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BACTERIOLOGICAL ANALYSIS OF SOME LONG CORES OF MARINE SEDIMENTS

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Until recently only the uppermost layers of marine sediments have been generally available for scientific investigations since most coring instruments obtained samples less than a meter long. The early bacteriological investigations of Certes (1884), Russell (1892), Fischer (1894), Gazert (1912), and Drew (1912) were confined to surface samples obtained by dredging. Lloyd (1931) was the first to report quantitative data on the vertical distribution of bacteria in subsurface layers of marine sediments. Similar investigations were made by Reuszer (1933), and Waksman et al. (1933). ZoBell and Anderson (1936) and ZoBell (1938) enumerated both aerobic and anaerobic bacterial populations. The longest core on which quantitative results have been published was 68 centimeters long (ZoBell and Anderson, 1936) although Waksman et al. (1933) have reported the occurrence of anaerobes down to a depth of 90 centimeters.

There are certain problems whose solutions require information on the depth to which bacteria may be present and active in marine sediments. For this reason the bacteriological analysis of as deep strata

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as possible is desirable. As an example of such problems it is sufficient to mention the controversy over the role of bacteria in petroleum genesis; certain geologists believe bacterial activity is limited to an initial period before the source material is buried deeply (White, 1935), while others believe bacteria remain active during the entire process (McCoy and Keyte, 1934).

Emery (1939) developed a heavy gravity coring tube which is a greatly enlarged modification of the Ekman (1905) mud sampler. Through the cooperation of Dr. F. P. Shepard and Dr. R. Revelle several cores of considerable length, obtained with this instrument on cruises of the "E. W. Scripps" were made available for bacteriological analysis. The results of these analyses are the subject of the present paper.

EXPERIMENTAL

The coring device consists of three principal parts: an upper iron pipe on which 200 to 500 pounds of streamline lead weights are placed; a valve to prevent loss of the core; and a lower iron pipe two inches in diameter and six to twenty feet long, the length used depending on the type of sediment expected. A special nose used on the end of the lower pipe serves to decrease the frictional force between the inside of the core tube and the mud. This instrument has taken cores up to 370 centimeters in length and has been used successfully in water over 4000 meters deep. The apparatus has actually penetrated as much as 770 centimeters into the sediment, but because of frictional forces a smaller and smaller percentage of each succeeding layer is collected as the corer moves through the mud (Emery and Dietz, 1939). Consequently the depth of the sample below the surface of the core as used in the following discussion and tables does not represent the actual depth below the surface of the sediment *in situ*. The actual depth will be greater than that reported by an amount depending in part on the ratio of the length of penetration of the core barrel to the length of core recovered, and in part on the nature of the sediment itself.

Usually the sediment was removed from the core tube immediately after being brought on board the vessel and cut into three- to five-inch sections which were stored in glass-covered pint jars. Samples for bacteriological analysis were obtained by dissecting out a radially central portion of mud from the inside of the freshly cut sections, using aseptic technique during all manipulations. These sub-samples were stored in sterile tubes or bottles in a refrigerator at 0° C. until returning to the laboratory on shore where the analyses were made. Core F 72, however, was analyzed on board immediately after it was obtained.

Ten grams of the sediments were weighed into tared dilution bottles

containing 90 cc. of sterile sea water. The bottles were then shaken vigorously for at least five minutes to obtain a uniform suspension. One-cc. portions of appropriate dilutions of the suspension were used to inoculate Petri dishes to determine the aerobic population, and oval tubes (Rittenberg et al., 1937) to determine the anaerobic population. A 1 : 100 dilution was the lowest used in making aerobic counts. If no colonies developed on either plate inoculated with this dilution the count was recorded as <50. A nutrient medium of sea water plus 0.3 per cent bacto-peptone, 0.2 per cent each of proteose-peptone and beef extract, 0.025 per cent $FeCl_3 \cdot 6H_2O$, and 1.5 per cent agar at a pH of 8.0 was used. The inoculated plates and oval tubes were incubated at 20° C. for two to four weeks before the colonies were counted.

All the samples examined, with one exception, were terrigenous deposits (Murray and Renard, 1891). According to the system of classification in use at the Scripps Institution (Revelle, 1940) these sediments are green clays or silts. Their median particle diameters vary from core to core and within individual cores, ranging from 2.5 to 44 microns. Their water contents vary with depth of burial and particle size, ranging between 30 and 70 per cent. Their organic nitrogen content falls between .10 and .32 per cent. The one pelagic deposit examined, F 72, consists of red clay overlying a very fine, dry, gray-green clay. Its median diameter ranges from 2.5 to 3.5 microns.

