ON THE OXIDATION OF ORGANIC MATTER IN MARINE SEDIMENTS BY BACTERIA

By

SELMAN A. WAKSMAN AND MARGARET HOTCHKISS

*Woods Hole Oceanographic Institution,**

New Jersey Agricultural Experiment Station

and

New York Medical College and Flower Hospital

The question as to whether the organic matter in the sea bottom is inert or is available as a nutrient for the bottom fauna and bottom-inhabiting bacteria is a problem of special interest in the cycle of life in the sea. A number of marine animals, making up the benthos or the bottom fauna, must depend upon this organic matter for their nutrition while the sea bottom also harbors an extensive bacterial population which require the energy to be derived from various nutrient elements for cell synthesis and respiration.

It has been definitely established that different marine sediments vary considerably in content of organic matter, depending on such factors as the nature of the bottom material, its distance from land, the topography of bottom, and the depth of water (*11*). Little is known as to whether this organic matter can be further decomposed once it has settled to the bottom. In his studies on the decomposition of Zostera plants in the sea, Boysen-Jensen (*4*) found that there was first a marked reduction in the nitrogen content to be followed later by a gradual increase in nitrogen. This led him to conclude that non-nitrogenous substances are decomposed to a greater extent than the nitrogenous matter by the bacteria in the sea bottom. The bottom fauna itself seemed to digest largely the non-nitrogenous bodies, such as the pentosans, while the nitrogenous compounds accumulated. The nitrogenous substances were considered to be transformed to humic compounds, which are attacked much less readily; and so the rôle of the bacteria in building up the complex organic nitrogenous compounds out of the mineralized forms of nitrogen was assumed to merit consideration. Boysen-Jensen separated the bottom sample into the "brown layer," or the uppermost 1 cm., and the lower 2 cm. layer; by digesting the sediment with pancreatic enzymes, he found that the upper layer of 120 sq. cm. surface of sea bottom contained 44–68 mg. of digestible

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nitrogen and the lower layer usually none. This method of determining how available the nitrogenous constituents of the sea bottom may be is open to criticism, because, under natural conditions, the digestion process is carried out by bacteria or by benthotic animals which will digest the carbohydrates even more readily and the small amounts of nitrogen consumed by these organisms would be built up into their cell bodies rather than be liberated in a mineralized form; a fact which was recognized by Boysen-Jensen.

From the bacteriological point of view, it is important first of all to determine to what extent bacteria are able to attack the humus complexes in the bottom material and how rapidly the essential nutritive elements, especially the nitrogen, are made again available for plant synthesis. The ratio of carbon to nitrogen in the marine humus has been found (*4*, *11*) to be more or less constant, varying from 8:1 to 12:1. Under purely artificial conditions of decomposition, for instance when marine mud was air dried, remoistened and placed in a glass vessel, it was found (*11*) that 150 gm. of mud gave off, in 14 days, 55.8 mg. of CO_2 as carbon and 11.1 mg. of ammonia as nitrogen; this showed that the organic matter itself was not absolutely inert, but could be gradually, even if only very slowly, decomposed.

The above experiments carried out by the use of different methods, although artificial in nature, pointed definitely to the fact that the organic matter in the marine bottom is not absolutely inert but can be slowly made available. It remains to be determined to what extent this organic matter can undergo decomposition in a sea water system, by the natural bacterial population of the sea, and how this process affects the important elements carbon and nitrogen in the organic matter of the sea bottom.

Among the different methods used in studying the decomposition of small amounts of organic matter in sea water, the consumption of oxygen has been found to be most convenient (*12*). Therefore, it might be possible to utilize the rate of oxidation of the organic matter in the sea bottom as a measure of its availability. The application of this method to the study of certain types of bottom material, notably muds, is complicated by the fact that these can also absorb oxygen chemically. Moore, for example, observed (*9*) that there was an increase in the amount of immediate chemical absorption of oxygen by marine mud, to a depth of 5 cm.; but below that depth the chemical absorption of the oxygen became constant and amounted to 0.3–0.5 mg. of oxygen per 1 gm. of dry mud. He further stated that the biological oxygen absorption was slower, although no data were given to substantiate this. ZoBell and Anderson (*15*) reported that 1 gm. of dry mud absorbed from sea water 0.2–4.8 mg. oxygen in 6 hours, but biological absorption of oxygen continued at a slower rate almost indefinitely; both chemical and biological oxygen absorption were found to be much greater in the surface layers of mud than in the deeper layers. No details were

given concerning the method for determining the biological absorption of oxygen, while in both contributions even the chemical absorption was only vaguely defined and described.

Various studies are on record concerning the absorption of oxygen by bottom material in lakes and in other bodies of water. Alsterberg (*2*) suggested that the disappearance of oxygen from the depth of a lake is due to the presence of easily oxidizable organic substances, often planktogenic in nature, in the bottom material. Kusnetzow (*7*) explained the disappearance of oxygen from the deeper portion of a lake as a result of the activities of the bacteria oxidizing methane, which had its origin in the anaerobic decomposition of the organic matter in the bottom material. He further reported (*8*) that the oxygen changes in a lake could be used as a measure of the intensity of the microbiological processes taking place in the bottom material.

