REPEATED RE-USE OF SEA WATER AS A MEDIUM FOR THE FUNCTIONING AND SELF-CLEANSING OF MOLLUSCAN SHELLFISH

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(With 8 Figures in the Text)

Beds of molluscan shellfish are frequently located in or near estuaries which receive sewage discharged from neighbouring towns. Each shellfish passes through its system many times its own volume of water every hour (see Fox, Sverdrup & Cunningham, 1937; Galtsoff, 1946), and the organic matter and a proportion of the bacteria present in the polluting sewage pass into the digestive tract. Thus dissection of a mussel so as to reveal the rectum shows a striking contrast between the dark brown or black colour in a mussel taken from a polluted bed and the clean appearance in one which has been immersed for some time in pure sea water. Shellfish sometimes contain pathogenic bacteria of the typhoid-paratyphoid group and may thus give rise to these diseases in people consuming them, particularly as large proportions of both mussels and oysters are eaten raw. For this reason emphasis has been placed in recent years on the importance of subjecting shellfish before marketing to a period of immersion in pure sea water so that the contents of the digestive tract, including bacteria of human faecal origin, may be evacuated during normal metabolic processes.

At the cleansing stations operated or supervised by the Ministry of Agriculture and Fisheries at which mussels are purified before being released for sale to the public, the process of purification adopted is that recommended by Dodgson (1928) and approved by the Ministry of Health. In this process a batch of mussels supported on wooden grids in an open tank is subjected to two successive periods of immersion, each lasting for about 16–20 hr., in sterilized fresh sea water. The sea water is sterilized in a separate tank by addition of a quantity of bleaching powder equivalent to three parts of chlorine per million. After standing overnight the residual chlorine is discharged by addition of a suitable quantity of a solution of sodium thiosulphate, and the dechlorinated water is then transferred to the tank containing the mussels. After each period of purification the water is allowed to flow back to the sea and a fresh batch of sea water is used for the next cycle of cleansing. As a result of several years' experience of this method of treatment Dodgson concluded that it was reasonable to expect cleansed mussels to contain not more than five lactose-fermenting bacteria per c.c. of flesh.

At the existing cleansing stations, which are near the coast, a plentiful supply of sea water can be obtained by pumping. There are, however, a number of places which are convenient for landing of mussels, but which are so far from the sea that it is impossible at certain times to obtain water of the required salinity. At one such place, for example, investigation has shown that at a point as near the mouth of the estuary as it would be possible to build a cleansing station the salinity of the water for several days during each fortnightly cycle of tides is below the critical value (specific gravity 1·016*) required for the correct functioning of mussels. For this reason the Ministry of Agriculture and Fisheries asked the Water Pollution Research Laboratory to investigate the possibility of treating sea water in such a way that it could be re-used for cleansing successive batches of shellfish. It would then be possible to take in a quantity of sea water when the salinity was satisfactory and to re-use the water so as to be independent of fresh supplies.

The investigation described here, in addition to pointing the way to possible development on the large scale, has led to a number of observations of general interest.

PRELIMINARY SMALL-SCALE EXPERIMENTS

For experiments on a small scale glass battery jars measuring 8 × 15 in., and 15 in. deep were used. A single layer of thirty mussels was supported on a metal grid (coated with bituminous paint as a protection against corrosion) which stood on the bottom of the jar. This grid was about 1½ in. high so that faeces and other dejecta fell through the grid to the bottom of the jar leaving the supernatant liquid clear. For each period of cleansing 30 l. of sea water, after being suitably treated according to the nature of the experiments, were added to the jar, thus allowing 1 l. of water for each mussel.

At the end of each period of purification the supernatant water above the grid was siphoned into an empty jar, the residue of the water below the grid being filtered through fine-mesh bolting silk to remove faeces and other deposits. The filtrate was then added to the bulk of water in the same jar and the deposits, including faeces, were discarded.

Chemical changes in sea water during purification of mussels

Preliminary observations in which a batch of mussels was immersed in fresh sea water in a battery jar for a period of 48 hr. at a temperature of 15–16° C. showed that the concentration of dissolved oxygen decreased to a very low figure, and the pH value of the water was lowered; these changes would be expected to

* Where accuracy was required in the course of this investigation salinity was determined in the laboratory by titration with silver nitrate solution, as described by Oxner & Knudsen (1920), using Knudsen's *Hydrographical Tables* (1901) to calculate results. As pointed out by Dodgson (1928), however, determination of specific gravity by hydrometer, though less accurate, is more suited to requirements in large-scale cleansing practice, and the errors likely to arise are not significant for this purpose. The value so obtained is the weight of a given volume of water referred to the weight of the same volume of distilled water at 4° C. Provided the temperature is recorded the hydrometer reading can be converted to the corresponding salinity by means of Knudsen's Tables. Thus at 15° C. a specific gravity of 1·016 corresponds to a salinity of 21·95 g./1000 g.

result from a normal process of respiration, dissolved oxygen being replaced by carbon dioxide. It was observed that the water, initially slightly cloudy, became brilliantly clear after an hour or so, through the filtering action of the mussels. Increases in ammonia content and in organic matter as measured by the permanganate oxygen demand were small.

It may be noted, however, that the excretory system of the lamellibranchs probably discharges into the water small quantities of urea and various other nitrogenous substances, including possibly uric and hippuric acids (cf. Field, 1922).

SMALL-SCALE TRIALS WITH RE-USED WATER

Experiments by Sherwood (quoted by Dodgson, 1928) have shown that mussels maintained in water of which they have themselves previously exhausted the dissolved oxygen become progressively more sluggish. It appeared, therefore, that if sea water were to be repeatedly re-used the depletion in dissolved oxygen resulting from the functioning of one batch of mussels should be remedied before the water was used for the next batch.

Tests were made in which 30 l. of sea water, previously de-oxygenated by being used for purifying a batch of mussels, were aerated in a battery jar by a current of diffused air supplied by a small electric blower at a rate of approximately 0·05 cu.ft. of air per min. through an Alundum* diffuser plate measuring 3 in. in diameter. The results showed that a short period of aeration was sufficient to re-oxygenate the water. Thus in one test the content of dissolved oxygen rose in 30 min. from 8 to 94 %, and in a second test it rose in 40 min. from 27 to 97 %, of the saturation value. It was concluded that a period of aeration of 1 hr. would be sufficient to ensure adequate re-oxygenation of used water.

A series of purifications was then carried out in the same water in a battery jar using the technique outlined previously. The water was taken from the estuary at Conway and had a specific gravity of 1·024 at 5° C. Each batch of mussels was subjected to two successive periods of immersion in the water, each period lasting 16–22 hr. The water was aerated with diffused air for 1 hr. between each cycle of cleansing. During each cycle of cleansing samples of water were taken (*a*) after aeration and before immersion of the mussels, and (*b*) at the end of the period of cleansing before the supernatant water was disturbed. On these samples determinations were made of pH value, specific gravity, temperature, dissolved oxygen content, chlorine demand, and amount of oxygen absorbed from acid permanganate in 4 hr. The metabolic activity of the mussels was assessed by the quantity of true and of pseudo-faeces and of byssus formed, by ability to clear the water of a suspension of fuller's earth, and by the rapidity with which the mussels responded to shock ('knock response').

The water periodically abstracted for chemical determinations was replaced by the addition of an equivalent volume of water from a jar containing sea water at the same stage of re-use. This was obtained by running a parallel series of experiments for this purpose.

* A proprietary brand of porous inorganic material.

In all, thirteen successive batches of mussels were cleansed in this way. Throughout the series the salinity of the water ranged from 29·4 to 35·5 (g./1000 g.) and the temperature ranged from 4·5 to 19·0° C. Values for dissolved oxygen, pH value, chlorine demand, and oxygen absorbed from acid permanganate are shown graphically in Fig. 1. It is evident that during each cycle of cleansing the metabolic

