	230 2400 210	70-700 680-7,500 70-630	<20 <20 <20

Sample	Water				
	Bacteria/100 ml				
	Tray	Total Coliform		E.coli	
		MPN	+95%	MPN	+95%
At the start of depuration	0=7	540	1-180-1,400	130	30-310
After 21 hours	Sump 0=7	94 79	28-220 25-190	17 11	5-46 2-25
After 42 hours of depuration	I=1 0=7	<2 <2	- 0.5-7	<2 <2	- 0.5.7

Key I=Inlet of Tray 0 = Outlet of Tray

Note: That initial contamination was very high and that flow rate doubled for this trial when compared with Experiments 1 and 2

The results show that the oysters successfully depurated total coliforms, E.coli and Faecal Streptococci at 10°C and Total Coliforms and E.coli at 14°C (the Faecal Streptococci were not present in significant numbers in the experiment at 14°C). The water samples show that the UV lamp successfully removed *E.coli* and Coliforms from the water. There is some evidence for the oysters shedding bacteria into the water at the start of depuration. This effect was not detectable after 21 hours. The trend of increased concentration of bacteria in the oysters in the lower trays is not significant. However if substantially more trays were added to the stack the effect should be investigated.

The results of depuration at 18°C are less satisfactory although there was at least 4-20 fold reduction in Total Coliforms and E.coli and a 360 fold reduction in Faecal Streptococci. The initial contamination of the oysters was extremely high and the flow rate had been doubled for that experiment. A longer period may have resulted in effective depuration. The causes of incomplete depuration - high temperature, extreme contamination, high flow rate or other factors - remain uncertain.

5.3.3 Resuspension of Particulates

The results of this experiment are shown in Table 13 overleaf. These show that the bacterial load of the particulate matter underneath the oysters is quite high and that if this matter is re-suspended it can be reingested by the oysters and bacterial re-contamination can occur. The results of previous Seafish work (6) indicated that potentially hazardous particulates from the surface of the oysters can be reingested by the oysters if suspended in the water column of the depuration plant.

The results of the high flow rate purification trial shown in Table 12 are also of some concern as there is indication of contamination passing down the stack in the water during the first half of the purification cycle. This may be a feature of the extremely high initial levels of contamination or result from the high flow rate disturbing and suspending particulates in which case the delay in purification could be explained by the time period required for those particulates to settle within the system or be absorbed by the oysters.

Table 13
Re-suspension of particulates Experiment.
This experiment was carried out after Experiment 1.

Sample	Oysters				
	Bacteria/100g flesh				
	Total Coliform		E.coli		Faecal Streptococci
	MPN	±95%	MPN	±95%	
Sample taken from Tray 1 after normal refill and drain down	20	4-34	20	4-34	<20
Sample taken from Tray 1 aftrer running with the UV lamp off and particulates stirred up for 3 hours	70	10-170	50	5-130	<20

Water				
Sample	Bacteria/100 ml			
	Total Coliform		E.coli	
	MPN	+95%	MPN	+95%
Midstream sample taken during drain down	<2		<2	
Sample from sump after mixing of particulates	17	5-46	17	5-46
Sample from bottom of tray containing particulates	920	300-3,200	32	9-78

6. Oxygen Consumption of Oysters in Depuration Plants

6.1 Method

These results were derived using data on oxygen levels in the experimental and commercial depuration plants. The oxygen consumption was calculated for each observation of oxygen levels by the following formula:

$((O_2)U - (O_2)D) \times F/TDWO = O_2$ consumption mg/g dry weight oyster/hr

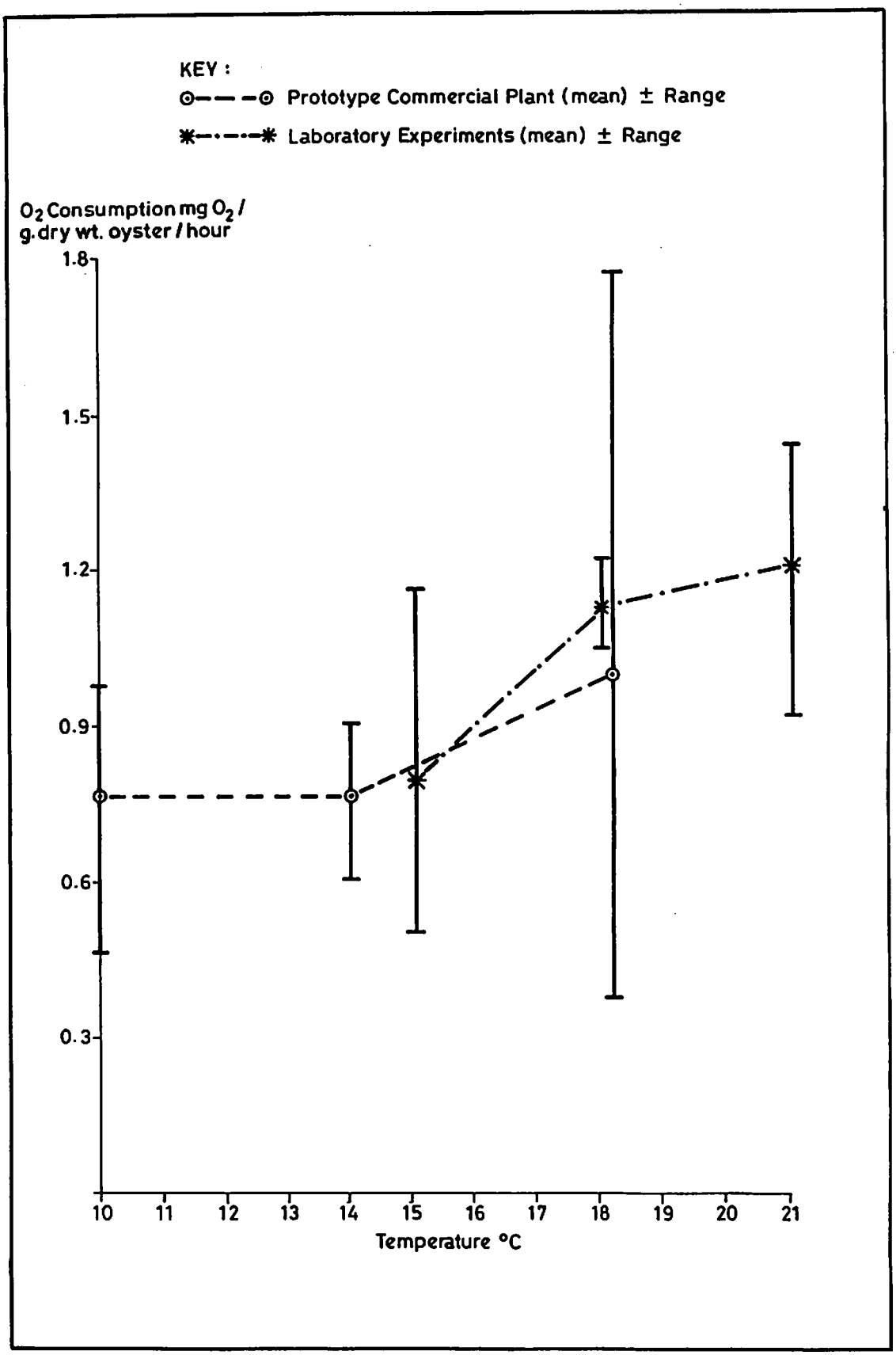
Where:

(0_2) U	=	Concentration of oxygen upstream of oysters mg/litre
(0_2) D	=	Concentration of oxygen downstream of oysters mg/litre
TDWO	=	Total Dry Weight Oyster
F	=	Flow of water (litres/hour through the system).

This method ignores oxygen uptake through the surface of the water.

6.2 Results

The results of these calculations are shown in Figure 18 overleaf for both laboratory systems and commercial prototype. The ratio of dry weight oysters to water is similar in both systems at 4-5 g/litre in the commercial system and 6.5 g/litre in the experimental system. The results are comparable, although there is more variance in the results for the commercial prototype. This method does not take into account the different respiration rates per gram oyster. Small oysters would be expected to respire more vigorously per gram than larger oysters and there may well be seasonal and other factors such as the water condition and the stress to which the oysters have been subjected.



Oxygen Consumption in mg O_2 per g. dry weight oyster per hour / temperature in °C.

Fig.18

7. Conclusions and Recommendations

1. The laboratory investigations provided some, but by no means conclusive, indications that there is a slight reduction in the activity and depuration of Pacific oysters when they are held at double the depth of layer currently recommended by MAFF.

   (i) there was some reduction in neutral red uptake from the surrounding water;

   (ii) there was some indication of lower oxygen consumption, and;

   (iii) there was some indication of higher bacteriological counts after purification.

   Considered separately each of these slight indications would not be considered significant but together they justify the recommendation that the currently recommended density of 530 oysters/m^2 (50 oysters/ft^2) should not be exceeded, at least until further work justifies any increase.

2. The laboratory investigations indicated that for the type of plant envisaged with re-aeration between boxes and assuming a maximum operating temperature of 21°C a water flow of 2 litres/oyster/hr should ensure adequate oxygenation. This applies only to the type of plant described in this report as the flow requirement will vary with plant layout and aeration system.

3. Based on the above, a prototype vertical stack purification plant utilising artificial seawater has been designed, built and tested. The plant features:

   (i) a stack of 7 boxes above a sump with water circulation pump, UV steriliser and heater incorporated,

   (ii) a cascade into one end of each box from the box above, flow along the length of each box through the oysters, and cascade into the box below,

   (iii) flow control devices to minimise turbulence, create an even flow through the oysters and efficient re-aeration via the cascades.

   (iv) the raising of the oysters above the base of the boxes and separate drainage of each box back to the sump leaving the detritus from purification on the base of each box, and

   (v) a nominal capacity with a single stack of boxes of 875 oysters in 300 litres of water, or double that with two stacks.

4. In initial testing of the plant demonstrated that:

   (i) the re-aeration system was effective and the resulting levels of dissolved oxygen throughout the system remained high,

(ii) purification was effective at the nominal flow rate and normal levels of contamination, although delay in purification was encountered with very high levels of oyster contamination (which coincided in the trials with a double flow rate) and,

(iii) the concern in the design to avoid the resuspension of detritus and subsequent re-contamination of the oysters during purification was justified as that did occur when the detritus was deliberately disturbed during the trials.

5. Further testing and development of the prototype will be required to establish a standard design of plant and its operating criteria.

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Appendix I

Bacteriological Methodology

Appendix I

Bacteriological Methodology

Bacteriological analysis for Total coliforms and *Escherichia coli* in both water and oyster flesh was carried out using the method described by West and Coleman (8). This is a Most Probable Number (MPN) technique; it is considered to be a more sensitive technique than pour plates or roll tubes. For faecal streptococci plate techniques were used.

Each sample of oyster flesh consisted of 10 meats pooled. The meats were removed from the oysters using an aseptic technique, placed in sterilised jars, and transported in chilled containers to the Public Health laboratory where the anlaysis was carried out.

Three replicate samples of oyster flesh were taken before each depuration experiment. After the experiment oyster samples were removed from positions in the depuration tanks as described in the report. Water samples were also taken in sterilised jars from positions in the plant as described in the report. The water samples were also analysed by the West and Coleman (8) MPN method. These techniques were used for all bacteriological assessments throughout the experiments.

Appendix II

Development of Prototype Vertical Stack Purification Plant

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Development of Prototype Vertical Stack Purification Plant

1. Introduction

Where quantities of bivalve molluscs to be depurated are small a vertical stack system is often used. This consists of boxes containing the shellfish supported one above the other in a frame sat over a sump. Water is pumped up, via a u.v. sterilizer, to the top box from where it cascades down from one box to another until finally returning to the sump. In order to progress trials with oyster depuration and to extend the range of standard plant designs, Seafish considered the development and construction of such a unit.

2. Existing Units

Vertical stack units are usually used for oyster depuration but in many cases are also used as storage facilities, whether or not purification is required. The basic design concept is good, it being a modular unit using a minimum of floor space, but there were several aspects of water flow and method of operation that concerned Seafish.

2.1. Water Cascade: in most systems water flows from one end of the box to the other, the water outlet consisting of two holes drilled in one end. In practice the water cascades directly over the oysters in the box below and may reduce the activity of individuals.

2.2 Water Flow: adequate dissolved oxygen levels throughout the boxes is an essential factor for effective purification. There was some concern with regard to the potential for water `dead spots' within the boxes and also the adequacy of the prescribed flow rates of 1 to 2 system water changes per hour.