Methods. The measurement of oxygen consumption has been of value in previous biological studies from this laboratory and it was decided that this method should be adapted to the present problem. In brief, this method consisted in the addition of the material to be studied to identical glass stoppered bottles which are incubated, usually submerged, at a predetermined temperature. At definite incubation times duplicate bottles were removed, the oxygen determined by the Winkler method, and the results expressed in cubic centimeters of oxygen per bottle. The bottles used in this work contained 225 cc. of liquid.

In attempting to use this method for the oxidation of marine bottom material, the method had to be variously modified: (1) It was necessary to compare the specific behavior of different types of bottom material. (2) It was desirable that the water in which the mud was suspended should be relatively inert and not exert too great an influence on the oxygen balance. (3) It was essential to determine how great an effect on oxygen consumption was exerted by the non-biological constituents of the bottom material. (4) The Winkler method being indirect, it was of prime importance to make sure that the results obtained were not due to chemical interactions of the reagents and the samples used.

The bottom material was obtained in a sterile glass tube which was lowered in a brass tube surrounded by a heavy weight; this is similar to the corer employed by Moore and Neill (*10*). The glass tubes with the mud cores were stoppered at each end with sterile rubber stoppers, kept on ice on the ship, and used as soon as possible after their return to the laboratory. The cores were carefully separated into two layers; the surface, brownish, freshly sedimented layer designated as the A horizon, and the sub-surface darker layer or B horizon. Quantities of mud varying from 0.1 to 0.5 gm., on a dry basis, were used; while for the sand 2 to 5 gm. portions were added to the 225 cc. bottles. The dry content of the material was calculated from

a separate aliquot sample taken for moisture determination. The final results were recorded on the basis of one gram portions of dry material.

Although it was possible to use sea water newly procured as a menstruum in which to suspend the mud, the rapid oxygen consumption resulting from the oxidation of the organic matter in such sea water (*12*) might have been confused with the oxygen consumption due to the added bottom material. The sea water menstruum was therefore stabilized by ageing. This was accomplished by securing a quantity of water, taken from Woods Hole Harbor at a point distant from the pier, filtering it through a fine silk net (#20) and placing it in 18 liter glass bottles in the laboratory for at least 5 days. Before use the water was aerated to re-saturate with oxygen.

A series of experiments with sterilized and unsterilized material showed that there was very little oxygen consumption under aseptic conditions and gave ground for the belief that "chemical" oxidation was of little significance as compared with "biological." As a consequence, the containers with mud and water were placed under conditions favorable to bacterial activity. Room temperature was used, and oxygen determinations were made at the start and throughout the duration of the experiment, which was usually 5 to 15 days. When bacterial numbers were determined, by the plate method, a sample of water was usually taken above the settled bottom material.

After a few determinations had been made by adding the Winkler reagents directly to the bottles of water and mud or water and sand, it was apparent that the oxygen values obtained reflected an oxygen consumption in excess of the probable biological consumption. This was because the mud particles reacted with the iodine liberated. To eliminate this interference, the supernatant liquid was obtained by siphoning into smaller oxygen bottles (130 cc. capacity) and these were used for the oxygen determinations. It was found that siphoning did not interfere with the oxygen content of the water. The supernatant liquid from above the mud always gave a higher oxygen content than similar material which had not been freed of mud. The difference was sometimes as little as 0.03 cc. oxygen or as great as 0.19 cc. oxygen per bottle.

Experimental. In the course of the development of a standard method to be used in handling fresh bottom materials, data concerning several factors of interest in laboratory experiments on decomposition were gradually collected.

One of these preliminary experiments showed the extent of oxygen consumption which might be expected with dried material. Two dried mud samples, which had been previously obtained (*11*) from *Atlantis* stations 1329 and 1331 and found to contain 2.45 and 1.46 per cent organic carbon and 0.285 and 0.143 per cent nitrogen respectively, were studied. The oxygen consumption during 54 hours was compared with that of dry sand

bottom from George's Bank containing 0.4 per cent carbon and 0.045 per cent nitrogen. The organic matter of both mud and sand was oxidized, although not very rapidly (Table I). In the case of sample 1329, 0.5 gm. of dry mud consumed 0.14 cc. oxygen, while 2 gm. of mud consumed 0.32 cc. oxygen in 54 hours. The corresponding amounts of oxygen consumed by mud 1331 were 0.12 and 0.32 cc. Although there was a difference in the

TABLE I

OXIDATION OF DRY MARINE MUD AND SAND IN SEA WATER

Oxygen content of water, cc. per bottle

Mud Material	*Amount used*[1]	*Incubation, hours*			
	gm.	*0*	*6*	*30*	*54*
Water alone	—	1.21	1.19	1.16	1.15
1329 mud	0.5	1.20	1.18	1.10	1.01
1329 mud	2.0	1.14	1.13	0.99	0.83
1331 mud	0.5	1.18	1.16	1.09	1.03
1331 mud	2.0	1.12	1.12	0.96	0.83
Sand	2.0	1.20	1.20	1.08	0.99
Sand	5.0	1.24[2]	1.21	0.97	0.84

[1] Per 225 cc. oxygen bottle.

[2] Higher oxygen content above the control is due to a few small bubbles of air remaining in the sand.