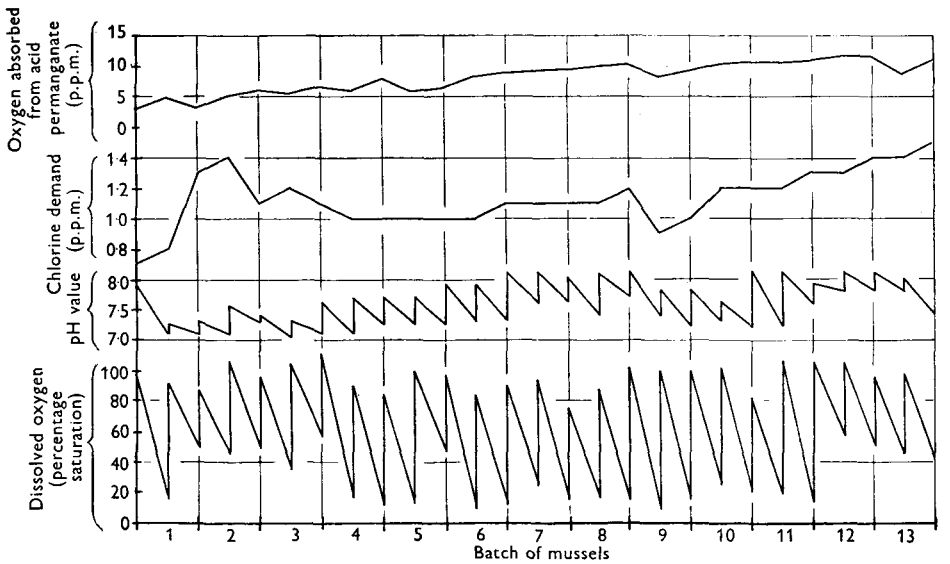

Fig. 1. Changes in dissolved oxygen content, pH value, chlorine demand, and value for oxygen absorbed from acid permanganate, in sea water repeatedly re-used for cleansing mussels. (Water aerated but not chlorinated between successive cycles of cleansing.)

activity of the mussels resulted in depletion of dissolved oxygen, which was restored by aeration before the beginning of the next cycle, and in the lowering of pH value, which increased again during the subsequent aeration. The chlorine demand fluctuated considerably but did not increase progressively and the value for oxygen absorbed from permanganate, though it increased somewhat throughout the course of the experiment, indicated that accumulation of organic matter was not appreciable. Thus the aggregate changes in the chemical characteristics of the water were of a low order. Moreover, the functioning of the mussels, assessed by their ability to use the dissolved oxygen in the water and by observation of the amount of byssus, faeces, and pseudo-faeces formed, and of response to external stimuli, was as vigorous at the end of the series as at the beginning.

Samples of mussels from each batch, taken before and after purification, were submitted to bacteriological examination to determine the count of *coli-aerogenes* bacteria, using the technique described by Clegg & Sherwood (1947). Results (Table 1) show that rather more than 50 % of the mussels were virtually free from bacteria when cleansed in re-used water, and that 90 % of cleansed mussels contained less than 5 lactose fermenters per ml. of body tissue.

Samples of the water were taken for bacteriological examination at the end of each cycle of cleansing. Results showed that the numbers of bacteria did not

Table 1. *Results of bacteriological examination of mussels cleansed in re-used water in small-scale experiments (water aerated but not chlorinated between successive cycles)*

Results as colony counts (per ml. of body tissue) of *coli-aerogenes* bacteria on MacConkey agar at 37° C.

	No. of mussels examined	Average bacterial count	No. of mussels free from bacteria	No. of mussels showing a count of 5 or less	Percentage of mussels showing a count of 5 or less
Before cleansing	78	252	—	—	—
After cleansing	78	3·3	41	70	90

increase progressively, but that they were subject to unpredictable fluctuations. Thus the plate counts on nutrient agar at 22° C. ranged from 400 to 3000 per ml., the presumptive count of *coli-aerogenes* bacteria ranged from 0 to 160 per ml. and in twelve of the nineteen samples tested the count of these organisms was not greater than 1 per 100 ml.

Three series of small-scale experiments were run on similar lines to those described above,* ten batches of mussels being progressively cleansed in each series. The series followed each other at intervals of about 1 month and, although the temperature varied between 10 and 19° C. throughout the series, it was usually between 13 and 15° C.

Results of bacteriological examination of mussels from each of these three series showed that the average counts of *coli-aerogenes* bacteria ranged from 68 to 104 per ml. of flesh in the untreated mussels; after cleansing the average count ranged from 0·5 to 3·0.

The bacterial content of the water throughout each series may be gauged from the results (Table 2) of examination of samples taken at the end of each cycle of cleansing. Counts of *coli-aerogenes* bacteria throughout each series are shown graphically in Fig. 2. It was concluded both from these experiments and from similar experiments at lower temperatures that re-used water may at times show a high count of intestinal bacteria. Previous experiments by Sherwood† have shown that re-used sea water does not support the growth of *coli-aerogenes* bacteria. Whether the count is maintained at a low level or is subject to large fluctuations appears to depend on the gathering grounds from which the mussels are collected. Those used for the three series in Fig. 2, for example, were collected from rather widely separated beds. Possibly the degree to which mussels kept the water free from bacteria was a function of the age of the mussel or of the state of its nutrition.

RE-USE PROCESS WITH CHLORINATION

The experiments described above had established a principle on which the re-use process might be based. The bacteriological findings suggested, however, that there should be two lines of defence against the danger of pathogenic bacteria being

* In these experiments fifty mussels were immersed in jars holding 50 l. of sea water and instead of making up the volume periodically a mussel was abstracted so as to maintain the volume of water per mussel at approximately 1 l.

† To be published shortly.

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Table 2. *Results of bacteriological examination of re-used sea water in small-scale experiments (water aerated but not chlorinated between successive cycles)*

Plate counts (per ml.) on nutrient agar and counts of *coli-aerogenes* bacteria at 37° C. (most probable number per 100 ml.).

	Series 2	Series 3	Series 4
	Plate counts at 20° C.		
Minimum	620	110	173
Maximum	69,000	41,000	12,000
Mean	22,700	9,170	3,310
	Plate counts at 37° C.		
Minimum	15	1	8
Maximum	238	460	275
Mean	73	107	57
	Counts of *coli-aerogenes* bacteria		
Minimum	11	0	0
Maximum	900	170	17
Mean	131	25	8

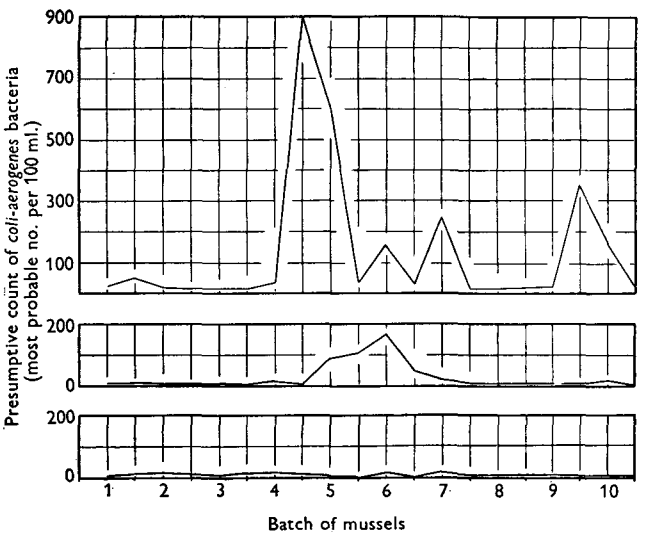

Fig. 2. Fluctuations in numbers of *coli-aerogenes* bacteria in sea water re-used for cleansing successive batches of mussels in three series of small-scale experiments. (Water aerated but not chlorinated between successive cycles of cleansing.)

carried from shellfish to consumer—first, the natural ability of shellfish to free themselves from intestinal bacteria if placed in water of appropriate salinity, and secondly, treatment of the water (and incidentally the surfaces of the tank and the pipes) to ensure that each batch of mussels is immersed in water which is itself free from intestinal bacteria. Mussels cleansed in re-used water which was not sterilized attained a high degree of purification, and clearly such mussels would be much less likely to convey infection to the consumer than those taken direct from a bed in an estuary. On the other hand, since such water may at times

contain a large number of intestinal bacteria there is always the danger that pathogenic bacteria discharged to the water by a contaminated batch of mussels may be ingested and conveyed to the consumer by a following batch. The probability of this occurring may be slight, but it was considered advisable to investigate the possibility of treating sea water with chlorine after each period of re-use.

