DECOMPOSITION OF ORGANIC MATTER IN SEA WATER BY BACTERIA

II. INFLUENCE OF ADDITION OF ORGANIC SUBSTANCES UPON BACTERIAL ACTIVITIES[1]

SELMAN A. WAKSMAN AND CORNELIA L. CAREY

Woods Hole Oceanographic Institution and Department of Soil Microbiology, New Jersey Agricultural Experiment Station

Received for publication December 8, 1934

It has been shown previously (Waksman and Carey, 1935) that sea water contains sufficient organic matter in true solution to support an extensive bacterial population. It remained to be determined what forms of organic matter are more readily subject to bacterial attack and to establish the relation between bacterial multiplication and organic decomposition. Various organic substances, simple and complex in nature, were added to sea water and the response of the bacterial population determined.

In a preliminary experiment, some floating marine sediment collected by Dr. Stetson of this Institution was used. This sediment was obtained in Nantucket Sound (Hedge Fence), with a pan sitting 4 to 6 feet above the bottom; the total depth of the water was 12 feet. The dry material analyzed 18 per cent loss on ignition, 5.6 per cent organic carbon, and 0.68 per cent nitrogen, giving a carbon-nitrogen ratio of about 8:1. Two small quantities of this material, in a fresh state, equivalent to 72 and 87 mgm. of dry matter, were added to 200-cc. portions of water, previously kept for forty-eight hours in the laboratory in glass containers and the bacterial changes in which were known. The water was now again incubated at 20° to 22°C., for twelve days, and the bacterial changes determined (table 1). The results

[1] Contribution No. 68 of the Woods Hole Oceanographic Institution and Journal Series Paper of the New Jersey Agricultural Experiment Station, New Brunswick, N. J.

show that the sediment offers a favorable substrate for bacterial development.

UTILIZATION OF DIFFERENT ORGANIC SUBSTANCES BY BACTERIA IN SEA WATER

Freshly collected water was filtered through ordinary qualitative paper and divided into several lots. In order to eliminate the controlling effect of phosphate, all received, with one exception (control water), 10 mgm. K_2HPO_4 per 1000 cc. of water. Varying amounts of different organic substances were added, and the water distributed into a series of specially cleaned glass-stoppered bottles and flasks. The bottles, to be used for oxygen

TABLE 1

Influence of marine sediment upon bacterial multiplication in sea water

Bacteria in 1 cc. of water

PERIOD OF INCUBATION OF WATER	WATER ALONE	MARINE SEDIMENT, 72 MGM.	MARINE SEDIMENT, 87 MGM.
days			
Start	636		
1	144,000		
2	173,500	*	*
3	88,000	1,012,000	1,725,000
6	20,500	257,000	785,000
14	15,600	128,500	86,500

* Sediment added at this time.

determination, were incubated under water and the flasks, for the study of bacterial changes, in a dark closet (table 2). The fresh water contained about 600 bacteria in 1 cc. A normal course of bacterial development and oxygen consumption took place in the control water, the addition of K_2HPO_4 having comparatively little effect. The marine sediment was again found to favor bacterial development, as shown both by increased numbers and oxygen consumption. The glucose was attacked readily by the bacteria, but not as rapidly and completely as the alginic acid (as shown by the lower oxygen consumption). This is no doubt due to the fact that the latter contained a small amount of nitrogen as an impurity, while the glucose, being free from nitrogen, brought about a nitrogen minimum as a result of the introduction into the water of a large amount of available energy. Fucus and its constituents, as well as the copepods and asparagine were utilized readily. The fresh diatom material was also decomposed rapidly; the lower numbers of bacteria and limited oxygen consumption were due to the fact that only an

