

# DECOMPOSITION OF ORGANIC MATTER IN SEA WATER BY BACTERIA

## I. BACTERIAL MULTIPLICATION IN STORED SEA WATER<sup>1</sup>

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The organic matter in the sea is unevenly distributed between the living plant and animal population, the suspended detritus, the organic matter in the sea bottom, and that present in the water in true and in colloidal solution. The living forms and the detritus make up only a small part of the total organic matter in the sea; the ratio between the organic matter in solution and that present in the total plankton was found to range from 7:1 (Gran and Ruud, 1926) to 300:1 (Krogh, 1934a).

The chemical nature of the organic matter in the marine bottom material was shown (Boysen-Jensen, 1914; Waksman, 1933) to be similar to that of humus in land soils, both in chemical composition and resistance to decomposition. However, the origin, chemical nature and transformation of the organic matter dissolved in the water are still open to question. According to Pütter (1907, 1924), algae give off into the water, in the process of their nutrition, large quantities of carbohydrates; it was suggested that this may possibly be a result of changes brought about by the bacteria adhering to the algae. Petersen (1911) was of the opinion that the dissolved organic matter does not come from plankton algae but from benthos formations. Pütter's ideas (1909) concerning the rôle of the dissolved organic matter in the nutrition of marine animals also met with considerable criticism.

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Krogh (1934a) found that the organic matter, as measured by the total carbon and nitrogen, is uniformly distributed in the sea, from the surface of the water downward; one cubic meter of water was shown to contain, on the average, 0.244 gram of nitrogen and 2.36 gram carbon. Although these results have not yet been confirmed and further extended, they point to certain important facts concerning the nature and distribution of the dissolved organic matter, namely: (1) the uniformity of its concentration in the water; (2) its specific chemical nature, as shown by a definite relation between the carbon and the nitrogen (about 10:1); (3) its non-availability as a nutrient for plant and animal life (it has even been considered as resistant to bacterial attack). Krogh (1930) emphasized that this organic matter must be looked upon as having gone out of organic circulation, thus representing slowly accumulating waste products; at least a fraction of it was believed to consist of "humus," highly resistant to further decomposition. The formation of stable organic compounds was also shown (Birge and Juday, 1934) to take place in the water of inland lakes, following the decomposition of plankton. The presence of true "humus acids" or their salts in solution in lake waters has been suggested by Ohle (1933).

A definite relation has been shown to exist between the transformation of the organic matter in the water and bacterial multiplication. When sea water is brought to the laboratory and allowed to remain undisturbed, rapid multiplication of the bacteria takes place (Gran and Ruud, 1926; Föyn and Gran, 1928); this results in the decomposition of a part of the organic matter in the water, accompanied by the absorption of oxygen (Knauthe, 1898; Fox, 1905; Winterstein, 1909; Harvey, 1925) and the evolution of carbon dioxide (Atkins, 1922) and ammonia. In addition to decomposition processes, bacteria also play an important function in synthesizing new complexes in the sea. Vernon (1899) found that in the purification of ocean waters by bacteria, a part of the ammonia is removed by these organisms and synthesized into cell substance. This has been confirmed recently by Butterfield and Purdy (1931), who look upon bacteria as important agents in absorbing dilute food from the water and concentrating it in their bodies.

The function of bacterial activities in the transformation of organic matter in sea water is thus shown to comprise two distinct processes: (1) the decomposition of the organic matter, resulting in the liberation of at least a part of the elements in forms ( $\text{CO}_2$ ,  $\text{NH}_3$ ,  $\text{PO}_4$ ) available for diatom and algal nutrition; (2) the assimilation of some of the dissolved organic substances and their transformation into bacterial cells, which in their turn can be used as sources of food for marine animals.

