BACTERIOLOGICAL STUDIES OF OYSTER CONDITIONING

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ABSTRACT

Preliminary experiments have been made to determine the best methods for conditioning oysters to rid them of coliform bacteria. When kept in tanks of sea water, oysters did not improve in sanitary quality even when the water was chlorinated. At chlorine concentrations sufficiently high to kill the coliform bacteria, the oysters did not pump the water through their systems. At lower concentrations, failure to eliminate the coliforms was due to recontamination because of the inability to get rid of these bacteria in the water in the tank.

When oysters were kept in flowing, clean water which was free of chlorine and coliform bacteria, the total bacterial count was reduced rapidly, and the coliform bacteria were completely eliminated within a very short time. In some instances oysters were coliform-free within 30 minutes when treated in this manner.

Although much more work is necessary to determine ideal conditions for eliminating coliform bacteria from oysters on a commercial scale, it appears that a system which employs flowing water probably will be the most satisfactory.

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The problem of utilizing shellfish from areas which are known to be only slightly polluted with domestic sewage has been considered by sanitarians for some time. A large supply of oysters is available from such areas providing they can be rendered safe for human consumption. The possibility of ridding oysters of undesirable bacteria by conditioning them in chlorinated water has been given some attention (Galtsoff, 1946, and U. S. Public Health Service, 1944). There are other methods which might be used, however; and this report describes bacteriological studies of oyster conditioning by the use of flowing water and in tanks containing sea water with low concentrations of chlorine and with chlorine-free water.

A recommended method of oyster conditioning requires that the oysters be held in tanks containing chlorinated sea water. The required initial free chlorine content is not less than 0.5 p.p.m., and a residual of not less than 0.05 p.p.m. must be maintained throughout a 48-hour period of conditioning. In order to test the effectiveness of this procedure, two types of bacteriological investigations were instituted by the U. S. Fish and Wildlife Service. The first was designed to determine the ability of the oyster to rid itself of coliform bacteria without chlorination. The second was to evalu-

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ate chlorination per se as an effective means of freeing oysters of these micro-organisms. This is a report of the experimental findings.

Bacteriological investigations were made on oysters maintained in flowing water in a Galtsoff device whereby the oyster effluent could be readily cultured. When exposed to large quantities of coliform bacteria, the oysters apparently managed to rid themselves of these micro-organisms within a very short time, usually becoming coliform-free within 30 minutes.

When stored in an aquarium with water containing the maximum concentrations of free chlorine (0.03 p.p.m.) which permitted the normal functioning of the oysters, coliform bacteria were retained over a period of 48 hours, and there was no apparent reduction in the total bacterial content of the shellfish.

The findings indicate that the described method of conditioning oysters by chlorination is probably ineffective.

In 1915, Johnstone published the results of his investigations of mussel purification. He found that, when polluted mussels were placed in 10-liter tanks filled with sea water flowing at the rate of one liter every 5 minutes, the bacterial content was reduced 90 percent in 2 days. The same results obtained when polluted mussels were transferred to unpolluted water and held over a period of six tides. He also reported that sea water containing 1 p.p.m. of free chlorine was effective in freeing mussels of sewage bacteria. He recommended the use of 5 p.p.m. in practice, since this amount of chlorine did not appear to interfere with the normal functioning of the mussels. At this concentration, 3 days were required for the elimination of the coliform bacteria. He concluded that exposure to running sea water or storage in chlorinated sea water would remove 90 percent of the objectionable bacteria. He postulated a twofold mechanism for chlorination:

> First, it renders the sea water sterile, and the sterile sea water mechanically removes the bacteria;
>
> Second, the free chlorine destroys the bacteria enmeshed within the shellfish.

Wells (1920) observed that food particles are eliminated within 5 hours after they have been filtered by the gills of the oyster. He concluded that, if conditions are maintained so that the oyster itself removes its own pollution and no further contamination occurs, a slightly polluted oyster can cleanse itself within 24 hours. Later (1929) he stated that continual spraying of oysters with chlorinated sea water offered security of the public health. His method required the maintenance of an excess of chlorine in the daytime, with a sufficiently low concentration at night to permit the normal functioning of the oyster.