2.3 Depth of Water: from an operational viewpoint the height of the system is restricted by the need to remove the top box. In order to maximise on the number of boxes in a stack therefore the boxes are shallow. This could result in oysters not being totally immersed.

2.4. Bottom Clearance: the oysters sit directly on the bottom of the boxes and are kept clear of faeces, mud and silt.

2.5. Drainage: it is important when draining down the boxes that the faeces etc, are not disturbed which could cause recontamination of the oysters. In existing systems a small bleed hole is drilled in the bottom of each box. This drains continuously during purification but has little effect on the water cascade due to its low flow rate. Its purpose is twofold. If the pump failed it was considered preferable to let the system drain and not leave the shellfish in stagnant water. In practice this really applied to lobster storage for which these systems could sometimes be used and not mollusc purification. The bleed hole was also the means of draining the system down. In theory boxes were completely drained and the flow of water was such that faeces would not be disturbed from the bottom of the box. In practice this method of drainage is too slow and operators may pull boxes out of a stack with oysters still partly immersed, disturbing faeces and potentially recontaminating. As an alternative the operator may put a bung into the box end to allow more rapid drainage. This also increases the potential for re-contamination due to sudden change in water flow around the hole. The bleed hole itself, if used correctly, could also cause recontamination as any material drawn through the hole will fall onto a box of immersed oysters below.

2.6 Water Temperature: this is an important factor in maintaining the balance between shellfish activity and dissolved oxygen levels. Pacific oysters *(C. gigas)* are now commonly farmed and purified in the UK. Recent work by Seafish has shown that a water temperature of 8°C is preferable to the 5°C currently specified by MAFF. Water heating may be required to maintain the temperature.

2.7. Handling: Pacific oysters are easily damaged and difficult to wash. Seafish considered it worth considering means of reducing handling and therefore damage when operating the purification unit.

3. Development of Prototype Unit

3.1 Box type: to reduce cost it was decided that a standard plastic box be used. The requirements considered included internal depth, loading, water flow and ability to fit in a rack system. The Allibert box no. 12030 of 30 litres capacity and currently used in some stack systems was ultimately chosen (Figure 1). This has internal working dimensions of 620 x 370 x 120mm.

3.2 Sump: the sump must have sufficient capacity to contain all the seawater with the boxes drained down and sufficient water when in use to maintain the circulation pump suction. For the prototype this was constructed in painted marine ply and GRP (Figure 2). The sump was made to take two vertical stacks of boxes and was 1.24 metres x 0.92 metres x 0.6 metres deep.

Although the sump could take two such stacks this was not considered for trials purposes and consequently it was partitioned. The working volume, directly below the boxes then had a capacity of 300 litres.

3.3. Support frame: Dexion galvanised mild steel angle (75mm x 50mm) was bolted together to form a frame with a capacity of seven boxes in a vertical stack. The frame was mounted on top of the sump and gave the unit an overall height of 2.1 metres. The boxes were mounted on Dexion supports and could be removed individually from the stack (Figures 2 and 3).

3.4. Circulation system: a Berisford PV21 flooded suction pump with magnetic drive was used with flow control valve, flowmeter and 15 watt UVAQ ultra-violet light sterilizer. A heater (3.5) was also fitted. Pipework was in ¾" ABS plastic with water pumped from the sump up to the top of the unit and into the top box. The circulation system was mounted on a sheet of painted marine ply attached to one side of the unit and clear of any water spray or splashing (Figure 4).

3.5 Heater and control: a 3KW in-line heater was fabricated and fitted into the water circulation system. This was made in 100mm ABS tube with a domestic, titanium, hot water element fitted. The required water temperature was maintained using a Digistat thermostatic control unit (Figure 6).

3.6 Box Modification - Water Flow: the problem was that of maintaining a positive water flow through each box without causing undue disturbance to the oysters. A number of different options were considered and tried. The one finally used was to cascade water from the handhold in one end of the box into the end of the box below from where it flows through the box and out of the opposite end and so on down the stack. This is down diagrammatically in Figure 5. This involved boxes having to be staggered in their vertical stack and also the fitting of fabricated stainless steel plates at each end of the box. To ensure that water cascading into the box did not fall directly onto the oysters a `V' shaped plate was attached to the box at one end (Figure 7). This contained the turbulence caused by the water cascade (Figure 8) and created a water flow across the box. Where the water cascaded out at the opposite end a smaller plate was attached to direct water in a uniform cascade and prevented it from running under the box (Figure 9). The plates were bolted onto the box with the handle areas sealed with mastic. The small drain holes in the box rim and stacking pillars were also blocked with mastic. These modifications allowed a suitable water depth when loaded of 120mm.

3.7 Box Modification - False Bottom: to keep oysters clear of faeces, mud etc., two options were considered. One was to use an open mesh plastic floor mat in the bottom of the box (Figures 1 and 10) and the other to insert a tray (Figure 11), of open mesh design inside the purification box. Most existing floor mat is quite deep (25mm) and too deep for the limited water depth available in the boxes used. Allibert `Allimat' was only 10mm deep and was used. An Allibert tray No.41015 was also found to fit inside the purification box. This had the advantage that with the system drained down the tray of oysters could be lifted clear of the faeces in the purification box bottom without direct handling of the oysters. Oysters could then be hosed down in the tray with the purification box washed out separately. The disadvantage of course was that it reduced the oyster capacity by up to 20%.

3.8 Box Modification - Drainage: the potential problems of box drainage are discussed in 2.5 and the use of a `bleed' hole in the box bottom has been discounted as not necessary. A number of possible options were considered and the method chosen was one of drilling two 12mm holes in the bottom of the nesting pillars of the box at one end (Figure 9). By splitting the drainage to two holes it was possible to have a reasonable drain down time of four minutes whilst minimising the potential for recontamination as a result of changes to flow conditions in each box. By inserting a section of plastic guttering beneath the drain holes from the box the water was sent directly to the sump (Figures 12 and 13). This allowed for individual boxes to be drained down instead of the whole system. This is necessary if the purification unit is to be used as a storage facility when molluscs from an individual box may be required.