TABLE 2

Influence of different organic complexes upon bacterial activities in sea water

NATURE OF COMPLEX ADDED PER LITER OF WATER	PERIOD OF INCUBATION					
	1 day	2 days		6 days		15 days
	Bacteria in 1 cc.	Bacteria in 1 cc.	Oxygen*	Bacteria in 1 cc.	Oxygen	Bacteria in 1 cc.
			cc. per liter		*cc. per liter*	
Control water	229,500	800,000	4.49	187,000	4.20	48,000
K_2HPO_4 alone	222,000	735,000	4.50	77,000	4.06	11,850
Marine sediment, 10 mgm.	970,000	975,000	4.37	695,000	3.55	
Glucose, 4 mgm.	1,135,000	1,070,000	3.98	430,000	2.41	470,000
Alginic acid, 4 mgm.	3,240,000	1,080,000	3.26	955,000	0.99	1,010,000
Cold water extract of Fucus, 10 mgm.†	4,520,000	3,815,000	0.84	1,135,000	0	
NH_4OH extract of Fucus, 10 mgm.‡	2,020,000	30,350,000	0	26,300,000	0	
Copepods, 50 Calanus	17,450,000	2,800,000	0.72	3,550,000	0	49,500
Asparagine, 4 mgm.	4,750,000	16,300,000	0	895,000	0	163,500
Fresh, dry Fucus, 10 mgm.	4,520,000	4,605,000	1.55	840,000	0	1,950,000
Pure culture of diatom suspension, equivalent to 3 mgm. dry matter	4,510,000	3,000,000	2.93	295,000	1.89	3,000

* The oxygen content of the freshly taken water was 5.10 cc. per liter of water.

† Dry matter basis.

‡ On basis of dry organic matter, most ammonia removed from extract by heating.

equivalent of 3 mgm. of dry diatom substance, representing 1.5 mgm. of organic matter, was added to each 200-cc. bottle of water.

Glucose was selected for a detailed study of the relation of bacterial multiplication to the decomposition of a known organic compound in sea water. When glucose alone is added to sea water, the available nitrogen soon becomes a limiting factor for bacterial multiplication; a small amount of ammonium sulfate was, therefore, also added to some of the samples of water, at the rate of one part of nitrogen to 10 parts of glucose. Three different concentrations of glucose were added to portions of fresh, paper-filtered water, namely, 3.3, 10.0 and 26.7 mgm. per liter, distributed into glass-stoppered bottles and incubated under water at 20° to 22°C. At definite intervals of time, some of the bottles were removed and analyzed for bacterial numbers and for oxygen concentration. Because of the fact that these cultures were kept in the dark, algal growth was eliminated; further, because of a lack or only limited development of autotrophic bacteria, which could easily be demonstrated by the lack of formation of nitrite and nitrate, oxygen consumption in the water may be taken as an index of glucose decomposition.

The results presented in table 3 show that just about twice as much oxygen was consumed upon addition of glucose to the water, but without added nitrogen, as in the control water. The addition of larger amounts of glucose had no further effect upon oxygen consumption and very little upon bacterial multiplication. This is due to the fact that when glucose alone is added to the water, the bacteria depend for the nitrogen required for their metabolism only upon the organic matter normally present in the water. Hence, the decomposition of added glucose is controlled by the rate of the decomposition of the organic matter present in the natural water. However, when available nitrogen was also added, the bacteria decomposed much larger quantities of glucose, as determined both by the increase in oxygen consumption and by the rate of bacterial multiplication. A few determinations were also made of the ammonia content of the water. Although no quantitative data can be presented here, because the results were not checked sufficiently, they pointed definitely to the fact that while the bacteria in the unmodified water liberated appreciable amounts of nitrogen as ammonia, the glucose-containing water was free from ammonia; in the case of the water to which both glucose and a nitrogen source were added, rapid disappearance of the ammonia nitrogen took place.

Simultaneous with the decomposition of glucose in sea water, the decomposition of glutamic acid was also determined. In the decomposition of 3.3 mgm. of this compound per liter of water, the bacteria consumed 1.92 cc. of oxygen in 3 days and 2.93 cc. in 7 days, i.e., exactly the same amounts as in the decomposition of a similar concentration of glucose plus available nitrogen. The rate of bacterial multiplication was also similar.