#### EXPERIMENTAL

##### *Course of bacterial development in stored sea water*

The bacterial content of the water was determined by means of the plate method, which gives only the viable bacteria capable of developing on a specific medium. The results thus obtained represent only a fraction of the total number of bacteria, viable and dead, present in the water. A direct microscopic examination gave about 200 times as many organisms, the ratio becoming narrower upon addition of fresh organic matter. With due recognition of the limitations involved in the use of the plate method for measuring the abundance of bacteria in a natural substrate such as sea water, it was felt that this method is still most reliable for comparative purposes. An agar medium, previously found (Reuszer, 1933) to give good comparative results for determining numbers of bacteria in fresh sea water, was used. Several dilutions of the water were prepared and, whenever possible, only those plates which had 20 to 200 colonies were selected for counting. The plates were usually incubated for two to seven days, at room temperature ( $22^\circ$  to  $25^\circ\text{C}.$ ), unless otherwise stated. Oxygen was determined in the water by a modification of Winkler's method and ammonia by Krogh's method (1934b).

In a preliminary experiment on bacterial multiplication in stored sea water, samples of water were obtained near the dock at Woods Hole Harbor. The water was filtered through paper and kept in the laboratory exposed to light. Rapid multiplication of the bacteria took place, the numbers increasing from 85 cells in 1 cc. of fresh water to 1070 in twenty-four hours and to 157,000 cells within four days. It was soon found, however, that light

had an injurious effect upon bacterial development. In subsequent experiments, therefore, the water was kept in the dark, either in flasks plugged with cotton or in glass-stoppered bottles immersed under water.

The results of a typical experiment on bacterial multiplication in stored sea water, without and with the addition of different organic substances, are shown in figure 1. One portion of water was left untreated. One portion received a trace of an iron-ligno-

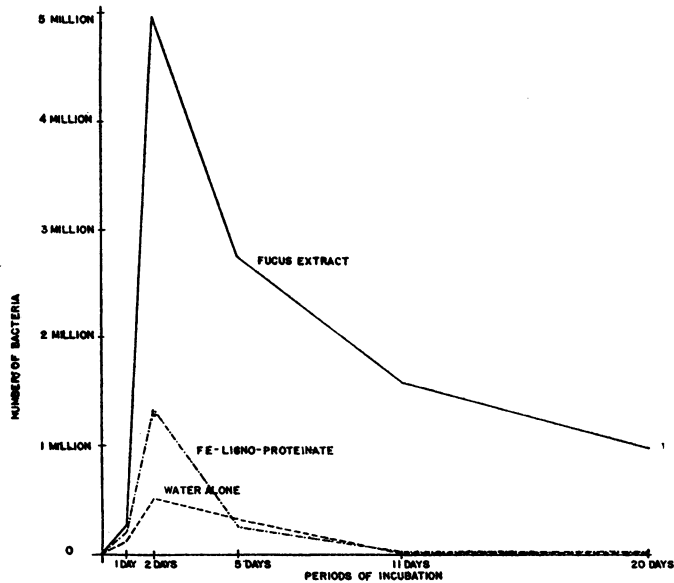


FIG. 1. INFLUENCE OF DIFFERENT FORMS OF ORGANIC MATTER UPON THE RATE OF BACTERIAL MULTIPLICATION IN SEA WATER

protein complex, introduced by adding 1 gram of this preparation to 500 cc. of fresh water, allowing to stand over night, and removing the undissolved organic matter by filtration through paper. A third portion received a small portion of a neutralized alkali extract of Fucus material. The rate of bacterial multiplication in stored water attained a maximum within very few days, usually 2 to 3; it then began to diminish rapidly, soon reaching a certain level or equilibrium which was considerably above that found in natural sea water. The amount and nature of the organic matter

in the water had a decided favorable effect upon the rapidity and extent of bacterial multiplication. Although the ligno-protein preparation was only sparingly soluble, it brought about an increase in bacterial numbers. The effect of the Fucus extract was still more marked, largely because of the greater availability of this form of organic matter, which offers an excellent source of energy for marine bacteria.