The drainage holes were positioned such that in use, 8% of the water in the box did not drain down which represented 4% of the unit water capacity. Water is also lost through evaporation and some splashing and in practice a make up of between 5% and 10% is required if re-using seawater. This water contained the faeces and mud at the bottom of the box (Figure 14) and prevented the material from draining down into the sump. It could only be removed therefore by washing out once the box had been removed from from the purification unit. Because the oysters were sat either on matting or a box the water level in the box was below the oysters before the box was removed from the stack.

The drain holes were sealed by means of rubber bungs. The individual hole size of 12mm was such that if one of the two bungs came out during purification the water flow out of the hole to the sump was less than that running through the system. Water would continue to flow through the cascade therefore although at a reduced rate.

3.9 Unit Capacity

Using the MAFF recommended loading density of 530 oysters per square metre the Allibert box has a capacity of 125 oysters (at an average weight of 100gm) or 100 if the tray insert is used. The prototype with its single box stack has a nominal capacity of 875 oysters which would double to 1750 if both stacks were fitted.

3.10 Water Flow Rate

Concern with water flow through the boxes is discussed in 2.2 and on the basis of water changes per hour is dependent upon the system water capacity. With a sump capacity of 300 litres the water flow through the boxes is 0.2 cm/second at a single water change per hour and 0.3 cm/second at one and a half. These compare well with flows in other proven systems developed by Seafish and coupled with the box modifications discussed in 3.6 allows a uniform water exchange with good re-oxygenation between boxes. Water change rates of 2 changes per hour with this system are not at present considered to be necessary when used for oysters.

4. Plant Operation

Clean boxes are filled with washed oysters (Figure 15) clear of the box bottom using matting or an additional mesh box (3.7). The boxes are then slid into the frame, care being taken to orientate them correctly. In practice it was found that numbering the boxes 1 to 7 made the task more straightforward.

Before turning on the u.v. unit and pump it is important to ensure that box drain bungs are inserted and that the sump is filled with sufficient seawater. If there is not enough seawater in the system then the sump and hence pump can run dry during filling, potentially damaging the pump. The flow valve can be turned open during filling but once complete must be turned down to the required water flow rate.

Under no circumstances should the heater be switched on until the system is running and the thermostat probe inserted into the water, usually in the top tray, otherwise the plastic heater casing will melt. This unit sufficed for the trials but in a commercial operation an alternative method, such as installing a heater in the sump, would be used. The Digistat control unit must be set to the required water temperature.

When draining down the heater must be switched off first and then the pump and u.v. The plastic guttering is inserted beneath the boxes to be emptied and the bungs removed. Once the water stops flowing out of the boxes the bungs should be replaced and the box removed from the frame. Oysters must be removed before washing to keep clear of faeces in the box bottom.