TABLE II

INFLUENCE OF STERILIZATION UPON OXYGEN CONSUMPTION BY MARINE BOTTOM MATERIAL

Material[1]	*Oxygen, cc. per bottle*				*Bacterial control after 120 hours*
	Start 1 hr.	*18 hrs.*	*42 hrs.*	*120 hrs.*	
Standard sea water	1.07	1.08	1.07	—	—
+ 2 gm. mud 1331	0.99	0.96	0.92	—	—
Standard sea water	0.94	0.94	0.94	0.94	0
+ 3.75 gm. Gay Head sand	0.91	0.92	0.91	0.91	0
+ 1.47 sandy mud	0.94	0.92	0.91	0.88	0
+ 0.47 gm. mud	0.87	0.79	0.76	0.65	+

[1] Amounts calculated on dry basis.

organic matter content of the two muds, as shown by the carbon contents, there was very little difference in the rate of oxygen consumption. In the case of sand, however, much less oxygen was consumed: 2 gm. of sand absorbed, in 54 hours, 0.16 cc. oxygen; 5 gm. absorbed 0.31 cc.

While experiments under aseptic conditions showed that sterilized material absorbed only a very small amount of oxygen from the water (Table II), the biological oxygen absorption by the same samples presented a different picture. This is shown graphically in Figure 34. The sand used was from 22 meters depth, 1.5 miles W. by N. from Gay Head (Marthas

Vineyard); it had 24.9 per cent moisture. The sandy mud was obtained from a depth of 37 meters, near Buoy No. 4 LB, 7 miles S.W. of Gay Head; it had 26.4 per cent moisture. The mud came from Buzzards Bay, 1 mile N.E. of Gong 6; it was at a depth of 16 feet and had a moisture content of 71.0 per cent. Two gram portions of the moist sand and 0.5 gm. portions of the moist sandy mud and mud were used per 225 cc. oxygen bottles. Calculations were made on the basis of one gram of dry material. In thirty one days, 1 gm. of the sandy bottom containing 0.8% organic matter (calculated from the nitrogen content) absorbed 0.44 cc. oxygen; the sandy

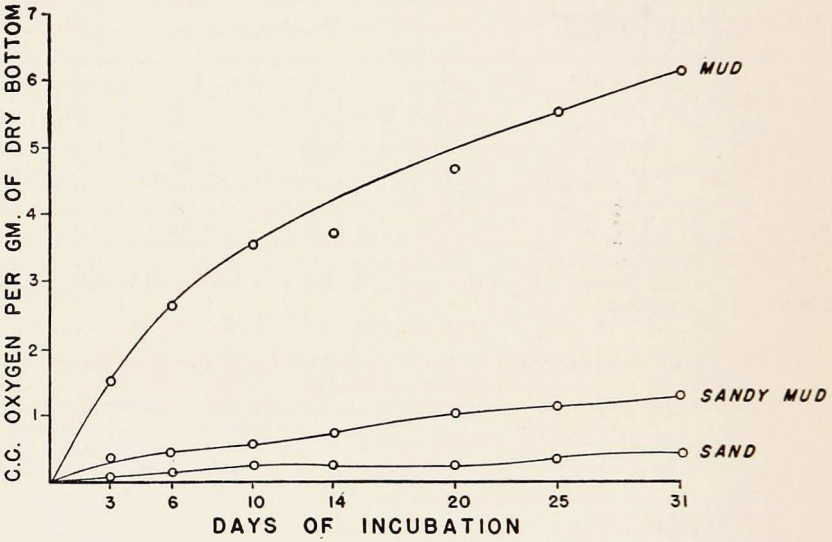

Figure 34. Course of oxygen consumption by different types of bottom material.

mud bottom containing 1.46% organic matter absorbed 1.31 cc. oxygen per gram; while the mud bottom with 5.82% organic matter absorbed 6.14 cc. oxygen per gram. The increases in oxygen consumption were greater than the increases in the organic matter content of the bottom material. This is shown by the ratio of oxygen in cc. to organic matter in mg., which was found to be 0.055 for the sand, 0.09 for the sandy mud, and 0.106 for the mud. The higher value for mud bottom points to the greater availability of its organic matter. Thus the degree of oxidation seems to depend on the presence of biological agents and the nature of the organic matter.

Some idea of the effect of different types of organic matter may be gained from a report on the experiments on oxygen absorption by material from different portions of the same core. Fresh cores of mud were obtained about

4 miles west by north of Gay Head. These cores were divided into 3 sections: 0–1 cm., 1–5 cm., and 5–20 cm. The moisture content of these sections of mud was 45.8, 33.7, and 26.7 per cent, respectively. One gram portions of the surface layer and 2 gm. portions of the lower layers of the fresh mud were added to a series of oxygen bottles; fresh sea water was used and the bottles were incubated for three days. The results presented in the following summary show that the surface layer of mud was somewhat more active biologically than the deeper layers, and this layer had the higher organic content.