A series of small-scale experiments was therefore run similar to those described above, but in which at the end of each cycle of cleansing the water was treated with hypochlorite equivalent to 2 parts of chlorine per million and allowed a period of contact of 1 hr. before being aerated with diffused air. Preliminary tests had

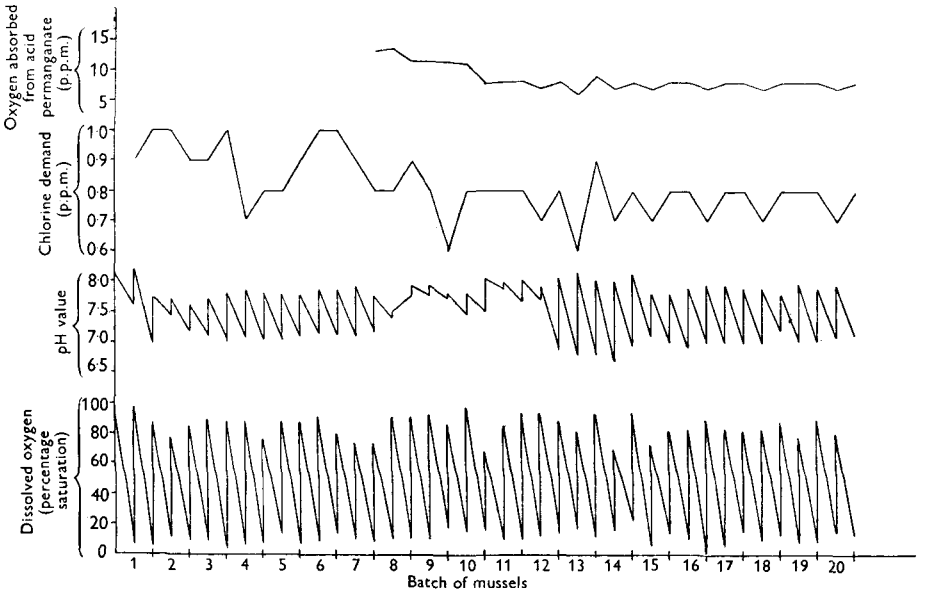

Fig. 3. Changes in dissolved oxygen content, pH value, chlorine demand, and value for oxygen absorbed from acid permanganate, in sea water repeatedly re-used for cleansing mussels. (Water chlorinated and aerated between successive cycles of cleansing.)

shown that in order to be sure that the activity of the mussels was not affected the concentration of residual chlorine in the water before it was added to the mussels should not be more than 0·05 p.p.m. The period of aeration was therefore prolonged until tests by the orthotolidine-arsenite method* showed that this condition was fulfilled. Twenty batches of mussels, each subjected to two cycles of cleansing, were purified in this way, and throughout the series no difference could be detected between the activity of these mussels and those cleansed in unchlorinated water. Values for dissolved oxygen, pH value, chlorine demand, and oxygen absorbed from permanganate in 4 hr., determined on samples of water taken at the beginning and at the end of each cycle, are shown graphically in Fig. 3.

* In the course of the investigation it was found that the arsenite modification of the orthotolidine method was advisable because of the presence of nitrite in the sea water which interfered with the ordinary orthotolidine test.

First series of semi-scale trials with chlorination

Results so far had been sufficiently encouraging to warrant trials of the process on the semi-scale. These were carried out in cement-lined brick tanks, each measuring approximately 12 ft. square by 3 ft. deep, and each with a total capacity of 2700 gallons; one tank was at a rather higher level than the other. The upper tank was used for sterilization of the sea water with chlorine and for dechlorination by aeration with diffused air. The lower tank was used for cleansing the mussels. The amount of sea water used in each experiment was 1500 gallons, which was sufficient to fill the upper tank to a depth of about 18 in. A diagram of the two tanks is shown in Fig. 4.

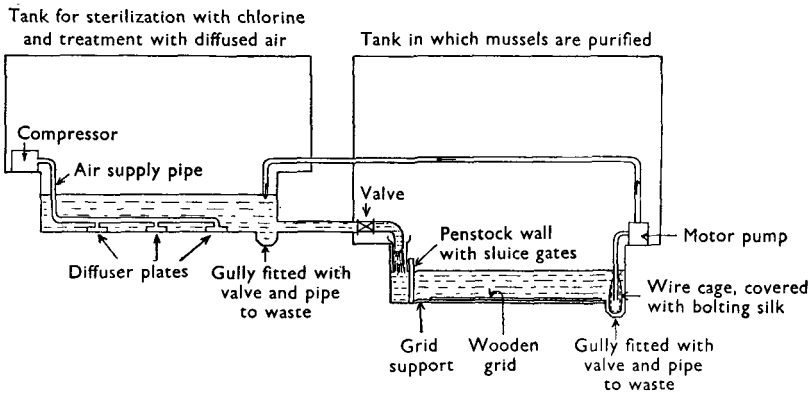

Fig. 4. Diagram of plant used in first series of semi-scale trials.

The water in the upper tank was aerated with air supplied from a $1\frac{1}{4}$ h.p. electrically driven compressor to nine Alundum diffuser plates distributed over the floor of the tank. In the lower tank approximately 4 cwt. of mussels were laid out on wooden grids resting on concrete supports. Dechlorinated water was transferred from the upper to the lower tank by opening the valve in the pipe connecting the two tanks (Fig. 4) and allowing it to flow in by gravity. The water entered the lower tank through a pipe behind a penstock wall, which prevented undue disturbance of the mussels by the rush of incoming water. The water then entered the main part of the tank through sluice gates in the penstock wall. At the end of a cycle of purification the waste water was pumped back into the upper tank, the suction end of the pump, which projected into the gulley along one side of the tank, being enclosed in a wire cage covered in bolting silk. This prevented faeces and other deposits from being pumped back to the upper tank. The residue of water in the gulley not picked up by the main suction was removed by means of a small hand pump, filtered through bolting silk, and transferred to the main bulk in the upper tank. Sufficient Chloros * to give a concentration of 2·0 p.p.m. chlorine was then added to the water and mixing was ensured by turning on the diffused air for 2 or 3 min. After a period of contact of 1 hr. the chlorinated water was aerated

* A proprietary solution of sodium hypochlorite containing approximately 10 % available chlorine.

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with diffused air until the concentration of residual chlorine was 0·05 p.p.m. or less. Meanwhile, the mussels in the lower tank were cleared of mud, faeces, débris, etc., by thoroughly hosing until this material had been washed through the supporting grids on to the floor of the tank, from which it was removed by flushing to the gulley and thence to waste. After each batch of mussels had been purified they were immersed in the freshly chlorinated water for a short period in order to sterilize the outside of the shells.

Twenty-six successive batches of mussels were cleansed in this plant. Fluctuations in content of dissolved oxygen and in pH value of the water were similar to those recorded for the small-scale experiments. The chlorine demand showed no tendency to increase throughout the series and never rose above 1·0 p.p.m. Addition of a constant dose of 2 p.p.m. chlorine at the end of each cycle of purification was therefore sufficient to ensure that there was residual chlorine available for disinfection of the sea water. Accumulation of organic matter in the water during the series of purifications, as judged by the value for oxygen absorbed in 4 hr. from acid permanganate, was extremely slight. This value increased only from 3·8 to 12·6 p.p.m. during the course of the twenty-six purifications. The temperature of the water ranged from 5·0 to 12·0° C. The mussels functioned normally in each cycle of purification.

Results of bacteriological examination

Five to ten mussels, taken from each of the last fourteen batches, after purification, were subjected to individual bacteriological examination. In fifty-eight mussels taken before cleansing from different batches during the series, the average count of *coli-aerogenes* bacteria was 150, and of faecal *Bacterium coli* (roll-tube counts on MacConkey agar at 44° C.) 39, per ml. of body tissue. Of 107 mussels tested after cleansing the average count of *coli-aerogenes* bacteria was 1·2, and of faecal *Bacterium coli* 0·23, per ml.; 93·5 % of cleansed mussels showed a count of 5 or less *coli-aerogenes* bacteria.

On seven occasions during the series the mussels to be cleansed by the re-use process were picked at random from those about to be cleansed for the open market in the large-scale tanks by the process referred to at the beginning of this paper. From bacteriological examination of cleansed mussels from both processes, therefore, a comparison of the two methods could be made. Results for counts of *coli-aerogenes* bacteria at 37° C. are shown in Table 3. Counts of faecal *Bacterium coli* were not determined on individual mussels, but on the pooled contents of five mussels. In thirty-two mussels taken from six batches before cleansing the average count was 43 per ml. of body tissue. In 120 mussels taken from ten batches after cleansing by the re-use process the average count was 0·2 per ml., and twenty pools of mussels out of twenty-four examined contained no faecal organisms; in 120 mussels cleansed in the large-scale tanks the average count was 0·6 per ml. and sixteen pools of mussels out of twenty-four examined were free from faecal organisms. These figures suggest, therefore, that mussels cleansed in continually re-used water which was chlorinated and re-oxygenated after each batch, compared favourably with mussels cleansed by the existing large-scale process.

Table 3. *Results of bacteriological examination of mussels cleansed (a) in semi-scale plant by re-use process, and (b) in large-scale tanks in sterilized fresh sea water*

Results as colony counts (per ml. of body tissue) on MacConkey agar of *coli-aerogenes* bacteria at 37° C.