Bacteriologically the nine terrigenous samples roughly fall into three groups characterized respectively by high, intermediate, and low bacterial populations. This classification does not give a sharp separation because certain of the cores might be placed in either of two groups, depending on the portion of the data stressed. The terms high and low populations refer only to the ranges observed in this investigation and are used merely for convenience in discussing the data; it should not be inferred they delimit the maximum and minimum possible bacterial contents of terrigenous sediments.

Table I shows the number of bacteria demonstrated at various levels in cores of green mud of intermediate population. The number of aerobes in the topmost layer is high, ranging between one million and eight million per gram wet weight. Immediately below the surface of the mud the number of bacteria found decreases rapidly, less than 10,000 per gram being present at a depth of 20 centimeters. Very few bacteria develop from sediment layers 70 centimeters or more below the surface, the number, with one exception, being below 500 per gram. A characteristic shown by FPS 149 which is common to almost every core examined is that at all levels the aerobes are more numerous than

the anaerobes. Only 1,500 anaerobes per gram were demonstrated at the surface of FPS 149; the number found dropping to less than 500 per gram in the first 30 centimeters and remaining very low throughout the rest of the core. It is unfortunate that the anaerobic populations of the other two cores were not determined.

Table II shows the vertical distribution of bacteria in two cores of green mud of low population. The cores in Table II are separated from those in Table I only because of the large differences in the aerobic population of the surface layers. In the deeper portions the numbers and vertical distribution of bacteria in the two groups of cores are quite similar.

The results of the counts on sediments of high population are given in Tables III and IV. In these cores the numbers of aerobes and anaerobes in both the surface and subsurface layers are considerably higher than in the cores discussed above. At the surface the counts are as much as tenfold greater and in the lower layers there are differences up to ten thousandfold. Core FPS 253 might have been included in the intermediate group but because of the large number of bacteria at the surface it is also considered a sediment of high population. It

TABLE I

NUMBER OF AEROBIC AND ANAEROBIC BACTERIA PER GRAM OF WET SEDIMENT DEMONSTRATED IN DIFFERENT STRATA OF CORES OF GREEN MUD OF INTERMEDIATE POPULATION

Sample	FPS 149		39-C-39		39-C-40A	
Location of station	32° 36.4′ N. 117° 27.8′ W.		33° 02.5′ N. 118° 01.5′ W.		33° 04.5′ N. 117° 55.5′ W.	
Depth overlying water	1190 meters		935 meters		916 meters	
Time stored before analysis	12 hours		4 hours		4 hours	
Depth of sample below surface of core in cm.	*Bacteria per gram*		*Depth of sample below surface of core in cm.*	*Bacteria per gram*	*Depth of sample below surface of core in cm.*	*Bacteria per gram*
	aerobes	*anaerobes*		*aerobes*		*aerobes*
0–2	7,500,000	1,500	0–3	1,850,000	0–2	2,415,000
2–5	250,000	2,250	3–8	230,000	2–5	55,000
5–9	200,000	7,200	8–18	2,900	5–8	15,800
9–13	100,000	1,350	18–28	600	10–12	800
18–22	20,000	470	48–58	<50	20–22	150
28–43	3,300	5	79–89	2,150	36–38	50
43–58	2,100	80	109–119	400	53–55	<50
58–74	100	10	137–150	50	74–76	400
74–89	200	10	170–180	250	91–94	<50
89–104	150	45				
104–119	150	10				
119–134	50	5				
134–149	100	5				
149–165	<50	0				
165–180	150	5				
180–196	200	5				

TABLE II

NUMBER OF AEROBIC AND ANAEROBIC BACTERIA DEMONSTRATED IN DIFFERENT STRATA OF CORES OF GREEN MUD OF LOW BACTERIAL POPULATION

Sample	39-C-1		39-C-4	
Location of station	32° 55.7′ N. 117° 36.8′ W.		33° 03.3′ N. 117° 25.5′ W.	
Depth overlying water	935 meters		465 meters	
Time stored before analysis	8 hours		6 hours	
Depth of sample below surface of core in cm.	*Bacteria per gram of sediment (wet weight) aerobes*	*anaerobes*	*Depth of sample below surface of core in cm.*	*Bacteria per gram of sediment (wet weight) aerobes*	*anaerobes*
0–5	180,000	7,500	0–3	240,000	17,500
5–17	60,000	6,500	3–8	155,000	12,000
30–43	1,100	30	33–46	300	10
56–69	50	0	71–76	350	65
81–94	100	0	109–122	50	0
145–158	<50	0			
185–198	<50	0			
211–224	100	0			
236–249	<50	0			

should be noted that the cores in this group were stored between 60 and 110 hours before analysis while those in the other two groups were all stored less than 12 hours.