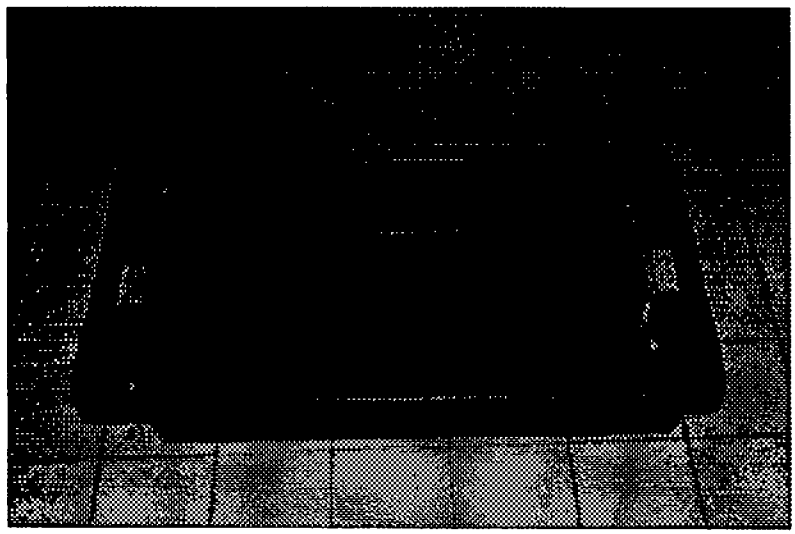


Figure 1 - Modified Allibert Box with Plastic Mat Insert

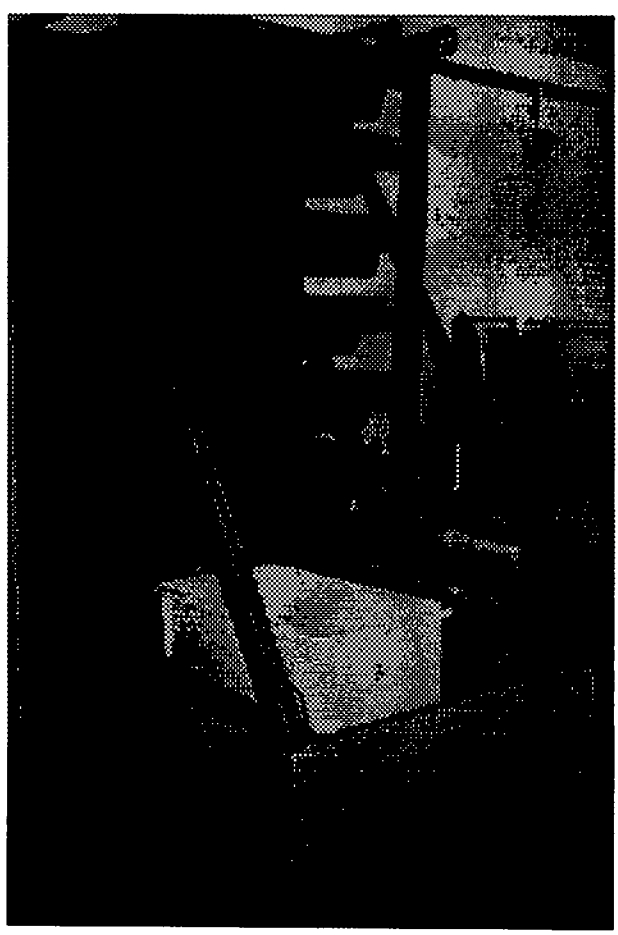


Figure 2 - Prototype Unit Sump and Support Frame

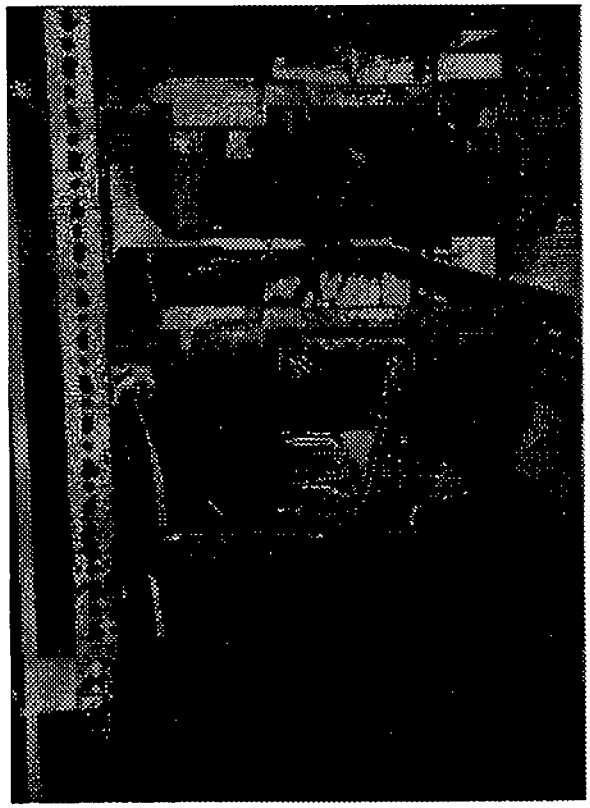


Figure 3 - Boxes Mounted on Frame

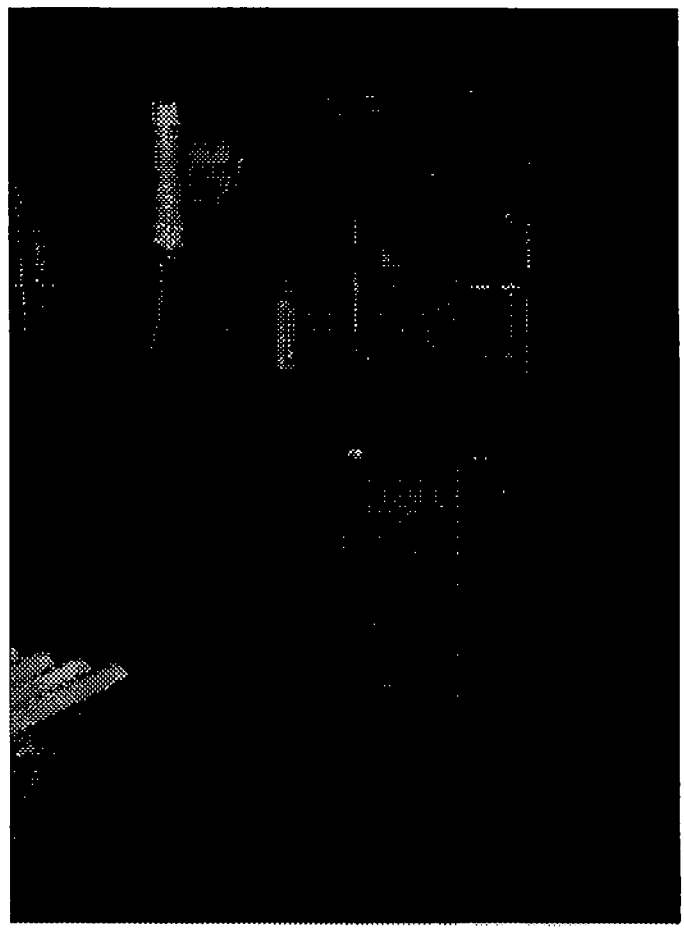
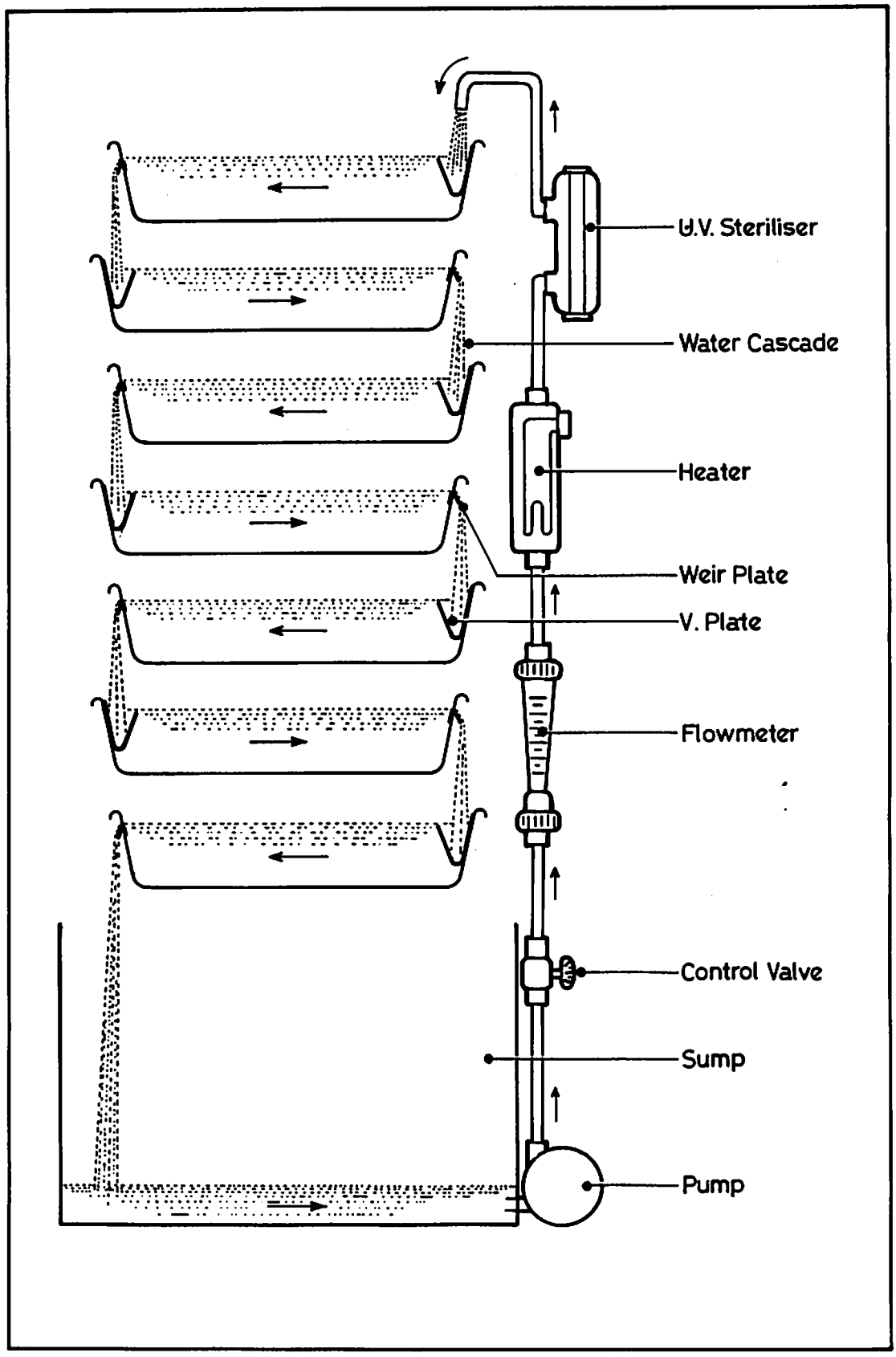