THE RELATION BETWEEN AVAILABLE NITROGEN AND GLUCOSE DECOMPOSITION IN SEA WATER

A further study of the decomposition of glucose in sea water brought out (table 4) the fact that sufficient nitrogen is liberated in one liter of water, as ammonia or as other available forms, to

TABLE 3

Bacterial activities in sea water receiving different amounts of glucose, with and without additional nitrogen

GLUCOSE ADDED	$(NH_4)_2SO_4$	BACTERIA IN 1 CC.				OXYGEN CONSUMED		
		Start	1 day	3 days	7 days	1 day	3 days	7 days
mgm. per liter	*mgm. N per liter*					*cc. per liter*	*cc. per liter*	*cc. per liter*
0	0	532	140,500	1,060,000	48,500	0.25	0.84	1.33
0	2.67	532			12,000			1.07
3.3	0	532	780,000	545,000	71,500	0.39	1.44	2.70
3.3	0.33	532		5,950,000			1.87	2.96
10.0	0	532	1,215,000	575,000	130,000	0.27	1.53	2.87
10.0	1.00	532		17,750,000	6,050,000		4.68	5.14*
26.7	0	532	1,370,000	480,000	96,500	0.39	1.35	2.82
26.7	2.67	532	14,600,000	7,100,000	515,000	3.62	5.14*	5.14*

* All oxygen in water consumed, anaerobic system.

enable the bacteria to decompose an additional 2.5 mgm. of glucose. The addition of nitrogen, in the form of ammonium sulfate, to the water had no further effect in bringing about any greater decomposition of the glucose, as long as the concentration of the latter did not exceed 2.5 mgm. per liter. When glucose was introduced in larger amounts, namely, 5 mgm. per liter, the addition of available nitrogen had a decided favorable effect upon its decomposition; however, in this case as well, only a certain concentration of nitrogen was required to favor the decomposition of the excess of glucose; any increase in the amount of ammonium sulfate added to the water had no further effect upon the process of decomposition. When still larger quantities of glucose were added to the water, at the rate of more than 5 mgm. per liter, another limiting factor soon manifested itself, namely, the rapid consumption of the oxygen. Fresh sea water contains just about sufficient oxygen to bring about the complete utilization of 5 mgm. glucose per liter; however, only a part of the carbohydrate (about two-thirds) is completely oxidized to liberate energy, while another part is utilized by the bacteria for the synthesis of their cell substance. Larger amounts of glucose give rise to an anaerobic system which is no longer characteristic of normal sea water.

TABLE 4

Influence of nitrogen upon the decomposition of glucose in sea water

GLUCOSE ADDED	$(NH_4)_2SO_4$	BACTERIA IN 1 CC.					BACTERIA IN 1 CC., BY MICROSCOPIC METHOD	OXYGEN CONSUMED			
		Start	1 day	2 days	3 days	5 days	3 days	1 day	2 days	3 days	5 days
mgm. per liter	*mgm. N per liter*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*cc. per liter*	*cc. per liter*	*cc. per liter*	*cc. per liter*
0	0	0.883	625	775	295	121.6	65,000	0.69	0.80	0.96	1.28
2.5	0	0.883	2,005	2,450	675	44.3	80,000	1.31	1.76	2.13	2.49
2.5	0.25	0.883	3,850	3,700	735	9.6	120,000	1.21	1.62	1.82	2.41
5.0	0.25	0.883	8,850	8,150	1,125	84.0	107,500	1.98	2.36	2.93	3.58
5.0	0.50	0.883	6,900	7,200	880	57.6	82,500	1.81	2.16	2.47	3.41
7.5	0.75	0.883	12,550	11,700	1,740	13.6	162,500	2.63	3.09	4.10	4.76
15.0	1.5	0.883	24,050	29,000	4,200	349.6	220,000	4.22	4.86	5.28*	5.30*

* Oxygen practically all used up, anaerobic system produced.

In view of the extensive multiplication of bacteria in stored sea water, especially in the water enriched with glucose, the total number of bacterial cells can readily be determined by direct microscopic counts. The ratio between the numbers of bacteria obtained by the microscopic and plate methods was, in the case of the unmodified stored water, between 200 and 300, similar to the ratio previously found (Waksman, 1934) for bacterial numbers in sea water and in plankton tow. The water receiving glucose gave a lower ratio, which tended to approach 100:1 or even less. In the presence of an excess of readily available energy material and at an active stage of bacterial multiplication, there was a greater proportion of viable bacterial cells, capable of developing into colonies on the plate.