Within certain cores the vertical distribution of bacteria departed from a general decrease in numbers with depth by a significant amount. In the two most striking examples observed (Table IV) the occurrence of layers of high population below layers of low population was associated with discontinuous changes in the physical properties of the sediment. In FPS 258, 270,000 bacteria per gram were found at a depth of 303–313 centimeters below the surface, while the number of bacteria in the layers above and below this depth was considerably

TABLE III

NUMBER OF AEROBIC AND ANAEROBIC BACTERIA DEMONSTRATED IN DIFFERENT STRATA OF CORES OF GREEN MUD OF HIGH BACTERIAL POPULATION

Sample	FPS 251		FPS 253	
Location of station	36° 13.0′ N. 121° 55.1′ W.		34° 49.0′ N. 121° 10.0′ W.	
Depth overlying water	676 meters		530 meters	
Time stored before analysis	108 hours		84 hours	
Depth of sample below surface of core in cm.	*Bacteria per gram of sediment (wet weight) aerobes*	*anaerobes*	*Depth of sample below surface of core in cm.*	*Bacteria per gram of sediment (wet weight) aerobes*	*anaerobes*
0–12	23,800,000	290,000	0–10	18,300,000	110,000
12–25	138,000	26,000	10–15	4,000	1,100
51–64	63,000	3,100	55–67	4,200	45
102–115	24,000	4,800	107–120	400	95
127–140	23,000	4,700	142–155	100	40

TABLE IV

NUMBER OF AEROBIC AND ANAEROBIC BACTERIA DEMONSTRATED IN DIFFERENT STRATA OF CORES OF GREEN MUD SHOWING ZONES OF HIGH POPULATION BENEATH ZONES OF LOW POPULATION

Sample	FPS 258	FPS 259
Location of station	34° 14.0′ N. 120° 02.5′ W.	34° 11.8′ N. 120° 02.0′ W.
Depth overlying water	566 meters	565 meters
Time stored before analysis	60 hours	60 hours

Depth of sample below surface of core in cm.	*Bacteria per gram of sediment (wet weight)* *aerobes*	*anaerobes*	*Depth of sample below surface of core in cm.*	*Bacteria per gram of sediment (wet weight)* *aerobes*	*anaerobes*
0–5	3,050,000	232,000	0–18	15,800,000	272,000
5–13	11,700,000	189,000	18–31	9,100,000	178,000
25–38	3,275,000	54,000	56–79	788,000	2,660
76–89	2,300,000	15,000	107–120	2,600	330
152–165	43,000	9,600	157–170	17,000	200
228–231	8,400	18,000	232–245	102,000	390
303–313	270,000	42,000			
342–355	15,000	2,200			

lower. A similar zone of high population was observed 232–245 centimeters below the surface in FPS 259. In exactly these same layers there was a very abrupt increase in the water content and decrease in the cohesiveness of the sediment. The zone of relatively high population at 78–89 centimeters depth in 39-C-39 has not as yet been correlated with an abrupt change in any other characteristic of the sediment.

Table V shows the numbers of bacteria found in the various layers of the only pelagic deposit examined. The numbers of aerobes and anaerobes were uniformly low throughout the length of the core with a maximum at the surface.

TABLE V

NUMBER OF AEROBIC AND ANAEROBIC BACTERIA DEMONSTRATED IN VARIOUS STRATA OF A CORE OF PELAGIC SEDIMENT

Sample	F 72
Location of station	30° 25.0′ N. 118° 32.5′ W.
Depth overlying water	3005 meters

Depth of sample below surface of core in cm.	*Bacteria per gram of sediment (wet weight)* *aerobes*	*anaerobes*
0–5	3,300	600
28–36	200	100
66–74	300	100
104–112	600	100
142–150	350	100
180–188	600	450
218–226	200	200

DISCUSSION

The vertical distribution of bacteria in the upper sixty centimeters of the terrigenous deposits of clays and silts examined is very similar in certain general features to that reported by previous workers. The large population at the surface, the rapid decrease in numbers of bacteria with depth, and the larger number of aerobes than anaerobes found in most cores in this investigation have also been reported by Reuszer (1933), ZoBell and Anderson (1936) and others. However, there are great enough differences in the numbers and distribution of bacteria in the various cores examined to make one question the advisability of generalizing such data. Without discussing the specific factors which account for these differences, it can be said that they are due to extreme vertical and lateral variability in the bottom materials themselves. It follows that "average" distribution curves of bacteria are of value only when they apply to a well-defined, homogeneous sediment.