Figure 4 - Prototype Circulation System



Water Flow Through Purification Unit Fig.5

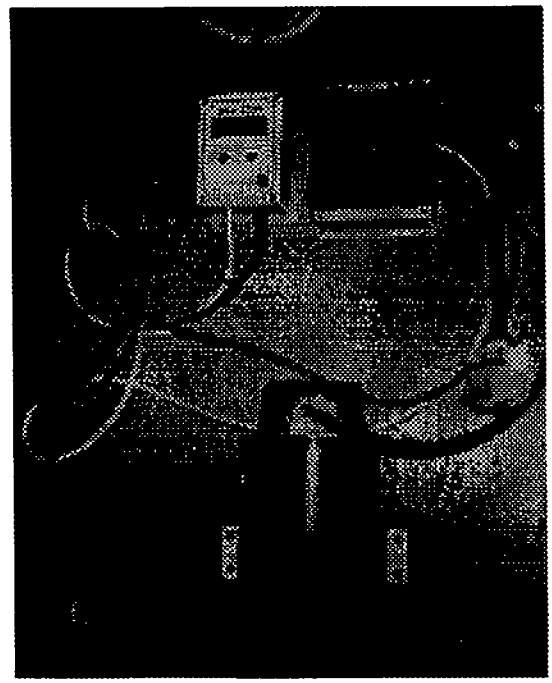


Figure 6 - Heater and Temperature Control Unit

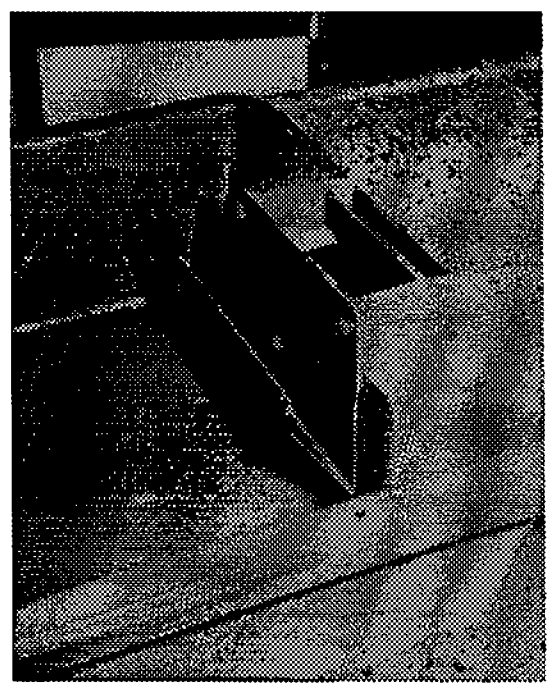


Figure 7 - V Shaped Water Containment Plate

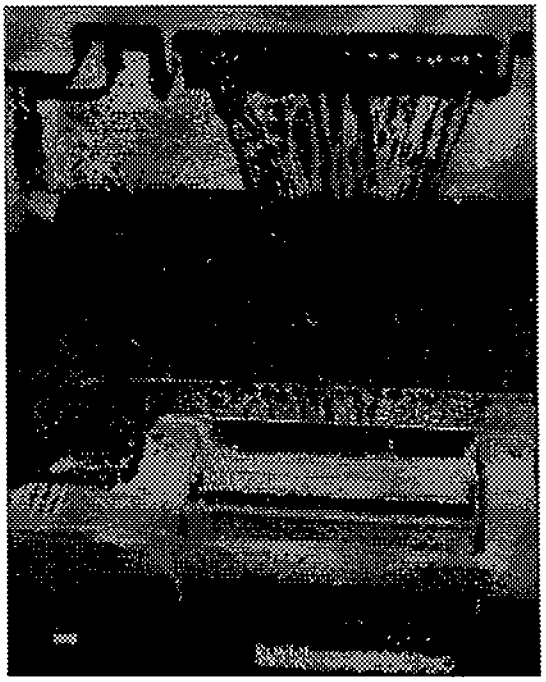


Figure 8 - V Shaped Plate Contains Water Cascade