The results presented in table 4 are sufficient to establish the relationship between the different bacterial processes, namely, decomposition of the organic matter in the sea water, bacterial multiplication, oxygen consumption and nitrogen liberation, the latter being determined by the extent of glucose decomposition (fig. 1). The consumption by bacteria of 1.28 cc. of oxygen per liter of water, in 5 days, is a measure of the decomposition of

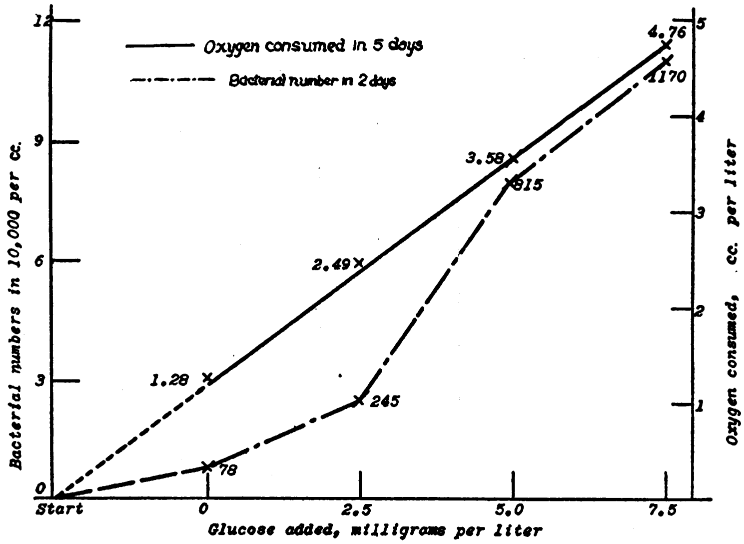

FIG. 1. DECOMPOSITION OF ORGANIC MATTER IN WATER, AS INFLUENCED BY ADDITION OF GLUCOSE

an equivalent amount of organic matter in the water, aside from that portion of the organic matter used by the bacteria for the synthesis of their cell substance; the latter may also be quite considerable, as shown by the microscopic counts. Using as a basis the results of Krogh (1934), namely that sea water contains about 5 mgm. organic matter per liter, it is a matter of simple calculation to determine that at least 25 per cent of the organic matter in the water is subject to rapid decomposition by bacteria; this is accompanied by the liberation of a corresponding amount of the nutrient elements (CO_2, NH_3, P_2O_5) stored in the organic matter and essential for plant life. The bacterial cells synthesized in the process can obviously become a source of food for animal life.

The results of the following experiment serve to emphasize further the fact that the rate of glucose decomposition in sea water is a function of the liberation of nitrogen in an available form. A quantity of water was collected from the Harbor at Woods Hole, at a depth of 4 meters, filtered through paper and kept at room temperature, in the dark, for 4 days; another

TABLE 5

Decomposition of glucose in sea water as an index of liberation of nitrogen in an available form

NATURE OF WATER	BACTERIA IN 1 CC. AT START	NO GLUCOSE			GLUCOSE ADDED		
		42 hours	4 days		42 hours	4 days	
		Oxygen consumed	Bacteria in 1 cc.	Oxygen consumed	Oxygen consumed	Bacteria in 1 cc.	Oxygen consumed
		cc. per liter		*cc. per liter*	*cc. per liter*		*cc. per liter*
Water from harbor, fresh	925	0.79	129,500	1.21	1.56	660,000	3.45
Water from harbor, 4 days old	28,700	0.26	11,500	0.44	2.40	310,000	4.08
George's Bank water, 5 meters, 12 days old	560	0.25	16,000	0.44*	2.76	100,000	4.70
George's Bank water, 56 meters, 12 days old	14,200	0.34	551,000	0.37†	3.41	2,026,000	4.27

* Same water freshly taken consumed, when incubated at 20° to 22°C. in the darkness, 0.64 cc. of oxygen per liter in 5 days.