Before cleansing		After cleansing				
				No. of mussels		Percentage of mussels
No. of mussels examined	Average bacterial count	No. of mussels examined	Average bacterial count	free from bacteria	showing a count of 5 or less	showing a count of 5 or less
Semi-scale plant with re-use process						
48	105	47	1·4	30	43	91·5
Large-scale process using fresh sea water for each batch						
48	105	47	5·0	29	38	80·9

Second series of semi-scale trials

During the course of the investigation a number of difficulties which would have to be overcome before the process could be conducted successfully on a large scale had been noted. A further series of semi-scale trials was therefore undertaken with the object of investigating these difficulties. Since on the large scale space would

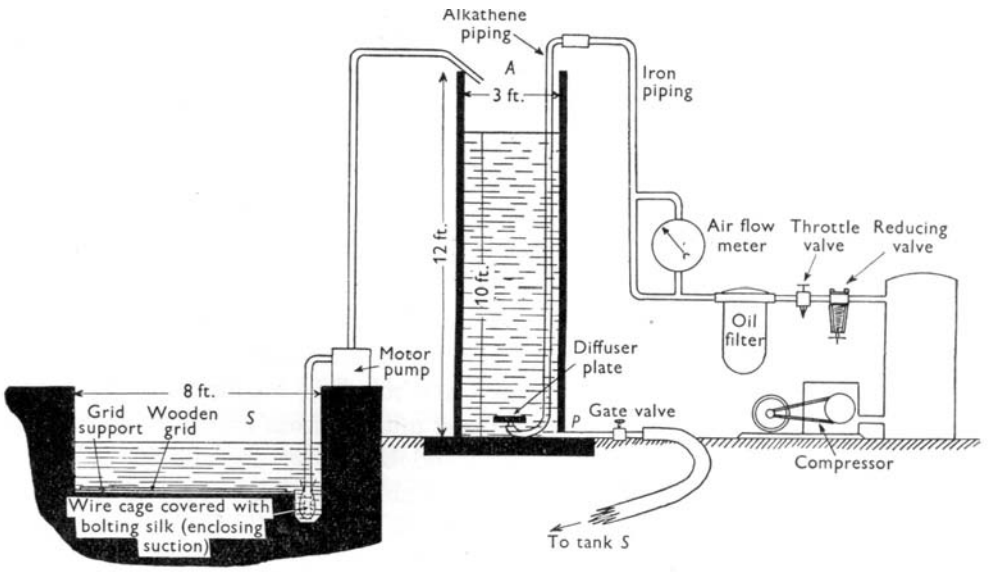

Fig. 5. Diagram of plant used in second series of semi-scale trials.

probably be saved by employing deep rather than shallow aeration tanks the plant used on this occasion included an aeration tank about 12 ft. deep. A suitable tank was made by cementing together three concrete pipes. These were erected vertically on a concrete base to form a tank 12 ft. high and with a capacity of 440 gallons when filled to the 10 ft. mark. The arrangement of the aeration tank and the mussel tank is shown in Fig. 5. At the base of the aeration tank a hole was drilled and

a length of 1 in. galvanized iron piping (*P*) was inserted so that the contents of the tank could be run off when necessary; this exit pipe could be closed by a gate valve. For cleansing the mussels the same tank was used as in previous semi-scale experiments (Fig. 4), but in order to accommodate it to the size of the aeration tank a brick partition was inserted and only a section of the tank measuring 8 ft. × 4 ft. 3 in. was used. On each occasion mussels weighing 1 cwt. were cleansed in this small tank. The cycle of operations was then similar to that in previous tests.

Air from an electrically driven compressor was passed through an Aerox filter to remove traces of oil before being passed through a 1 ft. square Alundum diffuser plate. The rate of flow was measured by an air-flow meter inserted between the air filter and the diffuser. The rate of aeration usually employed was between 0·15 and 0·45 cu.ft. of air per sq.ft. of tank area per min. In order to avoid complications due to rusting and the solution of iron, plastic ('Alkathene') piping was used in the aeration tank.

Trials were carried out with polluted water taken at high tide from the estuary of the River Ouse; the specific gravity of the water was 1·024 and the salinity 31·22 g./1000 g. It was thought advisable to improve the quality of the water before using it to purify mussels. For this purpose it was allowed to settle for 24 hr. in a concrete storage tank and the supernatant liquid was then pumped on to a batch of mussels used for the purpose of clarifying the water. Simple settlement removed a large proportion of the suspended matter, but the water was still turbid. The mussels clarified the water considerably as could be seen from measurement of turbidity by the Spekker absorptiometer. The effect of treatment on the quality of the water may be seen from the results of chemical analysis shown in Table 4.

Table 4. *Effect of preliminary treatment on chemical quality of polluted estuary water*

	Raw water	After settlement for 24 hr.	After further clarification by mussels
Suspended solids	35·8	11·4	Trace
Ammonia (as parts N)	0·07	0·07	—
Nitrite (as parts N)	0·025	0·023	—
B.O.D.*	10·6	8·0	3·0
O.A.†	4·7	4·3	3·8
Chlorine demand	—	0·8–0·9	0·4–0·6
Turbidity (as Spekker reading in 4 cm. cell)	0·212	0·169	0·030

Content in parts per million (except for Spekker reading)

* Biochemical oxygen demand in 5 days at 18·3° C.

† Oxygen absorbed from *N*/80 acid permanganate in 4 hr. at 26·7° C.

The clarified water obtained by this treatment was re-used for cleansing successively fifty-three batches of mussels.

During the first half of the series the fluctuations in pH value and in content of dissolved oxygen of the water were frequently comparatively small; on eighteen occasions the dissolved oxygen content at the end of the cycle of cleansing was 80 % or more of the saturation value. This appeared to be due to the fact that the

mussels were functioning sluggishly, as shown by the somewhat feeble formation of byssus, faeces, and pseudo-faeces.

The results of bacteriological examination, however, showed that the mussels were being cleansed satisfactorily. Thus, of 145 mussels subjected to examination before cleansing, the average count of *coli-aerogenes* bacteria at 37° C. was 57 per ml. of body tissue. Of 138 mussels tested after cleansing the average count was 0·8 per ml., 110 were sterile, and only five mussels gave a count greater than 5 per ml. Before purification the average count of faecal *Bact. coli* was 27 per ml. After purification 131 of 138 mussels tested contained no faecal *Bact. coli* and each of the remaining seven mussels had a count of only 2 per ml.

The temperature during this series of purifications was often appreciably lower than in previous investigations, and it seemed likely that this factor might be responsible for the sluggish behaviour of the mussels. The effect of temperature was therefore investigated.

EFFECT OF TEMPERATURE ON THE METABOLIC ACTIVITY OF MUSSELS AND ON THE CLEANSING PROCESS

The effect of temperature on the functioning of mussels and on the re-use process was investigated in battery jars using batches of forty mussels in 40 l. of sea water. In each series of purifications each batch of mussels was subjected to two cycles of cleansing and the water was re-used for cleansing ten batches of mussels. Investigations at each of the temperatures, 2, 4, and 6° C., in which battery jars containing mussels were put in a refrigerator at the desired temperature, were accompanied by a similar series of purifications at room temperature.

In the first group of experiments no chlorine was added throughout the series of purifications. The supernatant water at the end of the cleansing period (usually about 22 hr.) was siphoned off and aerated for 1 hr. with diffused air. The specific gravity of the sea water from the estuary, which was used in all these experiments, ranged throughout only from 1·024 in the experiments at room temperature to 1·027 in the experiments at 4° C. (salinity, 31·1–33·6 g./1000 g.).

Fluctuations in content of dissolved oxygen and in pH value of the water in the experiments at 2° C., together with the corresponding results for control experiments at room temperature, are shown in Fig. 6. It is clear that changes in the sea water produced by metabolic activity of the mussels were much less at 2° C. than at room temperature. At 4° C. the differences, though distinct, were much less marked than at 2° C.; at 6° C. the mussels appeared to be only slightly less active than at room temperature.

The content of ammonia (determined by distilling a sample to one-third of its volume and Nesslerizing the distillate) in the water at intervals during the series of purifications is shown in Table 5.

Differences in batches of mussels collected at different times probably account for differences in the amounts of ammonia excreted during the three series of purifications at room temperature. Part of the ammonia excreted may have been oxidized during the course of the experiments to nitrite and nitrate, for which no tests were made. In each series, however, the amount recorded at the lower

temperature was much less than that in the corresponding experiments at room temperature.

From each batch five mussels taken before and five taken after purification were submitted to bacteriological examination. A summary of the results is shown in Table 6. Evidently the degree of cleansing achieved at room temperature was not

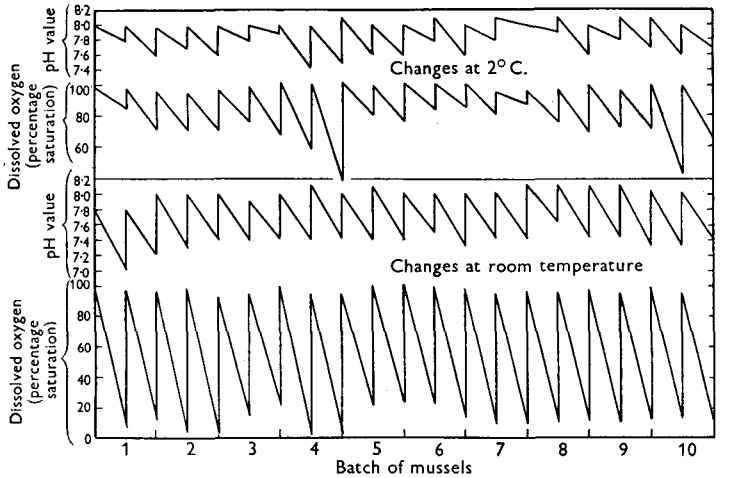

Fig. 6. Effect of temperature on changes in pH value and in dissolved oxygen content of sea water repeatedly re-used for cleansing mussels. (Water aerated but not chlorinated between successive cycles of cleansing.)