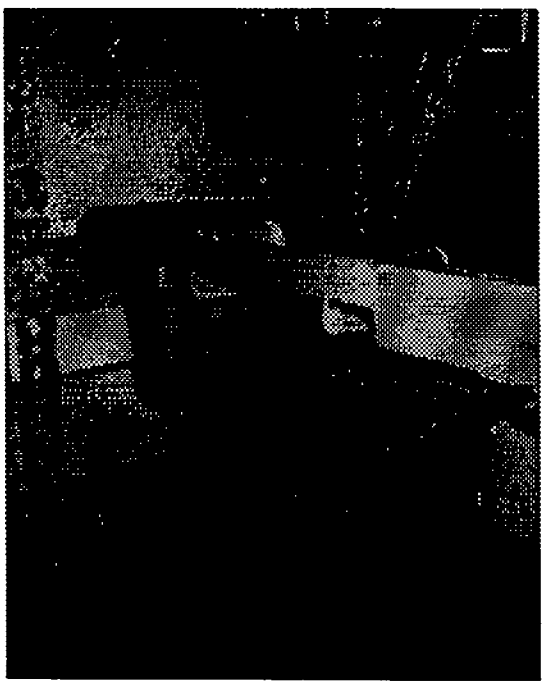


Figure 9 - Water Cascade Plate Note Bungs in Box Drains

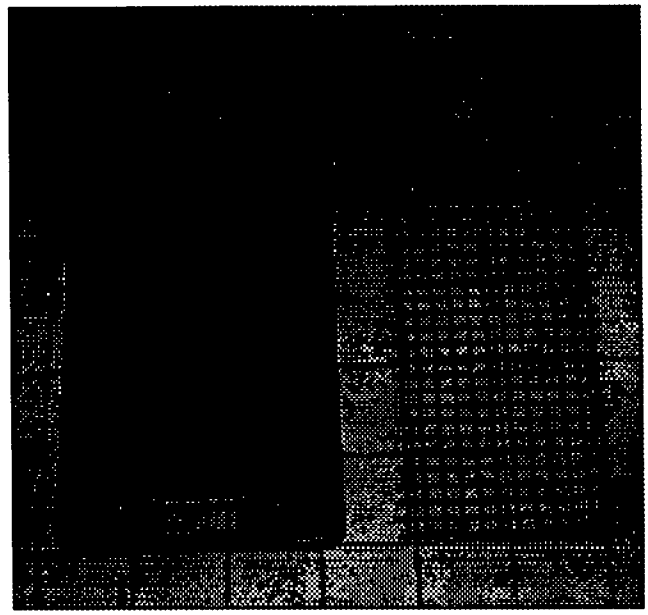


Figure 10 - Floor Mat Insert for Purification Box

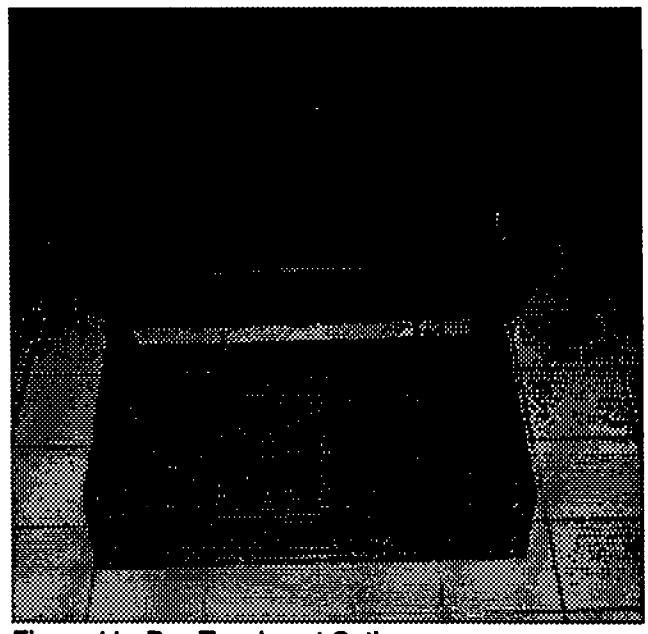


Figure 11 - Box Tray Insert Option

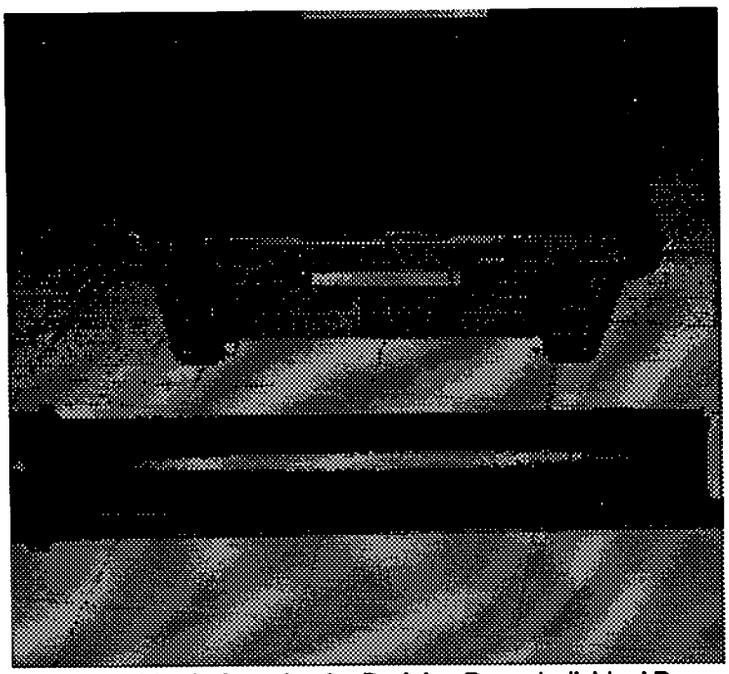


Figure 12 - Plastic Guttering for Draining Down Individual Boxes