Depth of layer cm.	*Mud used, dry basis gm.*	*Oxygen absorbed in 3 days cc.*	*Oxygen absorbed per 1 gm. of dry mud cc.*	*Nitrogen content of dry mud per cent*
0–1	0.54	0.17	0.315	0.095
1–5	1.32	0.35	0.265	0.087
5–20	1.47	0.40	0.272	0.070

In order to establish the relation of bacterial development to the oxidation of organic matter in the mud, the results of the following experiment may be cited. A mud core obtained in Buzzards Bay, near Weepecket Buoy, was divided into 3 sections. The upper layer of mud was suspended in some of the surface water and 5 cc. portions pipetted into a series of oxygen bottles; in the case of the other two layers 2 gm. portions of the moist material were used. The sea water menstruum was 24 hours old. There was a marked increase in bacterial activities in the water receiving marine mud. The surface layer of the mud was again the most active biologically. (Table III.)

TABLE III

BACTERIAL MULTIPLICATION AND OXYGEN CONSUMPTION OF DIFFERENT LAYERS OF A MARINE MUD PROFILE

Material used[1]	*0 days*		*1 day*		*5 days*		*Oxygen consumed per 1 gm. of dry mud*
	Bacteria in 1 cc.	*Oxygen, cc. per bottle*	*Bacteria in 1 cc.*	*Oxygen, cc. per bottle*	*Bacteria in 1 cc.*	*Oxygen, cc. per bottle*	
Standard sea water control	9,000	1.11	30,500	1.08	1,400	1.00	—
Standard sea water + 25 gm. dry mud 0–2 cm. deep[1]	4,000	0.95	83,000	0.74	19,800	0.49	1.84
Standard sea water + 1.05 gm. dry mud 2–6 cm. deep	7,500	0.80	114,500	0.68	15,800	0.31	0.47
Standard sea water + 1.05 gm. dry mud 6–10 cm. deep	5,500	0.85	116,000	0.69	9,700	0.19	0.63

[1] Nitrogen content of dry material, 0.146% for 0–2 cm., 0.180% for 2–6 cm., and 0.138% for 6–10 cm. layer.

A detailed study was made of the biological oxygen absorption by the A and B layers of a freshly taken mud profile. The position of the Station was off Gay Head. The A layer comprised the upper 2 cm. of mud and the B layer the lower 3 cm. (2–5 cm. depth). The nitrogen content of the mud was, on a dry basis, 0.176% for the A layer and 0.063 for the B layer. Cultured sea water was used and the oxygen determinations made in siphoned water. Different amounts of mud were added to the bottles. The curves presented in Fig. 35 are based upon the averages of the results obtained on the basis of 1 gm. portion of dry mud. The results again prove that the upper layer of mud exerts a greater oxygen absorption than the

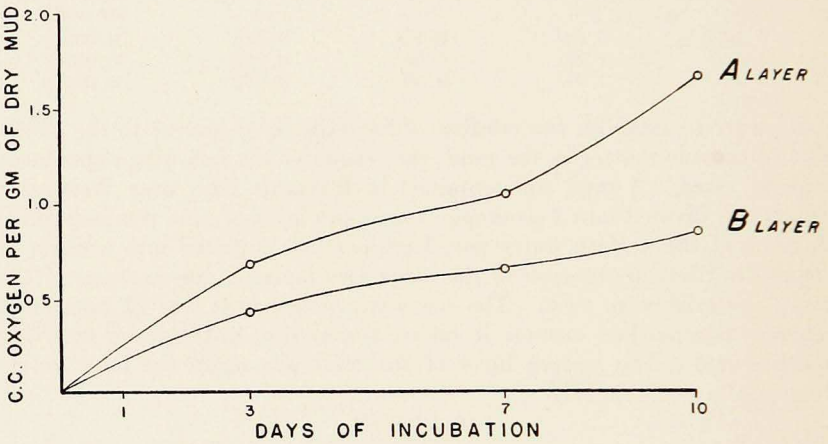

Figure 35. Course of oxygen absorption by surface (A) and sub-surface (B) layers of a Gay Head mud core.

lower layer and that the process, although slow, is gradual and continuous. The greater oxygen absorption by the mud from the upper layer may be due, in this case as well, to the greater organic matter content.

In order to demonstrate further the biological nature of the oxidation process taking place in marine bottom material added to sea water, a study was made of the influence of temperature upon oxygen consumption. Cultured water, 3 weeks old, and fresh mud taken off the coast of Cape Cod, in line of Provincetown were used. This mud contained 0.234% nitrogen, on a dry basis. Five cc. portions of a suspension of mud in sea water, equivalent to 0.26 gm. of dry mud, were added to a series of oxygen bottles. These were filled with cultured sea water and incubated, under water, at 3 different temperatures: the lowest, or refrigerator temperature, ranged, after the first 24 hours, from 2° to 6° C., with an average of 4° C.;

the medium or room temperature, ranged from 21° C. to 25° C., average 23° C.; the highest, or incubator temperature, was quite constant, after 48 hours, ranging from 31.0° C. to 31.9° C., averaging 31.5° C.

The results (Table IV) show the increase in oxygen consumption effected by the higher temperature of incubation, at which temperature, there was comparatively little oxygen consumed after 10 days and very few bacteria left in the water after 15 days. At the low temperature, there was an extensive population of bacteria in the water containing the mud, but these bacteria had a low oxygen absorbing capacity.