Table 5. *Effect of temperature on content of ammonia accumulating in sea water repeatedly re-used for cleansing of mussels*

Experiments at 2° C.			Experiments at 4° C.			Experiments at 6° C.		
	p.p.m. NH_3 in water			p.p.m. NH_3 in water			p.p.m. NH_3 in water	
Batch no.*	At 2° C.	In control at room temperature	Batch no.*	At 4° C.	In control at room temperature	Batch no.*	At 6° C.	In control at room temperature
1	<0·01	<0·01	1	<0·01	<0·01	1	<0·01	<0·01
3(*a*)	0·39	1·16	3(*a*)	0·56	0·92	3	0·28	0·36
3(*b*)	0·45	3·65	3(*b*)	0·76	2·10	—	—	—
6(*a*)	0·92	3·70	6(*a*)	0·96	2·20	6(*a*)	0·84	1·00
6(*b*)	1·54	3·60	6(*b*)	1·20	3·00	6(*b*)	0·92	1·10
9(*a*)	0·44	4·80	9(*a*)	1·20	3·20	9(*a*)	1·44	2·65
9(*b*)	0·40	4·60	9(*b*)	1·28	3·40	9(*b*)	1·90	3·00

* (*a*) denotes first cycle of cleansing; (*b*) denotes second cycle of cleansing.

significantly greater than that obtained at 4 or 6° C. On the other hand, at 2° C. the degree of cleansing was very low, cleansed mussels still containing an average of 58 *coli-aerogenes* bacteria, including 15 faecal *Bacterium coli*, per ml. of mussel flesh, although mussels from the same batches had been quite satisfactorily cleansed at room temperature.

Table 6. *Effect of temperature on proportion of bacteria removed from mussels cleansed in re-used sea water without chlorination*

(Colony counts on MacConkey agar.) Five mussels tested before and five after cleansing. Ten batches in each series.

Exps. at (° C.)	Average count before cleansing	Average count after cleansing at low temperature	Average count after cleansing at room temperature	Percentage of bacteria removed by cleansing at low temperature	Percentage of bacteria removed by cleansing at room temperature
		Coli-aerogenes bacteria (at 37° C.)			
2	104	58	1·9	44·2	98·2
4	112	2·5	3·0*	97·8	97·3
6	68	1·0	0·5	98·5	99·3
		Faecal *Bact. coli* (at 44° C.)			
2	51	15	0·5	70·6	99·0
4	70	0·7	1·1*	99·0	98·4
6	47	0·0	0·0	100·0	100·0

* Did not include one anomalous result in which a cleansed mussel showed a count of several hundred at 37° C.

A second group of experiments was run on similar lines to those just described, except that between successive cycles of cleansing aeration was preceded by chlorination. The dose of chlorine added was sufficient to provide a concentration of residual chlorine of 0·05–0·10 p.p.m. (the method of calculating the dose required is described later). A period of contact of 1 hr. was allowed before aeration was begun. Separate series of experiments were run at 4° and at 6° C. Changes in content of dissolved oxygen in the water showed the same general tendency as in the experiments without chlorination, indicating that the mussels were appreciably less active at 4° C. than at room temperature, but only slightly less active at 6° C. Results of bacteriological examination of mussels taken before and after chlorination throughout the series of purifications showed that there were no significant differences in the degree of cleansing achieved at 4 or 6° C. and at room temperature.

It was concluded from the results of experiments as a whole that at 2° C. cleansing was unsatisfactory, and that at 4° C. metabolism was definitely slower than at room temperature, though in the period of immersion normally allowed the mussels would cleanse themselves satisfactorily. It would, therefore, be advisable in large-scale practice to maintain the temperature of the re-used water at 6° C. or above. It might be necessary to provide artificial heating to ensure this.

FACTORS AFFECTING THE DISCHARGE OF RESIDUAL CHLORINE FROM CHLORINATED SEA WATER

During the course of the small-scale and semi-scale experiments in which a fixed dose of chlorine had been added, difficulties were not infrequently experienced in getting rid of the residual chlorine remaining at the end of the period of contact, aeration being continued until the concentration of residual chlorine fell to 0·05 p.p.m. or less. Thus in the first series of semi-scale experiments periods of aeration varied from $2\frac{1}{2}$ to 8 hr. On the large scale it would be necessary to run

the schedule of operations to a reasonably precise time-table, and to be confident of reducing the concentration of residual chlorine to 0·05 p.p.m. in a short time—say 1 hr. Possible methods of discharging residual chlorine were therefore investigated.

Preliminary experiments showed that if the residual chlorine was present as hypochlorite it was discharged more quickly from an aerated than from a quiescent liquid and that irradiation of the surface with sunlight or with ultra-violet light hastened the process considerably. When the chlorine was present as chloramine, on the other hand, its concentration decreased much more slowly and the effects of aeration and of light were comparatively slight.

Addition of thiosulphate

Dechlorination by addition of thiosulphate is quite satisfactory in the existing large-scale process, where fresh sea water is used for each batch of mussels. When the water is repeatedly re-used, however, complications arise from the effect of excess thiosulphate on the chlorine demand of the water, and from the effect on the pH value of the water of the sulphuric and hydrochloric acids formed by reaction between the chlorine and the thiosulphate. This reaction (cf. Partington, 1949) may follow several alternative routes, the second of the following three representing the change which takes place when thiosulphate is added to sea water containing excess chlorine:

(1) $2Na_2S_2O_3 + Cl_2 \rightarrow 2NaCl + Na_2S_4O_6$;
(2) $Na_2S_2O_3 + 4Cl_2 + 5H_2O \rightarrow Na_2SO_4 + H_2SO_4 + 8HCl$;
(3) $Na_2S_2O_3 + Cl_2 + H_2O \rightarrow Na_2SO_4 + 2HCl + S$.

Moreover, it must be remembered that in re-used water a variety of chlorine compounds contribute to the residual chlorine, so that it is impracticable to calculate the exact quantity of thiosulphate required to neutralize a given concentration of residual chlorine measured by the orthotolidine test. In practice an excess would be added and this would increase the chlorine demand. This in turn would have to be determined with accuracy on each occasion so as to be sure of adding the quantity of chlorine required to give the desired residual concentration.

Trials on the small scale confirmed these difficulties and led to the conclusion that treatment of sea water which involved repeated dechlorination with thiosulphate was inadvisable.

Addition of sulphite

Addition of excess sulphite to chlorinated sea water immediately removes residual chlorine: $Cl_2 + Na_2SO_3 + H_2O \rightarrow Na_2SO_4 + 2HCl$. In the re-use process, however, it is essential to remove the sulphite because of its toxicity. No data appear to be available for mussels, but according to Weigelt (1900) concentrations of sulphite equivalent to 0·5–1·0 p.p.m. SO_2 are highly toxic to trout and tench. It is reasonable to assume that concentrations of this order would tend to inhibit functioning of mussels. Experiments were therefore carried out to see how quickly excess sulphite was removed by oxidation during aeration of the water with diffused

air. It is difficult to detect and estimate small traces of sulphite in a complex material such as sea water, and the uncertainty of the methods available itself constitutes an objection to the use of this substance in large-scale practice, where it would be necessary to be certain that no sulphite remained in the water before running it on to the mussels.

So far as could be decided from these experiments, however, it appeared that the period of aeration required to eliminate the last traces of sulphite would be so prolonged as to render the use of sulphite for dechlorination impracticable on a large scale.

Removal with activated carbon

The use of activated carbon was tested in two ways. In the first series of experiments powdered activated carbon was added to chlorinated sea water and the concentration of residual chlorine was tested at intervals. The water was aerated throughout the period of test so as to keep the carbon in suspension. Comparatively large concentrations of activated carbon were required to effect a rapid decrease in the concentration of residual chlorine when this was present as hypochlorite. When the chlorine was present in the form of chloramine activated carbon was comparatively ineffective.