In 1930, Dodgson described a system of oyster purification which had been successful in England for 14 years. The oysters are placed on their sides in racks and are treated alternately with three sprayings of water and two 24-hour baths in sea water which is sterilized with 3 p.p.m. of chlorine and then neutralized with sodium thiosulfate. The final spraying is followed by an hour's bath in sea water containing 3 p.p.m. of chlorine. This is done to sterilize the conditioning tanks and the outside of the shells. Following the conditioning, the oysters are stored in sterile bags. It was found that increases in the length of time of conditioning did not appreciably enhance the purity of the shellfish. A standard of not more than five lactose-fermenting bacteria per milliliter of crushed whole oyster and its liquor was adopted.

The oysters used in our study were taken from Narragansett Bay. Upon arrival at the laboratory,[1] they were stored in a live trap until needed. A few days before each experiment, test oysters were brought into the laboratory and placed in glass aquaria through which sea water ran continuously.

The cultures employed were strains of coliform bacteria isolated from polluted oyster-producing areas in the Chesapeake Bay. They were characteristic Escherichia coli and retained their aerogenic properties when exposed to sea water.

The coliform scores were determined by the use of the standard fermentation tests (American Public Health Association, 1943) and Hoskins' (1940) most probable number tables. Plate counts were made with tryptone-glucose-extract agar (Bacto). All incubations were at 37° C. Fermentation tubes were incubated for 48 hours. Plates were counted at the end of a 24-hour incubation period.

The first set of experiments was designed to determine the extent to which coliform bacteria are eliminated by the oyster. The apparatus used was a modification of the one employed for the "drop count" method described by Galtsoff, Prytherch, Smith, and Koehring (1935). It consisted of a mixing chamber through which the sea water ran prior to entering a second chamber which housed the oyster. The volume of the mixing chamber was 1500 milliliters and that of the experimental chamber, 4500 milliliters. The rate of flow from the first to the second was 1020.4 milliliters per minute. The oyster was arranged with a rubber dam so that the water passing through it could be collected.

Before the experiment, samples of water were taken from each chamber for quantitative bacteriological determinations. Immediately following this sampling, a known quantity of coliform bacteria was added to the mixing chamber. Samples of the oyster effluent were collected after 5, 10, 30, 60, 120, and 180 minutes and after 24 hours. At the end of 48 hours, samples of water in the experimental tank and of that passing through the oyster were taken, and the oyster was removed for bacteriological examination. All samples were tested quantitatively for coliform content, and standard plate counts were made. The oyster meat was minced with the shell liquor and a known volume of sterile water, and portions of the mixture were cultured.

In the second set of experiments, eight oysters were placed in an aerated aquarium for 24 hours. At the end of this time, two oysters were removed for quantitative bacteriological tests. Following this, a known quantity of coliform bacteria was added to the aquarium water, and 3 hours thereafter, a second pair of oysters was removed for bacteriological examination. At this time a solution of chloramine-B was added to the water in a concentration which yielded 0.03 p.p.m. of free chlorine. This residual chlorine content was maintained for 48 hours. Thirty minutes after the chlorine had been added, a third pair of oysters was removed for bacteriological determination. After 48 hours' exposure to the chlorinated sea water, the fourth pair was subjected to bacteriological testing.

In the first set of experiments, determinations were made on four oysters. The data are shown in Table 1 (p. 10). They indicate that the majority of coliform bacteria are taken up rapidly by the oyster, since the discharge water contains relatively few of them. In each case there is a marked drop in the coliform content after 30 minutes, and from then on very few or none can be demonstrated in either the discharge water or the oyster. After 48 hours, coliform bacteria were demonstrable in only one of the four oysters, and the effluent from this specimen had

[1] These experiments were carried out at the Marine Biological Laboratory, Woods Hole, Mass.

been free of coliform bacteria after the first 30 minutes. The plate-count data are not included because of the wide fluctuations, which made any interpretation impossible.