TABLE IV

INFLUENCE OF TEMPERATURE UPON THE OXYGEN CONSUMPTION OF MARINE MUD

Temperature of incubation	*Period of incubation*	*Oxygen consumed, cc.*			*Bacteria in 1 cc. water (+ mud)*
		per bottle		*per 1 gm. of dry mud*	
		water alone	*water + mud*		
°C.	*days*				
4° C.	5	0	0.05	0.19	528,000
4° C.	10	—	0.15	0.48	—
4° C.	15	0.05	0.21	0.62	49,200
23° C.	5	0.04	0.24	0.77	182,000
23° C.	10	—	0.34	1.00	—
23° C.	15	0.12	0.47	1.35	4,200
31.5° C.	5	0.10	0.33	0.89	382,000
31.5° C.	10	—	0.49	1.49	—
31.5° C.	15	0.11	0.52	1.58	<100

It was found in this, as well as in other experiments, that when a rise in bacterial numbers accompanying active oxidation of organic matter in water or in bottom material is followed by a rapid drop in numbers, oxygen consumption continues, although at a slower rate, in the presence of the few bacteria capable of developing on an agar plate. Similar observations have been made by Allgeier, Peterson, Juday and Birge (*1*) who found, in the case of lake sediments, that after gas evolution had reached a maximum, during the anaerobic decomposition of these sediments, bacterial numbers diminished rapidly; this drop was not accompanied by any marked decrease in gas evolution. This continued oxidation, after bacterial numbers have been markedly reduced, may be due to the continued action of bacterial enzymes or activating factors, as suggested by Kreps (*3*), who emphasized that bottom deposits are particularly rich in such enzymes. It may also be due to the controlling effect upon the bacterial population by the animal population in the water, as suggested elsewhere (*13*). Of interest in this connection is the work of Butterfield (*6*) who found that in the presence of certain protozoa, a decline in bacteria took place, not accompanied, however, by a diminution in the oxygen consumption in the water.

What is the greatest oxygen consumption which can be obtained with fresh marine mud under optimum laboratory conditions? It seemed possible to determine this if a new supply of aerated water was used to replace the sample removed by siphon for oxygen determination. A series of oxygen bottles received 1.0, 2.5, and 5.0 gm. portions of fresh marine mud. One series of bottles was placed in the dark under water, in an undisturbed state, while the bottles in the other series were shaken for a half minute, by hand, several times daily. At intervals of 1 to 4 days, the supernatent clear water was siphoned off and analyzed for oxygen. The bottles were immediately refilled with fresh cultured water, which had been saturated

TABLE V

MAXIMUM OXYGEN CONSUMPTION OF FRESH MARINE MUD UNDISTURBED AND RE-SUSPENDED

Incubation days	*Oxygen consumed per bottle*						*Bacteria in 1 cc. of water*[3]	
	Bottles shaken			*Bottles unshaken*				
Amount of mud used per bottle[1]	*1 gm.*	*2.5 gm.*	*5 gm.*	*1 gm.*	*2.5 gm.*	*5 gm.*	*0*	*1 gm.*
1	0.53	0.79	1.08	0.30	0.43	0.51	51,200	279,000
3	0.51	0.76	0.95	0.29	0.32	0.32	51,900	89,500
5	0.24	0.49	0.57	0.31	0.48	0.49	—	—
10	0.42	1.12	1.30	0.64	1.26	1.51	56,900	161,000
15	0.14	0.65	0.74	0.28	0.70	0.88	—	—
22	0.18	1.08	1.09	0.27	1.01	1.51	12,500	12,000
30	0.03[2]	0.60	1.41	0.09	0.87	1.55		
Total	2.05[2]	5.49	7.14	2.18	5.09	6.77		

[1] Moisture content of fresh mud 47%.
[2] 26 days incubation.
[3] In supernatant water above the mud.

with oxygen, and incubated longer. The mud was obtained from Buzzards Bay, near Weepecket and contained 47 per cent moisture. The nitrogen content of the dry mud was 0.210%. The results reported in Table V represent the differences between the water controls and the water receiving the different amounts of mud. The total oxygen consumption per 1 gm. of dry mud, for a period of 28–30 days, was found to decrease with the amount of mud used; it amounted to 4.0 cc. when 1 gm. of moist mud was used per bottle; 3.99 cc. for 2.5 gm. mud, and 2.62 cc. for 5.0 gm. mud. Although the mud exerted a certain adsorptive effect upon the bacteria in the water, the numbers of bacteria were usually much higher in the water with mud than in the water alone.

A detailed study was now made of the oxidation of various types of bottom material collected from different depths of the sea and at different distances from land. Usually not over 7 days elapsed before taking the

samples and subjecting them to oxidation in cultured sea water. Soon after the cores were obtained, they were placed, in an undisturbed position on ice. When brought to the laboratory, the cores were removed aseptically from the glass tubes, and divided into two portions: the upper 1 to 4 cm. or A layer and the lower or B layer, the latter varying from 4 to 25 cm. in thickness. Aliquot portions of the bottom material were placed in oxygen bottles; these were filled with cultured water, 7–15 days old, and the bottles incubated at room temperature in the dark and under water. Usually 5 bottles were employed for each sample: one bottle was analyzed immediately after the sediment had settled for 1–2 hours; 2 bottles were analyzed after