In the second series of laboratory tests the chlorinated water was filtered through a bed of activated carbon consisting of a tube 1 in. in diameter filled to a depth of 2 ft. Results showed that in order to be sure of reducing the concentration of residual chlorine in chlorinated sea water to 0·05 p.p.m. or less very slow rates of filtration would be necessary; the efficiency of the filter was uncertain, being much greater with some batches of chlorinated water than with others. A large and expensive installation would probably be necessary in order to treat at such a slow rate the large volume of water which would be necessary in large-scale practice.

ADDITION OF CHLORINE TO GIVE A PRE-DETERMINED SMALL RESIDUAL CONCENTRATION

When chlorine is added to water containing small amounts of substances with which it reacts, the rapid absorption which takes place at first is usually followed by a much slower absorption over a long period. It is usual to assess the chlorine demand after a period of contact of 10 min. during which the rapid reaction is completed. It seemed likely that if a quantity of chlorine only slightly in excess of the demand were added to re-used sea water the excess would be absorbed during a reasonably short period of aeration. If, in fact, the chlorine could be added in large-scale practice to give with precision a pre-determined small residual concentration, this concentration could be fixed at a value shown by trial to be reduced to 0·05 p.p.m. or less during a period of aeration of 1 hr. The schedule of operations could then be timed with reasonable accuracy.

Experiments were carried out on a large scale at Lytham to see with what precision it was possible to treat sea water with chlorine to give the required residual concentration. Polluted sea water from the estuary of the Ribble was allowed to settle for about an hour to remove coarse suspended matter and was

then pumped into a large concrete tank with a capacity of 150,000 gallons. This water could be pumped to either one of two smaller concrete tanks, each with a capacity of a little more than 40,000 gallons. The suction end of the pump was located in a sump in the large tank and the chlorine was applied during pumping by leading a measured quantity of hypochlorite solution through rubber hose to the grating covering the end of the pump.

The apparatus used for applying the chlorine is shown in Fig. 7. A storage tank T_3 contained a measured quantity of Chloros diluted to 10 gal. with tap water. Diluted Chloros from this tank flowed through pipe P_2 into the inverted 10 gal. bottle T_4, displaced air escaping through tube A and the open screw clip S_1. When vessel T_4 was filled, clips S and S_1 were closed and clip S_2 was opened. The diluted Chloros then flowed into the funnel F_2 and through the 'S'-shaped hose H to L_2 where it was discharged to the funnel F_3 which was connected to a rubber hose leading to the pump suction. The rate of discharge of Chloros was dependent on the difference in level between L_1 and L_2 and the position of L_2 was adjusted to a point which previous trials had shown to give the rate of flow required. The lower end of the exit pipe G, delivering Chloros from the vessel T_4, was filed off at an angle of about 45°. Liquid flowed into the funnel F_2 up to the upper level of the slanting orifice so formed, when further outflow from T_4 was stopped by atmospheric pressure. Discharge of liquid from L_2, however, tended to lower the level L_1, bubbles of air entered T_4 through the slanting orifice at G, and a corresponding quantity of liquid was delivered into the funnel F_2. The effect of this process was to ensure that the liquid was discharged from L_2 under virtually constant head (the difference between L_1 and L_2).

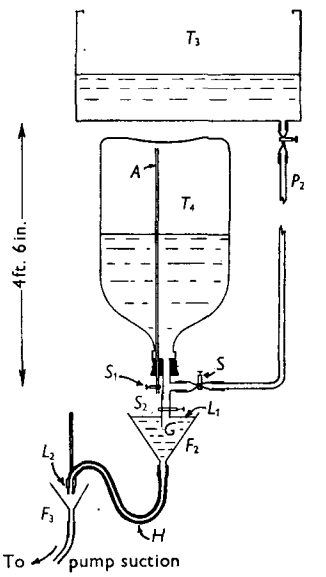

Fig. 7. Apparatus for applying measured quantity of chlorine during pumping of sea water.

Method of determining quantity of chlorine to be added

The first problem was to devise a small-scale test to find the amount of chlorine required to give a residual concentration of about 0·1 p.p.m. or less; having calculated from this figure the quantity of chlorine required for the whole bulk of water, this quantity could then be added at a controlled rate during pumping of the water. The accuracy of the method could be assessed by comparing the concentration of residual chlorine in the water in the tank with the expected value. Factors which it was thought might influence the results obtained were the temperatures and the intensities of light obtaining during the preliminary test and during the large-scale dosing.

After a large number of preliminary trials the method finally adopted was as follows:

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A sample of the water to be chlorinated was taken from the large chlorination tank and quantities of 250 ml. were poured into a number of 12 oz. stoppered glass bottles. A portion of the Chloros to be used in the large-scale dosing was diluted exactly 1:1000 with tap water. Different quantities of this dilute hypochlorite (e.g. from 0·5 to 3·0 ml. increasing in steps of 0·5 ml.) were added to the respective bottles, which were then placed in a tray of water from tank *B*, and were exposed to the air and light at the side of one of the smaller tanks. Residual chlorine by the orthotolidine test was determined (on the contents of each bottle) after 30 min., and the dose of Chloros required to give a residual value of 0·1 p.p.m. was noted. The corresponding quantity of Chloros which would be required to give the same residual value on the large scale was then calculated. This quantity was poured into tank T_3 (Fig. 7), and sufficient tap water was added to bring the total volume to 10 gal. Pumping of the 40,000 gal. of water from the large tank to one of the smaller tanks then began and the rate at which the diluted Chloros was applied was adjusted so that the whole quantity was added in rather less time than it took to fill this tank. At intervals up to a period of 1 hr. after completion of pumping, samples of water from the mussel tank were taken from points near each of the four sides and were tested for residual chlorine by the orthotolidine test.

From the results of thirty-four large-scale tests the following conclusions were drawn:

(1) The concentration of residual chlorine in the chlorinated water in the large-scale tank determined 20 min. after the tank was filled was sometimes appreciably greater than the value recorded in the preliminary bottle tests (after contact for 30 min.). Values determined 40 min. after the tank was filled were usually but not always lower, and values determined after 60 min. were consistently lower than those found in the preliminary bottle tests. Chlorination carried out in this way, therefore, provided a margin of safety against the occurrence in large-scale practice of a higher concentration of residual chlorine than was required.

(2) Within 20 min. after the water entered the tank the chlorine was uniformly mixed throughout the tank.

(3) When ammonia was absent the effect of strong light was to reduce the concentration of chlorine more quickly than occurred in less intense light.

(4) In the presence of ammonia complications arise through the presence of a break-point. With such samples it is possible to obtain the same concentration of residual chlorine with three different quantities of hypochlorite, one being above and the other two below the break-point. It was observed that when the dose was below the break-point the residual chlorine tended to persist for long periods even in the presence of strong light. When the dose was above the break-point the residual chlorine was discharged more easily, particularly in the presence of strong light.

(5) In order to ensure that the concentration of residual chlorine is not greater than that predicted by the preliminary tests, these tests and the large-scale chlorination should be carried out under reasonably comparable conditions of illumination.

Break-point chlorination of re-used sea water

The observation recorded above, that with fresh sea water containing ammonia, break-point chlorination gave residual chlorine which was relatively easily discharged, suggested that this method of treatment might prove effective with re-used sea water. It was found, however, that the type of curve given by re-used water varied very considerably with the different cycles of cleansing, and that most of the curves showing the relation between amount of chlorine applied and concentration of residual chlorine exhibited no break-point with periods of contact of 30 min.

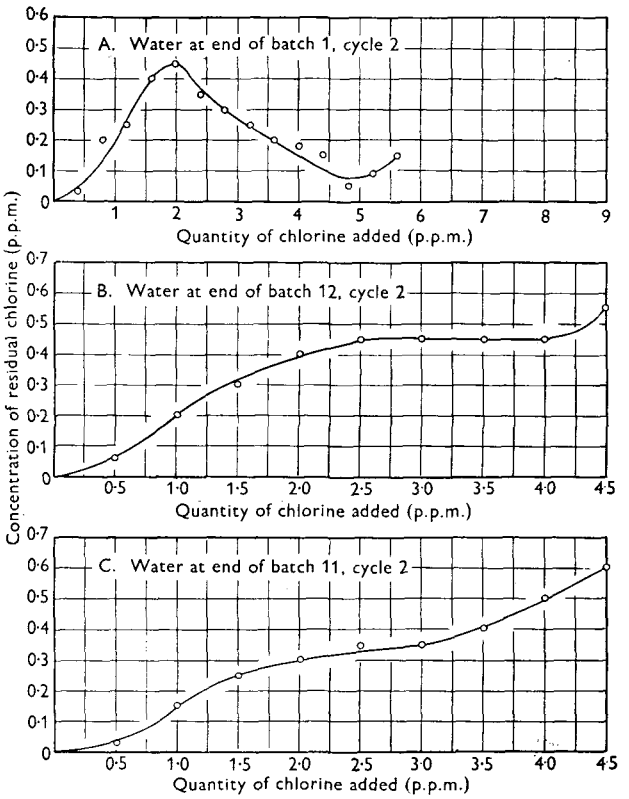

Fig. 8. Relation between quantity of chlorine added and concentration of residual chlorine. Types of curves obtained with re-used water.