† Oxygen consumption of fresh water 0.88 cc. per liter in 5 days.

quantity of water was taken fresh from the same place in the Harbor and also filtered through paper. Two other lots of water were obtained from George's Bank, at a depth of 5 and 56 meters, and kept in glass-stoppered bottles for 12 days at 6°C. These four lots of water received 6.7 mgm. K_2HPO_4 per liter and each lot was divided into 2 parts, one not receiving any glucose and the other receiving 10 mgm. glucose per liter. These lots were distributed in glass-stoppered bottles and incubated, under water, at room temperature. Bacterial numbers and oxygen consumption were determined after 42 hours and 4 days. The results (table 5) show that the water taken from the Harbor and stored for 4 days, at room temperature, liberated sufficient nitrogen to enable the bacteria to increase considerably the rate of glucose decomposition. The same is true of the water from George's Bank. Since this water had been incubated for 12 days before glucose was added, a much greater amount of the carbohydrate was decomposed without the addition of nitrogen.

TABLE 6

Influence of temperature upon bacterial activities in sea water

TEMPERATURE	GLUCOSE ADDED*	NUMBERS OF BACTERIA						OXYGEN CONSUMED			
		Start	1 day	3 days†	6 days	9 days	12 days	1 day	3 days	6 days	9 days
°C.		*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*cc. per liter*	*cc. per liter*	*cc. per liter*	*cc. per liter*
2	−	0.85		5.2	172.5	1,215.0	529		0.04	0.26	0.45
12	−	0.85	3.3	492.0	677.0	87.5		0	0.52	0.83	0.94
22	−	0.85	438.0	492.0	94.0	6.2		0.49	0.85	1.39	1.54
34	−	0.85	539.0	581.0	55.0	37.0		0.43	0.67	1.14	1.18
2	+	0.85		5.5	175.5	1,135.0	762		0.04	0.21	0.56
12	+	0.85	5.2	775.0	550.6	140.0		0	0.80	1.55	2.29
22	+	0.85	1,355.0	1,130.0	265.0	122.5		0.72	1.82	3.87	4.77
34	+	0.85	1,110.0	600.0	356.0	504.0		1.64	3.65	4.25	4.80

* Ten milligrams per liter of water.
† For the 2°C., read 2, 5, 8, 12 days.

INFLUENCE OF TEMPERATURE OF INCUBATION UPON BACTERIAL MULTIPLICATION AND OXYGEN CONSUMPTION IN WATER

Water obtained from Woods Hole Harbor was filtered and divided into two lots, one of which received 10 mgm. glucose per liter. Incubation took place in glass-stoppered bottles, in special water baths, kept at different temperatures. The results obtained in this experiment (table 6) tend to confirm the observations previously made, namely, that at higher temperatures, bacterial multiplication in stored sea water proceeds most rapidly and soon attains a maximum; this is followed by a rapid drop in numbers. At lower temperatures, however, the bacteria begin to develop more slowly but in time they attain as high a rate of activities as that observed at the higher temperatures (fig. 2). Similar results were obtained for bacterial multiplication in river water (Butterfield, 1933).

DISCUSSION

Bacteria are largely responsible for the mineralization of the organic residues of plant and animal origin in the sea; the energy thus liberated enables them to synthesize new complexes, largely in the form of bacterial cells or their decomposition products, which are found in the water in the form of suspended matter as well as in true solution. The organic matter in sea water is in a