TABLE VI

OXYGEN CONSUMPTION BY BOTTOM MATERIAL IN THE NEIGHBORHOOD OF WOODS HOLE

On basis of dry material

Sta. No.	*Position of station*	*Depth of station*	*Layer*	*Nitrogen*	*Oxygen consumed, cc. per 1 gm. in 4 days*
		meters		%	
1	Gay Head, 5¼ mile W by S	36.0	A	0.086	0.42
		36.0	B	0.070	0.29
2	Gay Head, ½ mile SE of Sta. 1	38.0	A	0.080	—
		38.0	B	0.067	0.18
3	Buzzards Bay 2 miles WNW of Weepecket Rock	15.7	A	0.257	1.10
		15.7	B	0.221	0.80
4	Same as 3, 1 mile NWW of Weepecket Rock	16.0	A	0.227	1.14
		16.0	B	0.182	0.93
5	Same as 3, 1/3 mile N of Weepecket Rock	15.3	A	0.255	0.82
		15.3	B	0.222	0.87
6	Same as 3, 1 mile NE of Gong 6	16.0	A	0.259	1.04
		16.0	B	0.222	0.94
7	Same as 3, Buoy 4LB	37.0	A	0.085	0.13
		37.0	B	0.048	0.05

4–7 days and the other two after 8–15 days; only the results of the final analyses are reported in the tables.

In the first experiment (Table VI), a group of bottom samples were collected from stations in the neighborhood of Woods Hole; the water was 7 days old, and the oxidation period only 4 days. In making the calculations, allowance was made for the chemical oxidation or oxygen consumption within 1 to 2 hours, and oxygen consumption by the water controls. The results reported in this and other tables thus represent the biological oxygen consumption due to the oxidation of the bottom material in the water; they are reported on the basis of 1 gm. of dry material. A marked correlation was found between oxygen consumption and organic matter content of the bottom material. The cores from stations 3–6 are higher in organic matter and show also more active oxygen consumption. By comparing the

average organic matter content of these stations with those containing less organic matter, using the results for the two layers, one finds that the organic matter in the former oxidizes more readily than in the latter.

Station Nos.	*Organic matter content* %	*Oxygen consumed, cc. per liter*	*cc. of oxygen / mg. of organic matter*
3–6	4.50	0.95	0.0210
1, 2, 7	1.45	0.21	0.0145

In the following two experiments, bottom samples were obtained at varying distances from land. In the first Atlantis cruise, the depths of the stations ranged from 104 meters to 1,250 meters. With the exception of the shallow station, coming from a depth of 104 meters, the organic matter from these bottom samples showed comparatively little oxygen consumption (Table VII). Although the organic matter content of these bottom samples was about 2 to 3 per cent, as calculated from the total nitrogen, there was only 0.07–0.27 cc. oxygen consumed per 1 gm. of dry material, in 14 days. These results suggest that the organic matter in the sea bottom at greater distances from land has a considerable resistance to decomposition. This was found to hold true of the oxidation of the organic matter in the bottom samples obtained on the second cruise of the Atlantis, when the samples came from even greater depths. The highest oxidation was 0.59 cc. per 1 gm. of dry bottom material, although this material contained 2.5 per cent organic matter. These results thus point definitely to the lower oxidizability of the organic matter in the deep sea bottoms.

A comparison of the data for the deeper stations, presented in Tables VII and VIII with those of the shallower stations (Table VI) points to another interesting difference: in the case of the bottom samples close to land, the upper layer of the bottom material gave greater oxygen consumption than the lower layer; however, in the case of the deeper stations (7, 8, 9 in Table VIII), the lower layer of bottom material frequently showed as great if not greater oxygen consumption than the upper layer.

In order to study further the oxidizability of the organic matter in sea bottom not far distant from land 3 groups of stations were selected as representing characteristic differences in depth and position. One group (stations 1–8) was obtained in line of the Isles of Shoals; the other group (stations 9–13) came from the 99 fathom hole in Massachusetts Bay, and the third group (stations 14–17) from a line off Provincetown. The results, presented in Table IX, show that the consumption of oxygen or the decomposibility of these bottom samples was greater than in the case of the bottom material which had been obtained at greater distances from land. There was also a certain definite correlation between the rate of oxidation and the depth or position of the bottom material which is brought out in

the group of samples from the 99 fathom hole (stations 9–13). In this hole the deeper stations gave higher organic matter content and the oxygen consumption by the bottom material was greater. A certain correlation

TABLE VII

OXYGEN CONSUMPTION BY BOTTOM SAMPLES FROM FIRST "ATLANTIS" CRUISE

On basis of dry material

Sta. No.	*Position of station*	*Depth of station*	*Layer*	*Nitrogen*	*Oxygen consumed, cc. per 1 gm. in 14 days*
	W Lat. N Long.	*meters*		%	
1	40.04′ x 70.50′	104	A	0.191	0.63
		104	B	0.154	0.65
2	39.56′ x 70.49′	175	A	0.046	0.10
		175	B	0.048	0.07
3	39.52′ x 70.48′	600	A	0.105	0.13
		600	B	0.087	0.15
4	39.50′ x 70.46′	914	A	0.106	0.07
		914	B	0.102	0.08
5	39.48′ x 70.47′	1,250	A	0.154	0.27
		1,250	B	0.139	0.17