Examples of different types of curve obtained with re-used sea water during the series of semi-scale experiments are shown in Fig. 8. Occasionally (but usually only at or near the beginning of a series when fresh or nearly fresh sea water was used) a fairly typical break-point curve was obtained as in A; more often the curve took the form shown in B or C. In contrast to a typical break-point curve which descends almost to the horizontal axis before rising again, the horizontal portion or the kink in these atypical curves occurred at a comparatively high level of residual chlorine. When doses slightly above this level were added a comparatively high concentration of residual chlorine resulted which was discharged only after

considerable periods of aeration, sometimes extending to several hours. It was decided that break-point chlorination was not applicable to re-used water of this type.

Method of chlorination and dechlorination finally adopted

It was concluded from the results of the trials described above that neither discharge of residual chlorine by addition of chemicals nor chlorination beyond the break-point with the object of eliminating combined chlorine was practicable with re-used sea water. On the other hand, it seemed that the re-use process might be run successfully if the quantity of chlorine added on each occasion were the smallest quantity (below the break-point) required to give a residual concentration only a little greater than 0·05 p.p.m. If the chlorine were added with sufficient precision the residual concentration would then be reduced to this level or below during the course of the subsequent aeration for 1 hr. Removal of residual chlorine in this way probably depended largely on its slow reaction with small quantities of the products of mussel metabolism present in the sea water, and the rate of removal therefore tended to vary with the activity of the previous batch of mussels.

FINAL SEMI-SCALE TRIALS

Experiments were run in the plant previously described, which included a deep aeration tank, air filter, and air-flow meter. A small dosing apparatus of the same pattern as that used in the large-scale trials was arranged to deliver the chlorine to the pump suction, the appropriate quantity being determined by preliminary bottle tests on the supernatant water at the end of each period of immersion of the mussels. In this way forty-two successive batches of mussels were cleansed in re-used water, each batch receiving two cycles of cleansing.

Results showed that the concentration of residual chlorine after contact for $\frac{1}{2}$ hr. in the semi-scale plant was usually smaller than that obtained in the bottle tests (on forty-six out of sixty-four occasions tested). After contact for 1 hr. in the semi-scale plant the concentration of residual chlorine observed exceeded the value obtained in bottle tests on only seven occasions out of eighty-four.

Since it is important that a margin of safety should be a constant feature in large-scale practice, the reason for the several occasions when the residual value in the semi-scale plant proved to be somewhat larger than was expected from the bottle tests was investigated. The commonest factor responsible for this was found to be the presence of too strong a light during the carrying out of the bottle test. Lack of laboratory accommodation had necessitated the use of a converted greenhouse for laboratory tests. During the winter months this greenhouse was protected from sunlight by the brow of a hill in the rear. In early spring, however, the sun was sufficiently high in the sky to expose the greenhouse to strong light. Under these conditions it was found that the amount of chlorine discharged during the manipulations necessary in the performance of the bottle tests was sufficient to make the values found for residual chlorine appreciably lower than when the same operations were carried out in a shaded room. Differences in the values

obtained under the two different conditions are shown in Table 7. When the light in the greenhouse was dimmed by means of sun-blinds no further difficulties in this respect were encountered.

Table 7. *Concentrations of residual chlorine in re-used sea water after contact for* 30 *min. with different amounts of chlorine added* (*a*) *in a greenhouse exposed to fairly strong light, and* (*b*) *in a partially darkened room*

During the period of contact the chlorinated water was shaded from the light in both series.

Chlorine added (as ml. 1/1000 Chloros per litre)	Concentration of residual chlorine (p.p.m.) after contact for 30 min.	
	Chlorine added in greenhouse	Chlorine added in partially darkened room
2·0	0·00	0·05
3·0	—	0·10
4·0	0·02	—
5·0	—	0·20
6·0	0·03	0·23
7·0	—	0·28
8·0	0·03	—
10·0	0·07	—

On one occasion difficulty in removing residual chlorine was due to another cause. Instead of using the dosing apparatus the plant operator had added the calculated quantity of Chloros from a measuring cylinder to the bulk of water in the aeration tank and mixing was ensured by turning on the current of air for 5 min. The concentration of residual chlorine after contact for 1 hr. was appreciably higher than had been expected, and it was found necessary to aerate for a comparatively long period to reduce it to 0·05 p.p.m. This confirmed observations in the laboratory that the expected residual concentration is only obtained if the chlorine is added very gradually so as to mix it intimately and uniformly with the whole bulk of water.

That small concentrations of residual chlorine of the order recorded in this series of experiments are effective in maintaining very low counts of *coli-aerogenes* bacteria in the water was shown by the results of bacteriological examination. Counts of the water before chlorination sometimes rose appreciably but, of seventy-seven samples of water tested after chlorination and aeration, fifty-four samples showed no *coli-aerogenes* bacteria and sixteen samples showed a count of only 1 per 100 ml. Ninety per cent of the samples therefore conformed to the bacteriological standard required for good drinking water. Only one sample had a count higher than 5 per 100 ml.

It is necessary to stress the fact that a good bacteriological condition of the water is maintained only if proper precautions are consistently adopted. Instances of two conditions which led to high counts in the water are worth recording: (1) during the cleansing of one batch some water was inadvertently lost from the

cleansing tank through a leaky valve. After chlorination and aeration the water in the aeration tank was made up to volume by adding about 100 l. of fresh water from the estuary. The *coli-aerogenes* count of the whole bulk of water was then found to be 14 per 100 ml. Addition of estuary water for make-up purposes should therefore be avoided so far as possible. (2) An open hatch in the wall of the hut enclosing the cleansing tank, and just above the tank itself, allowed rain to enter the hut when the wind was blowing in that direction. On one stormy day rain was blown in considerable quantities on to the floor of the hut and this trickled into the tank, carrying with it dirt and oil which had accumulated on the floor. The count of the water even after chlorination and aeration rose to 50 per 100 ml. on this occasion. This illustrates the necessity for keeping re-used water isolated from possible contamination of this kind. Success in keeping the bacterial count of the water low with only small concentrations of residual chlorine depends upon the absence of suspended organic matter which protects bacteria from the action of the chlorine. Mussels are highly effective in clearing water and so creating optimum conditions for the bactericidal action of chlorine. If, after a cycle of cleansing, the water is accidentally contaminated with organic matter and bacteria, these conditions are spoilt.

Results of bacteriological examination of forty batches of mussels are shown in Table 8.*

Table 8. *Results of bacteriological examination of mussels cleansed in re-used sea water in final series of semi-scale experiments*

Water aerated and chlorinated between successive cycles of cleansing. Results as number of bacteria (per ml. of mussel flesh) giving red colonies on MacConkey agar (*coli-aerogenes* at 37° C., faecal *Bacterium coli* at 44° C.). From each batch of mussels five were examined before and five after cleansing

Before cleansing		After cleansing				
				No. of mussels		Percentage of mussels
No. of mussels examined	Average bacterial count	No. of mussels examined	Average bacterial count	free from bacteria	showing a count of 5 or less	showing a count of 5 or less
		Coli-aerogenes bacteria at 37° C.				
210	85	210	3·3	161	184	87·6
		Faecal *Bact. coli* at 44° C.				
208	40	209	1·1	179	198	94·7

RE-USE OF SEA WATER FOR CLEANSING OYSTERS

Experiments on the cleansing of oysters, by a process similar to that used commercially for mussels, have been described by Webb (1930) and by Dodgson (1936). Webb found that oysters lying in polluted water did not themselves become

* Results for two batches were not included as the abnormally high counts suggested an error in bacteriological technique.

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polluted so regularly as did mussels under the same conditions. Individual oysters exhibited a wide variation in bacterial counts, some showing fairly heavy pollution, some remaining relatively clean. On an average, however, a batch of oysters was almost invariably less polluted than a batch of mussels taken from the same situation. Temperature was found to be a controlling factor in the degree of purification attained during immersion in sterilized sea water. Dodgson, describing the large-scale process for the purification of oysters at Brightlingsea, says that, whereas mussels function actively at temperatures little above freezing-point, oysters do not function at all below 40° F. (4·4° C.) and do not function vigorously below 54° F. (12·2° C.). For this reason tanks for use in winter must be covered and provided with means for heating.