Among the different factors influencing bacterial activities and the rate of bacterial multiplication in sea water, temperature and oxygen concentration are most important. Two quantities of

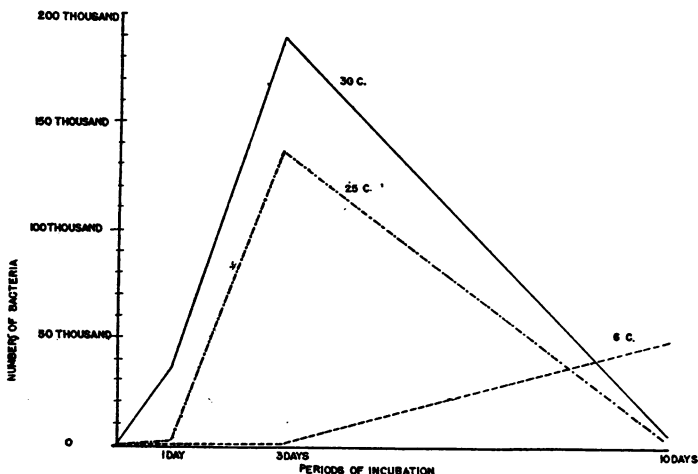


FIG. 2. INFLUENCE OF TEMPERATURE UPON THE RATE OF MULTIPLICATION OF BACTERIA IN STORED SEA WATER

water were collected from the middle of the Great Harbor at Woods Hole and about 1.5 miles off Quick's Hole, at a depth of 4 to 5 meters. The water was distributed into Erlenmeyer flasks, and these were incubated at three different temperatures, namely, 6°C., 25°C. and 30°C. At definite intervals, the numbers of bacteria were determined. The data reported in figure 2 represent averages of the results obtained for the two samples, since the numbers ran parallel in the water from both stations. The most rapid development of the bacteria took place at 30°C.; the maximum was attained within three days at this temperature, as well

as at 25°C. At 6°C., the rate of bacterial multiplication was very slow; however, the increase continued even after ten days incubation, so that on that day there were more bacteria in the water which had been kept at the lower than at the higher temperature, i.e., the maximum at the lower temperature was attained after a longer period of time. These results were confirmed by the use of water taken at a distance from shore.

The influence of depth of water upon bacterial multiplication, at 22° to 25°C., is shown in figure 3. There was an increase in the

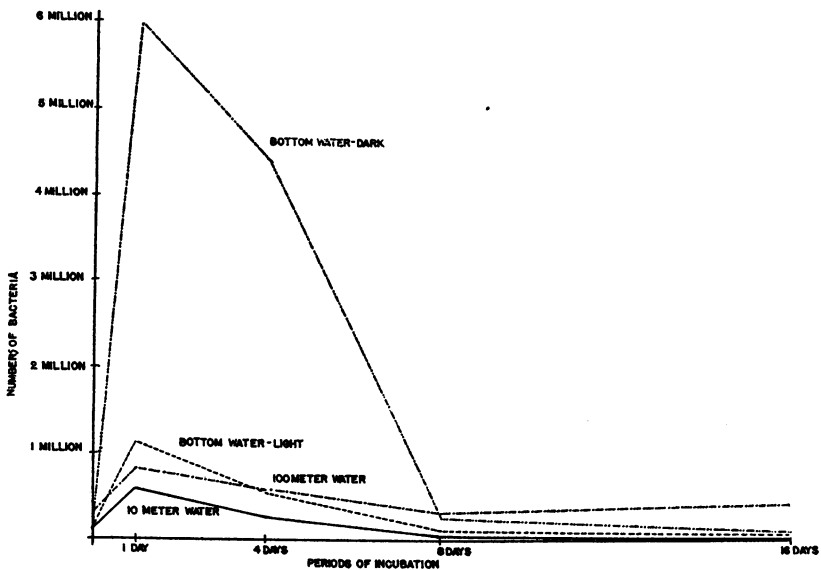


FIG. 3. INFLUENCE OF DEPTH OF WATER UPON THE RATE OF BACTERIAL MULTIPLICATION WHEN WATER IS KEPT AT 25°C.

rate of bacterial activities with an increase in depth of water. The actual numbers of bacteria found in the natural water were 2, 6 and 17 cells per 1 cc. of water, with increasing depths. The exceptionally high rate of bacterial multiplication in the water taken just above the bottom was no doubt due to contamination with some of the bottom material. The water exposed to light gave much lower numbers than the same water kept in the darkness.