TABLE VIII

OXYGEN CONSUMPTION BY BOTTOM SAMPLES OBTAINED IN SECOND "ATLANTIS" CRUISE

On basis of dry material

Sta. No.	*Position of station*	*Depth of station*	*Layer*	*Nitrogen*	*Oxygen consumed, cc. per 1 gm. in 15 days*
	W Lat. N Long.	*meters*		%	
6	40.14′ x 67.46′	1,134	A	0.030	0.31
		1,134	B	0.040	0.08
7	40.13′ x 67.39′	1,609	A	0.130	0.26
		1,609	B	0.112	0.41
8	40.09′ x 67.23′	2,100	A	0.133	0.39
		2,100	B	0.124	0.59
9	40.04′ x 66.67′	2,967	A	0.118	0.27
		2,967	B	0.089	0.34
10	40.25′ x 66.00′	4,000	B	0.034	0.11

was also found between the amount of organic matter in the bottom material and the rate of its oxidation.

In all the above experiments, the water was tested, at the end of the oxidation period, for ammonia, nitrite and nitrate.[1] In no case could these be detected save in mere traces. This might have been due to the fact that the incubation period of 14–15 days was not sufficient for the liberation of the nitrogen in appreciable or detectable amounts in the decomposition of

[1] The authors are indebted to Dr. Cornelia L. Carey for making these tests.

the mud. However, it emphasizes the fact that the nitrogen in the marine bottom is highly resistant and is not readily mineralized by the bacteria. Once the organic residues have settled to the bottom, the nitrogen has become so closely bound in an organic form as to be practically immune to microbial attack. Quite the opposite occurred in experiments on the decomposition of marine plankton (*5*, *14*).

In one experiment (Table V), the mud was analyzed for total nitrogen at the beginning and at the end of the decomposition period. There was an increase, on the basis of the dry mud, from 0.210% nitrogen in the fresh

TABLE IX

OXYGEN CONSUMPTION BY BOTTOM MATERIAL FROM A LINE OF STATIONS OFF MASSACHUSETTS BAY, ISLE OF SHOALS AND CAPE COD

Sta. No.	*Position*	*Depth*	*Layer*	*Nitrogen*	*Oxygen consumed, cc. per 1 gm. in 14 days*
	W Lat. N Long.	*meters*		%	
1	42°57′ x 70°31′	83	A	0.193	1.54
		83	B	0.139	0.94
2	42°57′ x 70°27′	100	A	0.209	1.55
		100	B	0.173	1.49
3	42°57′ x 70°25′	100	A	0.138	0.97
		100	B	0.048	0.46
4	42°57′ x 70°22′	132	A	0.252	1.19
		132	B	0.192	2.02
5	42°57′ x 70°20′	157	A	0.291	1.23
		157	B	0.254	1.36
6	42°57′ x 70°18′	161	A	0.306	1.00
		161	B	0.272	1.24
7[1]	42°59′ x 70°15′	155	A	0.288	1.59
		155	B	0.227	1.32
8[2]	42°59′ x 70°12′	175	A	0.326	1.86
		175	B	0.278	1.06
9[3]	42°30′ x 70°18′	90	A	0.081	0.06
10	42°30′ x 70°18′	145	A	0.179	0.87
		145	B	0.051	0.47
11	42°30′ x 70°18′	170	A	0.162	0.83
		170	B	0.176	1.88
12	42°30′ x 70°18′	170	A	0.245	1.33
		170	B	0.211	1.45
13	42°30′ x 70°18′	175	A	0.245	1.71
		175	B	0.228	0.94
14	42°00′ x 69°46′	150	A	0.081	0.62
		150	B	0.047	0.72
15	41°59′ x 69°41′	195	A	0.260	1.41
		195	B	0.261	1.43
16[4]	42°01′ x 69°38′	210	A	0.233	1.11
		210	B	0.234	1.46
17[5]	42°01′ x 69°43′	165–170	A	0.226	1.04
		165–170	B	0.204	1.23

[1] Atlantis Station 2648.
[2] Atlantis Station 2654.
[3] Stations 9–13 are from 99 fathom hole in Massachusetts Bay and close to it.
[4] Atlantis Sta. 2645.
[5] Atlantis Sta. 2646.

mud to 0.233% nitrogen[1] in the mud left after 30 days' oxidation. If one is to assume that this increase in the nitrogen content of the mud is accompanied by a narrowing in the carbon-nitrogen ratio and, therefore, by a corresponding decrease in the carbon content, the amount of carbon lost by oxidation would correspond roughly to 2.3 mg. carbon per 1 gm. of dry mud oxidized. The amount of oxygen theoretically required for the oxidation of this carbon would be 4.29 cc. The actual oxygen consumption varied from 2.62 to 4.00 cc. oxygen, depending on the amount of mud inoculum, as shown above. This brings added proof that the nitrogen was not liberated in a mineralized form since it could be accounted for almost quantitatively in the residual mud.

DISCUSSION

A knowledge of the abundance, chemical nature and rapidity of decomposition of the organic matter in the sea bottom represents a number of significant theoretical and practical problems. Among these, the question of the influence of the nature of the organic matter upon the occurrence of bottom-feeding or benthotic animals, as well as the decomposition of the organic matter by bacteria and the return of the elements into circulation are not the least important. To what extent does the content of organic matter in the sea bottom influence the abundance of bottom feeding animals? Is the occurrence of the latter connected with the amount of organic matter or with the nature of this organic matter? Is the organic matter subject to decomposition or oxidation by bacteria, and how can this best be measured?