In view of the possible importance of the oyster industry in this country in the future, preliminary experiments were undertaken to see whether adequate cleansing could be accomplished in sea water which was repeatedly re-used for successive batches by a process similar to that described for mussels. Owing to the difficulty and expense of obtaining large numbers of oysters these preliminary experiments were restricted to small-scale trials in battery jars. Since naturally polluted oysters were not obtainable in that locality (Conway) a batch of about 800 oysters was obtained for experimental purposes and the requisite number for each cleansing was artificially polluted before use by immersing the oysters in a battery jar containing sea water to which a drop of a mixed culture of coliform bacteria (taken from a positive tube of MacConkey broth in a routine presumptive test) had been added. The degree to which oysters were polluted by this method was found to be uncertain, and this uncertainty accounts partly for the large fluctuation in the numbers of organisms recorded in the bacteriological examination of untreated oysters. The plan of the experiments was otherwise similar to that previously described for the small-scale investigations with mussels. For the first six batches the water was re-oxygenated, after each cycle of cleansing, by aeration with diffused air, but was not chlorinated. For subsequent batches of oysters the water was treated with a dose of Chloros equivalent to 2 p.p.m. chlorine at the end of each cycle of cleansing, and after a period of contact of an hour was aerated until the residual concentration of chlorine had fallen to 0·05 p.p.m. Samples of water were taken at the end of each cycle of purification for examination by the usual chemical tests. During the course of the experiments the specific gravity ranged from 1·024 to 1·026, salinity from 30·68 to 33·10 g./1000 g., and the value for oxygen absorbed from acid permanganate in 4 hr. from 6·4 to 16·4 p.p.m. Fluctuations in the content of dissolved oxygen, in pH value, and in chlorine demand were similar in range to those observed in small-scale and semi-scale experiments with mussels.

In all, twenty successive batches of oysters were cleansed in the re-used water. From the seventh purification onwards, each batch was compared with a similar batch cleansed in fresh sea water which had been sterilized by chlorination and dechlorinated by aeration. From observations of the amount of faeces formed, and from the results of chemical examination of samples of water, there appeared to be no significant difference in the metabolic activity of oysters in the re-used

water and in the fresh sea water, nor did the re-used water show at the end of the investigation any appreciable accumulation of organic matter or other undesirable characteristics.

For assessing the degree to which the oysters were free from bacteria batches 1–4 inclusive and batches 7, 12, 15 and 20 were not included, as during these periods either conditions of temperature were not satisfactory or the oysters were too highly or too little polluted to be regarded as normal. In the remaining twelve purifications the individual counts of thirty-six untreated oysters tested on MacConkey agar at 37° C. ranged from 0 to 2680, with an average of 424, per ml. Counts of pools (three oysters in each pool) in MacConkey agar tubes at 44° C. ranged from 4 to 430, with an average of 94, per ml. After cleansing it was found that, of fifty-eight individual oysters tested on MacConkey agar at 37° C., thirty-nine were sterile, seven showed only 1 colony, five showed 2 colonies, four showed 3 colonies, two showed 4 colonies, and only one oyster showed more than 5 colonies per ml. of minced shell contents. In counts of pools on MacConkey agar at 44° C., nineteen of the twenty-four pools tested were sterile and none showed a count greater than 5 per ml. Results obtained in control experiments in which the oysters were cleansed in fresh sea water were of the same order, and it may be concluded that, on the whole, oysters were cleansed in re-used at least as satisfactorily as in fresh sea water.

CONTINUOUS RE-USE OF ARTIFICIAL SEA WATER FOR CLEANSING OF MUSSELS

It was felt that situations might sometimes occur in commercial practice in which supplies of sea water were periodically required, either to replace accidental losses from tanks of sea water being continuously re-used for the cleansing of shellfish or to maintain existing volumes against losses due to evaporation or incurred during normal large-scale manipulation. In such cases, if natural sea water were not easily procurable, it would be an advantage to be able to use artificial sea water. Similar considerations apply to laboratories situated inland in which maintenance of shellfish for experimental purposes is desired. Experiments were therefore carried out to see whether mussels would function satisfactorily in artificial sea water, prepared in the laboratory by dissolving in fresh water suitable quantities of the major constituents of sea water, and whether such artificial sea water proved to be satisfactory for repeated re-use.

Two parallel experiments, designed on similar lines to those of previous small-scale experiments in battery jars were carried out, using artificial sea water prepared according to two different recipes. The table on p. 455 shows the quantities (g.) dissolved in 30 l. of distilled water.

Recipe 1 was designed to provide the major constituents of ocean water (cf. analyses quoted by Harvey, 1928). Recipe 2 was based on figures given by Lyman and Fleming, quoted by Harvey (1945). Each type of artificial sea water was diluted to a specific gravity of approximately 1·023 so as to be comparable with that used in the previous small-scale and semi-scale experiments.

Fifteen successive batches of mussels were cleansed in each type of artificial sea

Constituent	Recipe 1	Recipe 2
NaCl	750	704
$CaCl_26H_2O$	39·6	33·2
$MgSO_47H_2O$	115·2	—
$MgCl_26H_2O$	86·4	149·4
KBr	3·6	2·8
K_2CO_3	5·5	—
KCl	16·8	19·9
Na_2SO_4	—	117·5
$NaHCO_3$	—	5·71
H_3BO_3	—	0·78
$SrCl_2$	—	0·72
NaF	—	0·09

water, the efficiency of the process being followed by the usual chemical and bacteriological examinations and by visual observation of the shellfish. For the first six batches of mussels the water was not chlorinated, but for the cleansing of each subsequent batch the water was treated with Chloros equivalent to 2·0 parts chlorine per million before each cycle of cleansing. Throughout the series of purifications in sea water of Recipe 1 the temperature ranged from 10·0 to 19·2° C., specific gravity from 1·022 to 1·023, and salinity from 28·80 to 30·72 g./1000 g. In sea water of Recipe 2 the temperature fluctuated from 9·8 to 19·6° C., specific gravity from 1·022 to 1·023, and salinity from 29·00 to 30·17 g./1000 g. Fluctuations in the content of dissolved oxygen, in pH value, in chlorine demand, and in the amount of oxygen absorbed from acid permanganate in 4 hr. were very similar in character and extent to those recorded previously in the small-scale and semi-scale experiments with natural sea water. Functioning of the mussels was normal throughout. Results of bacteriological examination showed that the degree of cleansing achieved by the mussels was consistently satisfactory throughout the series of purifications with both types of artificial sea water.

Results as a whole showed clearly that mussels were cleansed in each of these two preparations of artificial sea water as effectively as in re-used natural sea water or in the commercial process using fresh sea water for each cleansing.

SUMMARY AND CONCLUSIONS

When mussels are allowed to function in sea water the main changes occurring in the water are depletion of dissolved oxygen and lowering of pH value. Provided the faeces and pseudo-faeces are not disturbed the increase in the content of organic matter is not appreciable. If the supernatant water is removed and aerated with diffused air the water is re-oxygenated, the pH value is restored to its original level, and the water so treated may be re-used for immersing a fresh batch of mussels. The process of re-use may apparently be continued indefinitely.

Under these conditions a high degree of cleansing is achieved by the mussels, the count of *coli-aerogenes* bacteria being reduced to a small fraction of the original; counts of bacteria in the water, on the other hand, are subject to large fluctuations. It was considered advisable for this reason to chlorinate the water between each cycle of cleansing in order to immerse the mussels on each occasion in water which

itself was reasonably certain to be free from coliform bacteria, potentially including pathogens.

Experience showed that the greatest difficulty involved in the use of chlorine was in removing residual chlorine so that it would not inhibit functioning of the mussels when the water was re-used. After trials of various alternatives it was concluded that the most satisfactory method was to determine by means of small-scale tests the smallest quantity of chlorine (below the break-point) required to give a residual concentration of 0·05–0·10 p.p.m. in the re-used water and then to add the corresponding quantity with precision to the bulk of water by means of a dosing apparatus while the water was being pumped from the mussel tank to the aeration tank. After a period of contact of 1 hr. the water was aerated for a further hour. A series of trials in a semi-scale plant showed that this treatment ensured that residual chlorine in the water being added to the mussels did not exceed 0·05 p.p.m.; as a result the mussels functioned satisfactorily and the degree of cleansing attained was comparable with that attained in the existing mussel-cleansing tanks in which sterilized fresh sea water is used for each cycle of cleansing. Although the concentration of residual chlorine was small the mussels kept the water so clear that this concentration was effectively bactericidal and the bacterial quality of the water was usually comparable with that of good drinking water. Low temperatures retard the metabolic activity of mussels and below 4° C. this is so marked that the degree of cleansing achieved is unsatisfactory. For this reason it is recommended that the temperature of the re-used water should be maintained at 6° C. (43° F.) or above.

Oysters were found to be satisfactorily cleansed by a process of re-use similar to that adopted for mussels, provided the temperature of the water was maintained at 54° F. (12·2° C.) or rather higher.

Small-scale trials showed that artificial sea water, prepared by dissolving in fresh water suitable quantities of the major constituents of natural sea water, could be successfully re-used for cleansing mussels.

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