The bacterial population of the sediments buried under more than 60 centimeters is of special interest since no previous observations have been reported for these deeper layers. The data show that both aerobic and anaerobic bacteria are present at a considerable distance beneath the ocean floor. Even though cores as long as 355 centimeters were examined, a lower boundary of the bacterial population was not reached. The numbers of bacteria demonstrated in these lower levels was uniformly small in those samples analyzed immediately after collection. ZoBell (1938) has shown that the number of bacteria increase two- to fourfold after storage at 0° C. for 48 hours. The larger populations found in the samples stored for sixty hours or over may therefore be due in part to multiplication of bacteria after obtaining the core. Since only the stored cores showed high populations in the lower levels one might conclude that the populations *in situ* below 60 centimeters are always small. Only a small number of cores were examined, consequently this apparent relationship may be due to entirely different, unrecognized factors. The multiplication of bacteria in the sediments during storage shows that these muds are capable of supporting many more bacteria than are usually found, and it is possible that under certain circumstances the population *in situ* in deeply buried layers may approach that of the surface layer.

The small number of anaerobes found in the lower layers is quite puzzling. The green clays and silts contain an abundance of organic matter (Revelle and Shepard, 1939) and are very reducing in nature (ZoBell, 1935), often containing free hydrogen sulphide in the subsurface layers. These characteristics should all be conducive to an

abundant anaerobic microflora. Part of the reason for the paucity of anaerobes detected may lie in the method of making the counts, since the oval-tube procedure has the same limitations as any indirect method of enumerating bacteria based on the counting of colonies developing on a single medium. Very little information is available on the type of substrate best suited for growing the maximum number of a naturally-occurring mixed microflora of anaerobes. It is quite probable that the nutrient medium used in this investigation is not suitable for the bacteria existing on the relatively resistant organic compounds occurring in sediments which have been subjected to intensive aerobic and anaerobic decomposition.

The recovery of viable bacteria from deep layers of sediment is not proof of their activity in that material. It is well known that spores exist for long periods in a quiescent state and there is a growing belief that non-spore-forming bacteria may also have some type of resting stage. The isolation of aerobic, non-sporulating bacteria from the anaerobic environment of these sediments lends some support to this belief. As pointed out by ZoBell (1938), the small number of bacteria present in the lower layers may represent dormant survivals of the active population present when the sediment was first laid down. The opposite hypothesis, that most of the bacteria demonstrated are active *in situ*, and that the small numbers present (allowing for the limitations of the enumeration procedures) result from various unfavorable environmental conditions such as low temperature, low water content, and other inimical factors, seems equally probable.

The occurrence of discontinuous zones of bacterial populations in the same loci where there are abrupt changes in the physical and chemical properties of the sediments is a striking example of the relation between population and environment. It is of interest to point out that the observation of such biological zonation can serve to focus attention on changes in physical or chemical properties which might otherwise be overlooked.

Core F 72 is the only pelagic deposit represented in this series and to the best of the author's knowledge the data presented is the only report in the literature on the vertical distribution of bacteria in such sediments. The number of viable bacteria recovered from this material is much smaller than from the terrigenous deposits. Even though only one pelagic sample has been analyzed, it is believed that small bacterial populations will be characteristic of all such deposits. This conclusion is based on the very slow rate of deposition of these sediments, estimated to be less than a centimeter every thousand years, and on the correspondingly long period of oxidation undergone before the

material is buried beneath the ocean floor (Revelle, 1940). As a consequence most of the utilizable organic matter would be decomposed while the sediment was in contact with oxygen-bearing bottom water leaving very little for bacteria to multiply upon immediately below the surface of the deposit. It is hoped that more pelagic sediments will be available in the near future so that additional data can be gathered on this point.

SUMMARY

The numbers of aerobic and anaerobic bacteria present at various depths in cores of marine sediments up to 355 centimeters in length were determined.

Viable bacteria were found in the bottom of the longest cores examined although in most instances the number present was very small.

The numbers of bacteria found vary greatly from core to core at corresponding depths and also vertically in individual cores. A decrease in bacterial population with depth was usually observed. Many more aerobes than anaerobes were demonstrated even though conditions below the surface of the sediments appeared to be more favorable for anaerobic growth. The smaller number of anaerobes found may be partly due to limitations in the enumeration procedure.

In certain sediments, zones of high bacterial population were found beneath zones of low population. In two instances the location of such zones corresponded exactly with the loci of abrupt changes in other characteristics of the sediments.

The number of bacteria demonstrated in the one pelagic deposit examined was much smaller and more constant than in the terrigenous deposits.

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