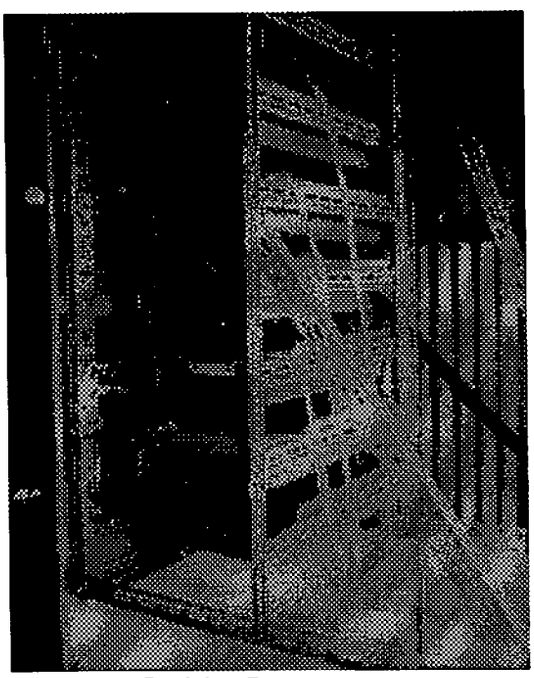
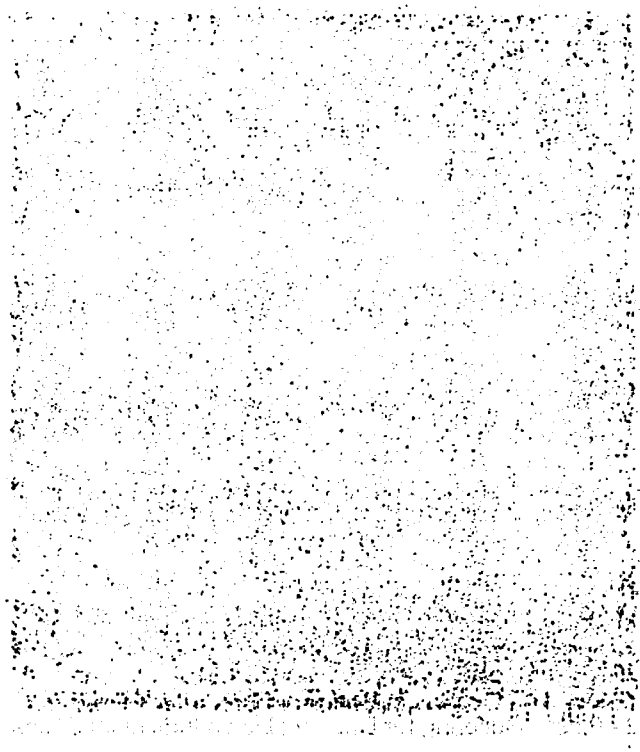
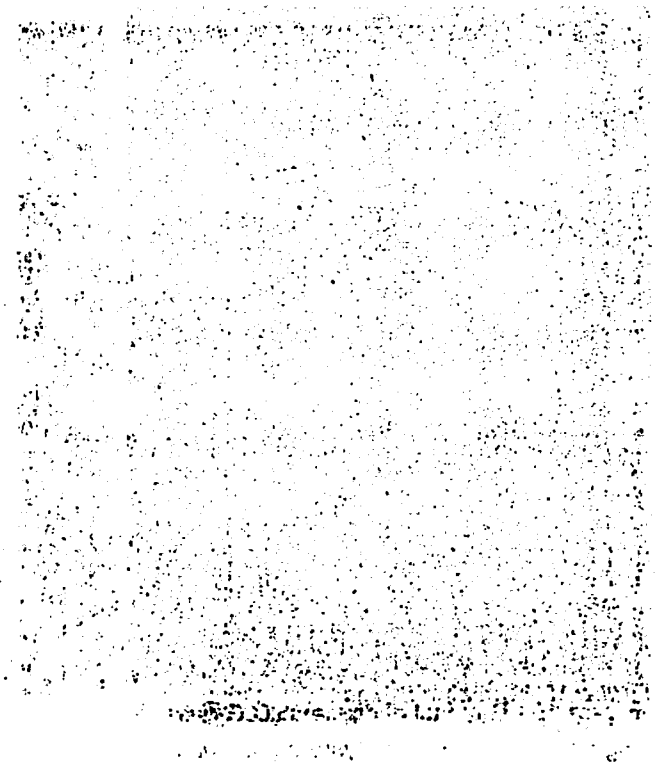


Figure 13 - Draining Boxes





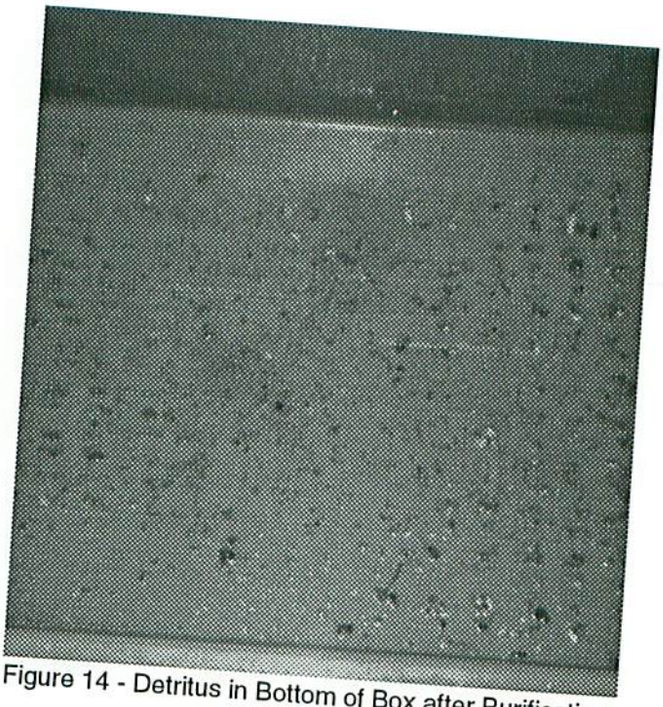


Figure 14 - Detritus in Bottom of Box after Purification

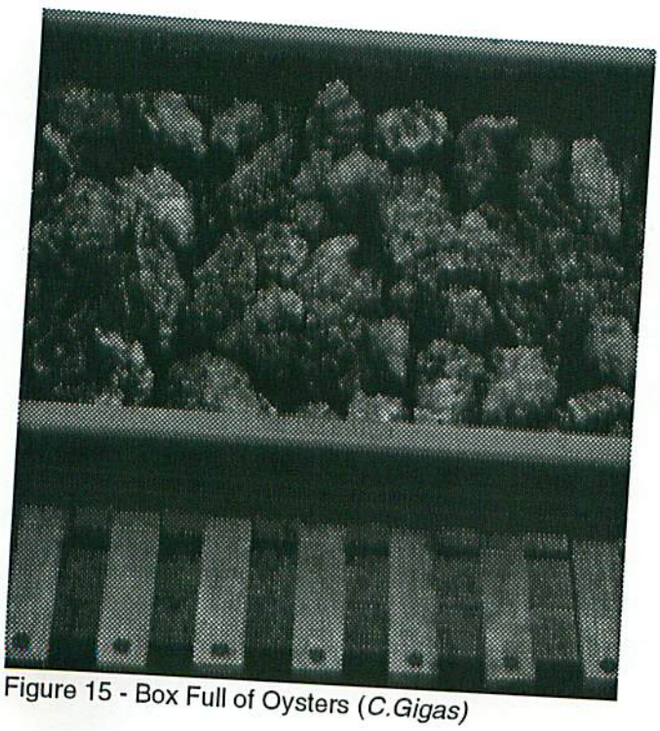


Figure 15 - Box Full of Oysters (*C.Gigas)*