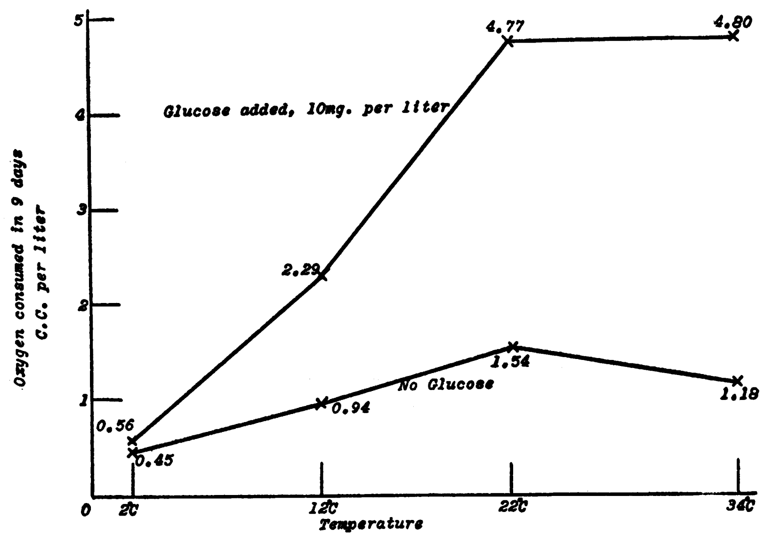

FIG. 2. INFLUENCE OF TEMPERATURE UPON BACTERIAL ACTIVITIES IN SEA WATER, AS MEASURED BY OXYGEN CONSUMPTION

state of dynamic equilibrium in which the residues and waste products of higher forms of life, bacterial activities and the dissolved organic complexes, are all involved.

Although the concentration of organic matter in sea water is small, namely about 5 mgm. per liter, as determined from the results of Krogh, it is not absolutely resistant to bacterial decomposition. When water is enclosed in a vessel, active bacterial multiplication sets in; this is accompanied by rapid decomposition of the organic matter. The rapidity of this process depends upon a number of factors, especially temperature, oxygen tension, abundance of organic matter and chemical nature of the organic matter itself. The determination of the rate of bacterial multiplication in sea water is the most sensitive index for the study of the changes produced in the organic matter content of the water as a result of bacterial activities; a slight change in organic matter concentration brings about an extensive change in the numbers of bacteria. This method can be supplemented by the determination of the biochemical changes in the water, especially oxygen absorption and liberation of nitrogen as ammonia.

Most bacteria present in sea water are aerobic in nature; sea water contains sufficient oxygen to permit oxidation processes to proceed uninterruptedly in the presence of concentrations of organic matter comparable to those ordinarily present in the water; the amount of oxygen consumed by the bacteria in the process of oxidation must, therefore, be parallel to the amount of CO_2 liberated. Since the organic matter of the water is of a more or less constant carbon-nitrogen ratio, as indicated by the results of Krogh, the decomposition of a certain amount of this organic matter should be accompanied by a parallel liberation of nitrogen, as ammonia or in some other available form. Since the decomposition of glucose added to sea water was found to be controlled by the amount of available nitrogen in the water, the liberation of nitrogen in the decomposition of organic matter in the water can be determined not only directly but also indirectly, namely by adding glucose to the water and measuring the rate of its decomposition.

A definite parallelism was obtained between the increase in bacterial numbers, oxygen absorption and liberation of nitrogen in an available form in stored sea water. This parallelism was modified considerably by the separation of the organic matter in the water by means of different filtration procedures. Water passed through a filter fine enough to remove the bacteria and reinoculated with cultured fresh water gave a higher rate of bacterial multiplication; the latter was not always accompanied by a parallel increase in oxygen consumption. Whether this was due to the modification of the organic matter by the filtration process, whereby the development of special groups of bacteria was favored, or to some other factor still remains to be determined.

The results presented here are sufficient to permit calculation of the relationship between bacterial development and organic matter transformation in sea water. Although bacterial multiplication and oxygen consumption run parallel in stored sea water, they are not directly proportional, bacterial multiplication preceding somewhat the consumption of the oxygen. The number of active bacteria varies considerably during the period of active multiplication, followed by their gradual destruction; the amount of oxygen used by a certain number of bacteria is not the same throughout their life cycle. The results presented in table 4 can be taken as a basis for computation. Without the addition of glucose, the bacteria increased from 883 to 625,000 in 1 cc. of water, in 24 hours; this was accompanied by the consumption of 0.69 cc. oxygen per liter of water, or 1.1 cc. of oxygen consumed per liter for an increase of 1 million bacteria per 1 cc. During the second 24 hours, the bacterial numbers increased by 150,000 and the oxygen consumption by 0.11 cc., or 0.73 cc. oxygen was consumed per liter for an increase of 1 million bacteria in 1 cc. The maximum rate of bacterial multiplication seems to have been reached about that time; the numbers later began to diminish rapidly, since, within the third day, there was a drop in numbers of 480,000; this drop was accompanied by an increase of 0.16 cc. oxygen consumption per liter. During the following two days, the bacterial numbers dropped by 173,000, while the oxygen consumption continued at the rate of 0.16 cc. per liter per day.