*Bacterial multiplication in filtered sea water*

Bacterial development in stored sea water takes place at the expense of the organic matter suspended or dissolved in the water. The addition of a small amount of organic matter of a type comparable in chemical nature to that which is found in the sea has a decided stimulating effect upon bacterial multiplication. In order to throw light upon the specific nature of the organic matter in the water and its transformation as a result of bacterial action, three methods of attack suggest themselves, namely, (1) the separation of the organic matter by filtration of the water through different types of filters; (2) the study of the decomposition of the organic matter in water obtained from different depths, in relation to the photosynthetic zone; (3) the decomposition of different forms of organic matter added to the water.

Several methods of filtration were used in these experiments: (1) filtration through ordinary filter paper, hardened paper, or glass filters, thereby removing the coarser suspended particles and most of the plankton forms; (2) filtration through Seitz and membrane filters, which remove the bacteria as well; (3) colloidal filtration, thus leaving in the water only the substances present in true solution (Krogh, 1934b). The water filtered through Seitz, membrane and colloidal filters was always reinoculated with either fresh water or water kept for eighteen to twenty-four hours in the laboratory, whereby an enriched water culture was obtained. The vacuum produced in the filtration of the water through Seitz or membrane filters reduced the oxygen tension of the water, so as to make conditions unfavorable for the growth of the normal aerobic bacterial population commonly found in sea water; the water thus filtered was subsequently shaken for thirty to sixty minutes, to resaturate it with oxygen.

A quantity of water was obtained from the Great Harbor and separated into four portions: one was filtered through ordinary qualitative paper and is spoken of as fresh water; one was passed through a sinter filter; one was filtered through a Seitz filter; and one was ultra filtered. The water was inoculated and distributed in glass stoppered bottles; these were immersed in a water bath at 20° to 22°C. After definite intervals, the oxygen content was

determined in some of the bottles, while others were used for bacterial counts and ammonia determinations.

Only the results obtained for the fresh and sinter-filtered water are reported in figure 4. A definite parallelism was found to exist between bacterial multiplication, oxygen absorption and ammonia formation. In the case of the fresh water, 1.26 cc. of oxygen per liter was consumed in eleven days, while in the sinter-filtered water it was only 0.56 cc.; the corresponding amounts of ammonia produced in the two samples of water were 86 and 44

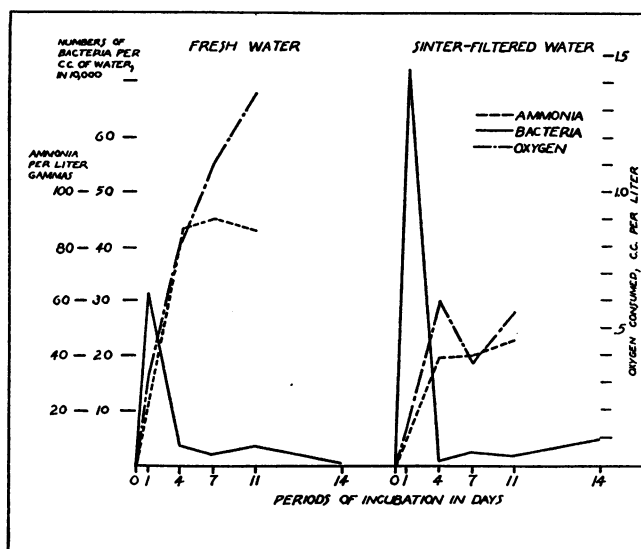


FIG. 4. INFLUENCE OF FILTRATION UPON BACTERIAL ACTIVITIES IN SEA WATER

gammas per liter.<sup>2</sup> This tends to show that the suspended material removed from the water in this experiment by sinter-filtration comprises a large part of the actively decomposing organic matter.