Table 1 - Bacterial Content of Oysters Held in Flowing Sea Water and of Their Effluent

Source of Samples	Most Probable Number of Coliform Bacteria per 100 ml.			
	1st Oyster	2nd Oyster	3rd Oyster	4th Oyster
a) Water in mixing chamber at start	7.3	43.0	120.0	39.0
b) Water in experimental chamber at start	7.3	150.0	120.0	23.0
c) Water in mixing chamber immediately after addition of pure culture of *E. coli*	-	-	59600.0	67300.0
d) Oyster effluent 5 mins. after c)	0	150.0	210.0	75.0
e) " " 10 " " "	0	150.0	1100.0	1100.0
f) " " 30 " " "	9.1	21.0	39.0	1100.0
g) " " 1 hour " "	-	0	9.1	0
h) " " 2 hours " "	-	0	3.6	0
i) " " 3 " " "	-	0	3.6	0
j) " " 24 " " "	-	0	3.6	0
k) " " 48 " " "	-	0	0	0
l) Whole oyster 48 " " "	0	0	0	23.0

The data obtained in the second set of experiments are given in Table 2. The oysters contained coliform bacteria initially, and these were not eliminated during the 24-hour acclimatization period. The effect of the addition of the

Table 2 - Effect of Chlorination on Bacterial Content of Oysters Held in Aerated, Standing Sea Water

Source of Samples	BACTERIAL COUNTS			
	1st Run		2nd Run	
	M.P.N. coliforms per 100 mls.	Plate Count per ml.	M.P.N. coliforms per 100 mls.	Plate Count per ml.
a) Two oysters taken after 24 hours in aquarium, immediately before addition of pure culture of *E. coli*	1100.00 9.1	245 110	3.6 1100.0	45 38
b) Aquarium water immediately after addition of pure culture of *E. coli*	3200.0	-	26400.0	-
c) Two oysters taken 3 hours after (b) and immediately before addition of chloramine-B	150.0 -	62 29	20.90 1100.0	73 70
d) Two oysters taken 30 mins. after addition of chloramine-B	7.3 -	40 34	1100.0 1100.0	37 34
e) Two oysters taken 48 hours after addition of chloramine-B	0 -	175 70	23.0 9.1	126 145

culture is not clear, since the oysters themselves varied considerably in their initial bacterial content. The addition of the chlorine solution and the maintenance of 0.03 p.p.m. of free chlorine for 48 hours yielded coliform-free oysters in one instance but not in the other. The plate counts showed an actual increase under these conditions in both instances.

The results of these meagre experiments lead to two probable conclusions regarding oyster conditioning. The first is that oysters having access to a continuous supply of running water are capable of freeing themselves of demonstrable coliform bacteria within a very short period, possibly within 30 minutes. Second, storage in a tank at a concentration of chlorine which permits the normal functioning of the oyster (Galtsoff, 1946) over a period of 48 hours does not result in coliform-free specimens, nor does it lower the total bacterial content of the oyster. Therefore, conditioning by the described method of chlorination is probably

inadequate for the production of coliform-free oysters. These results also confirm the work of previous investigators.

LITERATURE CITED

DODGSON, R. W.

1930. The problem of polluted shellfish and its solution. Journal du Conseil. 5: 149-166.

GALTSOFF, P. S.

1946. Reaction of oysters to chlorination. Research Report 11, U. S. Fish and Wildlife Service.

------ PRYTHERCH, H. F.; SMITH, R. O.; and KOEHRING, V.

1935. Effect of crude oil pollution on oysters in Louisiana waters. Bull. U. S. Bu. of Fisheries, No. 18.

HOSKINS, J. K.

1940. Most probable number for evaluation of Coli-Aerogenes tests by fermentation tube method. Pub. Health Reports. Reprint No. 1621.

JOHNSTONE, JAMES.

1915. The methods of cleansing living mussels from ingested sewage material. Proc. and Trans. of the Liverpool Biol. Soc. 29: 119-151.

WELLS, WILLIAM F.

1916. (A) Artificial purification of oysters - A report of experiments upon the purification of polluted oysters, etc. Pub. Health Reports. 31: 1848-52.

1920. (B) The purification of oysters as a conservation measure. Amer. Jour. of Pub. Health. 10: 342-44.

1929. (C) Chlorination as a factor of safety in shellfish production. Amer. Jour. of Pub. Health. 19: 72-79.

AMERICAN PUBLIC HEALTH ASSOC.

1943. Bacteriological examination of shellfish and shellfish waters. Amer. Jour. of Pub. Health. 33: 582-91.

U. S. PUBLIC HEALTH SERVICE.

1944. A manual of recommended practice for the sanitary control of the shellfish industry.