When glucose was added to the water, at the rate of 2.5 mgm. per liter, there was just about twice as much oxygen consumed as in control water; the rate of bacterial development was also higher, especially during the first three days of active multiplication. The consumption of 1.28 cc. of oxygen per liter of water indicates the complete decomposition of about 30 per cent of the organic matter naturally present in the water. Assuming a stable relation of the carbon and nitrogen in the water, one would expect that about 30 per cent of the nitrogen in the water, or about 75 gammas per liter, should also be made available. This amount of nitrogen is just sufficient for the complete decomposition of 2.5 mgm. of glucose, since the addition of available nitrogen had no effect in bringing about any greater decomposition of this amount of glucose; the assumption is therefor made that all the glucose is decomposed in 5 days, an assumption fully justified because of the aerobic system of the sea water and because bacterial numbers have already passed their maximum. In the presence of 2.5 mgm. of glucose, there was an increase in oxygen consumption of 1.21 cc. per liter, which is equivalent to about 1.5 mgm. of glucose; one may, therefore, conclude that only about 60 per cent of the glucose has been completely oxidized and the energy utilized for bacterial activities. The other 40 per cent of the carbohydrate had no doubt been changed largely into bacterial cell substance, as can be demonstrated by theoretical considerations: 1 mgm. of glucose contains 0.4 mgm. carbon; the carbon-nitrogen ratio of the bacterial cell is about 5:1; this would mean that the synthesized bacterial cells should require about 80 gammas of available nitrogen per liter, a figure close, if not identical, to that calculated from the decomposition of the organic matter in the water.

The above calculations are fully confirmed by the results obtained with increasing amounts of glucose. The addition of 5 mgm. of glucose per liter of water brought about the consumption of 3.58 cc. of oxygen in 5 days; by subtracting the amount of oxygen consumed in the control water, a difference of 2.3 cc. is obtained; this is just twice as much as the oxygen consumption in the decomposition of 2.5 mgm. glucose. The corresponding amount of oxygen used by the bacteria in the decomposition of 7.5 mgm. glucose was 4.76; allowing for the control water, it gives 3.48 cc., or just about three times as much oxygen consumed as for 2.5 mgm. of glucose.

These data (fig. 1) are sufficient to emphasize not only the parallelism in oxygen consumption and glucose decomposition, but also that between oxygen consumption and decomposition of organic matter present in natural sea water. When only 2.5 to 7.5 mgm. glucose were added per liter of water, the oxygen did not become a limiting factor. Further addition of nitrogen had no effect in bringing about any greater oxygen consumption, which leads one to conclude, especially since bacterial numbers were already diminishing rapidly, that all the glucose in the water had been used up. If the straight line relationship is true for the decomposition of the glucose added to the water and the oxygen consumption, there is no reason why it should not hold true for the decomposition of the organic matter of the water itself and oxygen consumption. An amount of organic matter, comparable to about 2.5 mgm. of glucose, or about 50 per cent of the total organic matter in sea water, is thus found to be readily subject to decomposition by bacteria. About 60 per cent of the organic matter decomposed was completely oxidized, as shown by the amount of oxygen consumed, and about 40 per cent of it was converted into bacterial cell substance and other products of bacterial metabolism.

Similar results were obtained in other experiments, as reported previously (Waksman and Carey, 1935, fig. 4). The oxygen consumed by the bacteria in the fresh water, in 11 days, was 1.26 cc. per liter; in the case of the sinter-filtered water, only about a half as much oxygen, or 0.56 cc., was consumed. The liberation of ammonia was parallel to the consumption of oxygen and confirms the above theoretical calculations. The liberation of about 80 gammas of ammonia per liter also points to the fact that about a half of the organic matter in the fresh water is subject to decomposition, since some of the nitrogen thus made available will be reconsumed by the bacteria.