<sup>2</sup> The results of the ammonia determinations reported here should be considered as preliminary in nature. They were not checked sufficiently, hence no emphasis can as yet be laid upon them. In other experiments, either no increase in ammonia was obtained at all or only widely varying results were obtained. Although there is no doubt that in the process of decomposition of the organic matter in the water, nitrogen is liberated in an available form or in a form readily assimilable by bacteria as will be brought out later, the exact nature of this nitrogen is still a subject for further study.



Extensive bacterial multiplication took place in both the Seitz and ultra-filtered water; this was accompanied by oxygen absorption. In shaking the Seitz-filtered water, considerable ammonia was absorbed from the laboratory air, while only a small amount of colloid-filtered water was available; hence the results of bacterial activities in these portions of water are not reported; the fact was demonstrated, however, that the organic matter present in the water in true solution can be readily utilized by bacteria.

To determine the influence of the oxygen concentration of the water upon bacterial development, a quantity of fresh water was passed through a Seitz-filter. One-half of the filtered water was allowed to remain undisturbed, while the other half was thor-

TABLE 1

*Influence of oxygen tension upon the activities of bacteria in sea water*

TREATMENT OF SEITZ-FILTERED WATER	PERIOD OF INCUBATION							
	Start		2 days			5 days		
	Bacteria in 1 cc. of water	Oxygen	Bacteria in 1 cc. of water	Oxygen	Oxygen consumed	Bacteria in 1 cc. of water	Oxygen	Oxygen consumed
		cc. per liter		cc. per liter	cc. per liter		cc. per liter	cc. per liter
Aerated thoroughly.....	107	4.75	912,000	4.61	0.14	1,426,000	3.01	1.72
½ aerated + ½ unaerated...	88	4.42	9,350	4.37	0.05	1,098,000	3.54	0.86
¼ aerated + ¾ unaerated...	78	4.34	8,000	4.24	0.10	812,000	4.13	0.21
Unaerated.....	69	3.95	2,950	3.88	0.07	5,150	3.84	0.11

oughly shaken to saturate it with oxygen. Four quantities of water were now prepared: (1) filtered, unaerated; (2) filtered, oxygen saturated; (3) 50 per cent unaerated + 50 per cent aerated; (4) 75 per cent unaerated + 25 per cent aerated. These four lots of water were distributed into glass-stoppered bottles, which were then placed under water at room temperature. The oxygen content and bacterial numbers were determined immediately, and after two and five days incubation (table 1). Bacterial development in the unaerated water took place only very slowly; oxygen consumption was limited. In the fully aerated water, bacterial multiplication was normal, reaching over a million cells per 1 cc. within five days; this was accompanied by

rapid oxygen consumption. In the mixed water, the rate of bacterial multiplication was reduced by a decrease in oxygen content; however, a gradual adjustment of the bacteria to the diminished oxygen tension was observed. This modification of the bacterial activities obtained by reducing the oxygen tension of the water is of such distinct interest that, before the subject has been studied in further detail and before the modifications in the physical and chemical conditions of the water, such as change in hydrogen-ion concentration, are better understood, no broad generalizations can be made.

The results of another experiment, in which a greater variety of filters were used and in which an attempt was made to determine the effect of substances removed from the filter itself upon bacterial activities, are presented in table 2. The first few hundred cubic centimeters of the filtered water were discarded, and two subsequent portions used. The bacteria were again found capable of utilizing the organic matter present in the water in true solution. It is of interest to note that in the normal water and in the water modified by the addition of organic matter, as well as in the water incubated at different temperatures, there was a definite parallelism between bacterial multiplication and oxygen consumption, but not in the water filtered through fine filters. This may either be due to the development of specific bacteria which grow on the particular forms of organic matter left in the water, or to some specific effect of the filter upon the organic substances in the water. Attention is also called to the high numbers of bacteria in the water passed through the Seitz and colloidal filters.