The determination of the organic matter content in the sea bottom represents little difficulty. It can be done roughly by measuring the loss on ignition, but more accurately by an analysis of the organic carbon or the total nitrogen content. Since the ratio between these two elements is more or less constant in the marine organic matter or marine humus, the determination of one of these two elements may be sufficient as a measure of the abundance of humus. The carbon can be multiplied roughly by 2 and nitrogen by 20, to give the total organic matter in the bottom material. A knowledge of the nature of the organic matter represents, however, a much more difficult problem. Chemical analyses, as attempted by one of us (*11*) or hydrolysis by means of proteolytic enzymes, as attempted by Boysen-Jensen (*4*), are either too complicated or are not comparable with marine processes. The measurement of the rapidity of oxidation of the bottom material, under conditions similar to those taking place in nature, namely in a sea water system with favorable oxygen tension, offers a much simpler method for comparing different types of marine bottom material. The oxidation of the organic matter in sea water was found to be a convenient

[1] The 3 samples of mud contained 0.230%, 0.238%, and 0.232% nitrogen.

method for measuring the relative availability or stability of this organic matter.

Three of the stations from which material was collected for the present investigation have also been used the year previously in a study made by Dr. Hjort and Dr. Bigelow concerning the relation of sea bottom to the occurrence of shrimps. It is of interest to compare, for these 3 stations at least, the abundance of organic matter, the rate of oxidation and the occurrence of shrimps:

Sta. No.	*Shrimp catch (1936)*	*Nitrogen content of mud bottom, dry basis, per cent*		*Oxygen consumption cc./1 gm. in 14 days*
		(1936)	*(1937)*	
	qts.	*A layer*		
2645	1.5	0.217	0.233	1.11
2648	16.0	0.295	—	1.59
2654	180.0	0.309	0.326	1.86

The above results show a very interesting correlation between the occurrence of shrimps and the rapidity of oxidation of the organic matter in the bottom material. The number of samples used for this comparison is too small, to justify any broad conclusions; but the results are highly suggestive.

Of particular interest in connection with these studies is the fact that the organic matter in the sea bottom is oxidized only very slowly by bacteria. It was found that 1 mg. of organic matter consumed only 0.1 cc. oxygen, when allowed to oxidize, under optimum conditions in the laboratory, for a period of 15 to 30 days. Assuming 50 per cent carbon in the organic matter, only about 10 per cent is found to become oxidized in this period of time. When this is compared with the rapid decomposition of the plankton material (*5, 14*), the differences are particularly striking. This points to great resistance of the organic matter in the sea bottom to decomposition.

Even more important than the limited decomposition of the organic matter is the fact that the nitrogen is not mineralized at all in the period of time used, namely in 15–30 days. The oxidation process is thus found to take place at the expense of the non-nitrogenous organic complexes in the bottom material. The fact that this organic matter is not uniform in composition, but is made up of a number of organic compounds has been demonstrated previously (*11*).

Of further significance is the fact that the rate of oxidation of the organic matter from bottom deposits nearer to land and from shallower basins is considerably greater than that from bottoms at greater depths and distances. The organic matter in these bottoms is much more resistant, the oxidizability being only one-tenth or one-fifth of that of the organic matter in shallower bottoms near land. This suggests that the organic matter of the latter is more "aged" or has had time to become more highly oxidized.

SUMMARY

1. A study has been made of the rate of oxidation of the organic matter in the sea bottom, as influenced by its abundance, nature of bottom material, distance from land and depth of bottom.

2. A convenient procedure was developed for this study. It consisted in placing definite amounts of fresh bottom material in oxygen bottles containing a known volume of "cultured" water (water kept for several days in the laboratory and resaturated with oxygen). The bottles were incubated for 7–15 days or longer; the clear water was then siphoned into smaller oxygen bottles and the amount of oxygen measured. The results have been calculated on the basis of 1 gm. of dry material. Comparisons were made between the organic matter content, as calculated from the total carbon or nitrogen in the material, and the amount of oxygen consumed.

3. Sandy bottoms were found to consume very little oxygen chemically, while mud bottoms consumed larger amounts. The biological consumption of oxygen by different types of bottom material was considerably greater than chemical absorption.

4. By allowing oxidation of the organic matter to proceed for 15–30 days, it was found that 1 milligram of organic matter in bottoms close to land would consume about 0.1 cc. of oxygen, under the most favorable conditions.

5. The organic matter in sea bottom at greater depths was oxidized to a much lesser extent than the organic matter in shallower bottoms, nearer to land.

6. The organic matter in the sea bottom was found to be much more resistant to biological decomposition or oxidation than had been previously determined for the organic matter in sea water or in the plankton organisms.

7. The oxidation of the organic matter, at least for 15–30 day periods, took place at the expense of the non-nitrogenous constituents; very little of the nitrogen was liberated in a mineralized form in that period of time.

8. It is suggested that the rate of oxidation of the organic matter in the bottom material be used as a measure of the availability or nature of this material.

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