The most important and crucial question may now be raised. Since extensive bacterial multiplication can take place in sea water, when some of it is enclosed in a glass vessel and allowed to remain undisturbed, why does the same process not occur in the sea itself, under natural conditions? No definite answer can as yet be given to this question until more is known of the factors controlling and favoring bacterial development in the sea. One may suggest, however, the following hypothesis: Under natural conditions, the organic matter in the water is in a state of equilibrium between formation and decomposition; this equilibrium may be considered as dynamic rather than static. New organic complexes are continuously formed in the water as a result of the decomposition of plant and animal residues; this tends to compensate for the gradual destruction of the organic matter due to bacterial activities. The bacterial population in the sea is kept down, under natural conditions, to a certain minimum, due, on the one hand, to the consumption of the bacteria by protozoa, copepods and other marine animals and, on the other hand, to the presence in the water of "certain controlling factors" injurious to free bacterial development. When sea water is placed in a glass container, the animals rapidly die out; any modification of the other controlling factors, whether organic or inorganic in nature, is still a subject for further study. These modifications, however, are sufficient to enable the bacteria to begin to multiply rapidly and utilize the organic substances present in the water both in suspension and in solution. The relatively large amount of organic matter found in solution in sea water may, therefore, not be due merely to its absolute stability, but to the fact that the specific conditions are not favorable for rapid bacterial multiplication. When more favorable conditions are created, as in the process of constant mixing of the water, greater bacterial multiplication and greater oxygen consumption takes place; one has to compare only the activities of the bacteria in the water on George's Bank with those of the Gulf of Maine, to find a certain justification for this assumption.

The specific nature of the controlling factors which prevent more abundant bacterial multiplication in the sea itself may vary. It is known that as a result of continuous growth of bacteria in the same liquid medium, products are formed which are unfavorable to their further development. Frequently the mere aeration of the culture is sufficient to favor bacterial multiplication. A further illustration is found in the case of land soils; when a soil is brought to the laboratory and mixed, much more abundant bacterial multiplication takes place than in the undisturbed soil; after a certain period of time, a new equilibrium is established.

The bacterial activities in sea water may thus be considered to be in a state of dynamic equilibrium. Any modification of this equilibrium, the exact conditions of which are not yet understood, brings about a rise in bacterial activities; this is accompanied by a rise in the rate of decomposition of organic matter in the water. This organic matter can, therefore, not be considered as absolutely resistant to bacterial action, but its presence in the sea is merely a result of specific conditions which control the bacterial equilibrium. When these conditions are modified and bacterial development favored, the organic matter can undergo rapid decomposition.

SUMMARY

1. The amount and specific nature of the organic substances added to sea water control the abundance of the bacterial population developing in the water.

2. The decomposition of a nitrogen-free organic material, such as glucose, added to the water is controlled by the amount of available nitrogen present in the water.

3. As a result of the decomposition of the organic matter present in sea water, some of the nitrogen is liberated in an available form; this can be measured not only directly as ammonia, but also indirectly, by the extent of decomposition of glucose added to the water. Any excess of glucose remains unattacked, unless some available nitrogen is also introduced.

4. When sea water is placed in glass containers and kept under favorable conditions, a large part (25 to 50 per cent) of the organic matter in the water is decomposed within 10 to 12 days, as measured by the amount of oxygen consumed and nitrogen liberated in an available form.

5. A tentative explanation is suggested for the stability and uniformity of the organic matter in sea water, based on the specific relationship of bacteria to the animal population of the sea and to the presence, under natural conditions, of factors unfavorable to rapid bacterial development.

In carrying out these investigations, the authors were assisted by several members of the Oceanographic Institution to whom grateful acknowledgment is made here. Dr. Ancel Keys obtained the samples of water from the deep sea station and prepared the ultra filtered water; he and Mr. Davis have also made the ammonia determinations. Dr. David Kirsh and Mr. C. E. Renn assisted in obtaining the samples of water and in making the oxygen determinations.

REFERENCES

BUTTERFIELD, C. T. 1933 Sewage Works Jour., **5**, 599–622.
KROGH, A. 1934 Ecol. Monogr., **4**, 421–429, 430–439.
WAKSMAN, S. A. 1934 Ecol. Monogr., **4**, 523–529.
WAKSMAN, S. A., AND CAREY, C. L. 1935 Jour. Bact., **29**, 531–543.