The influence of depth of water upon bacterial multiplication and oxygen consumption is illustrated in table 3. In the case of the water obtained from the Gulf of Maine Station, the greatest activities took place in the water from 40 meters depth and then diminished above and below that zone. No such regularity was observed in the water taken from George's Bank, probably due to the constant mixing of the water at this station. Water was also obtained by means of Nansen bottles from station 2247 (latitude 39° 43', longitude 69° 53'), to a depth of 1800 meters.

The greatest bacterial multiplication took place in the water taken at a depth of 50 meters, or within the photosynthetic zone;

TABLE 2

*Bacterial activities in sea water passed through different types of filters*

NATURE OF FILTER	BACTERIA IN 1 CC. OF INCUBATED WATER				OXYGEN CONSUMED ON INCUBATION		
	Start	2 days	5 days	7 days	2 days	5 days	7 days
					cc. per liter	cc. per liter	cc. per liter
Qualitative filter paper (No. 12).	810	1,145,000	73,000	15,400	0.84	1.37	1.46
Hardened filter paper.....	4,946	690,000	44,300	62,400	0.78	1.11	1.21
Seitz filter, first lot.....	650	210,000	942,600	432,000	0.82	1.34	1.43
Seitz filter, second lot.....	1,870	176,500	738,000	270,660	0.78	0.73	1.29
Membrane filter, first lot.....	846	705,000	23,000	42,700	1.17	1.02	1.05
Membrane filter, second lot....	1,320	395,000	15,000	3,230	0.68	1.14	1.22
Colloid filter.....	1,800	940,000	712,000	621,000			
Seitz filtered sterile water (uninoculated).....	0		0			0	

TABLE 3

*Bacterial activities in sea water taken at different depths in the Gulf of Maine and George's Bank*

POSITION OF STATION	DEPTH OF WATER	TEMPERATURE OF WATER	OXYGEN	BACTERIA IN 1 CC. OF INCUBATED WATER				OXYGEN CONSUMED ON INCUBATION			
				Start	2 days	5 days	10 days	2 days	5 days	10 days	
								cc. per liter	cc. per liter	cc. per liter	
Gulf of Maine	5	18.49	6.11	255	378,500	420,000	82,600	0.71	0.86	1.05	
	40	5.72	6.23	343	647,500	205,000	185,000	0.55	1.26	1.33	
	40° 34' x	229	6.19	4.28	31	454,500	185,000	336,000	0.47	0.45	0.69
	69° 19'	5*	18.49	6.11	255	309,000	400,000	19,000	0.56	1.05	1.25
George's Bank	5	7.76	6.81	617	492,500	245,000	132,600	0.21	0.64	0.85	
	20	7.64	6.62	525	388,000	70,000	143,000	0.37	0.46	0.76	
	41° 10' x	56	5.96	6.57	1,266	210,500	1,215,000	570,600	0.40	0.88	1.08
	69° 30'	5*	7.76	6.81	617	410,000	880,000	24,300	0.52		1.00

\* Small amount of bottom material, mud or sand, added to each bottle.

water obtained at greater depths gave, on incubation, lower bacterial activities.

## SUMMARY

A study has been made of bacterial multiplication in stored sea water, obtained from different depths and different regions, and modified by filtration through different filters. The results can be summarized as follows:

1. When sea water is placed in glass containers and stored in the laboratory or in the sea, rapid multiplication of the bacteria takes place.

2. The bacteria multiply at the expense of the organic matter present in the water in suspension and in solution, as measured by an increase in oxygen consumption.

3. The maximum development of bacteria takes place within two to three days, at 20° to 30°C. At lower temperatures, the rise in bacterial activities is much slower; however, in time they may attain as high if not a higher maximum than at the higher temperature.

4. The oxygen concentration of the water is highly important for bacterial activities; at a reduced oxygen tension, the organic matter of the water is attacked with considerable difficulty.

5. In the decomposition of the organic matter in the water by bacteria, a definite parallelism was obtained between bacterial multiplication, oxygen consumption and liberation of nitrogen in an available form.

6. These results lead to the conclusion that sea water contains sufficient organic matter in true solution, to support, under favorable conditions, an extensive bacterial population.

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