SOME CHEMICAL AND BACTERIAL CHARACTERISTICS OF BOTTOM DEPOSITS FROM LAKES AND ESTUARIES

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(With 2 Figures in the Text)

The composition of the deposits forming the beds of seas, lakes and estuaries has been studied for a number of purposes. One group of workers has been concerned with the subject as part of an ecological study of large bodies of water, other groups because of its relation to pollution or to navigation.

Investigations by a number of workers (cf. Pearsall, 1921; Moore, 1931; Twenhofel & Broughton, 1939; Pearsall & Lind, 1942; Pennington, 1943; Pearsall & Pennington, 1947) suggest that the organic matter in deep-water deposits represents mainly the remains of planktonic organisms, though small fragments of wood and dead leaves may occur in some samples. The productivity and the nature of the plankton appear to depend mainly on the concentrations of available nitrogen and phosphorus in the water. An increase in these concentrations may result, for example, from the breakdown of humus following forest clearing, from an increase in the population of grazing animals on the neighbouring land, or from pollution of the water by sewage. The organic matter accumulating in this way, including the bodies of dead planktonic organisms, is subject to bacterial decomposition during the process of deposition, and by the time the sediment has become consolidated this process of decomposition may have reached an advanced stage.

Polluting matter in a body of water may include material of faecal origin, organic matter of non-faecal origin, and inorganic substances with toxic properties. Since the organic matter may be greatly changed by processes of synthesis and decomposition before it becomes part of the bottom deposit, it follows that chemical analysis of such a deposit may not be capable of revealing whether or not the original pollution was faecal in character. The potentialities of a bacteriological examination for this purpose have hardly been investigated.

The Water Pollution Research Board of the Department of Scientific and Industrial Research (1938) compared the composition of deposits in localities comparatively free from pollution with that of mud in the estuary of the Mersey, which received considerable discharges of sewage and trade wastes. It was found to be impossible to decide, from the results of analysis for organic matter (determined from the weight lost on ignition of a weighed sample of the dried material), organic carbon, Kjeldahl nitrogen, and silica, whether a given mud was deposited in a polluted or an unpolluted estuary; no bacteriological examination was made.

In the present work samples of mud from a number of sources, subject to varying degrees of pollution, were submitted to chemical and bacteriological examination in order to compare their characteristics and to note possible indices of pollution.

EXPERIMENTAL

The samples collected were of three main types: (1) those from the Thames Estuary, known to be subject to severe and continual pollution; (2) those from tidal rivers other than the Thames, subject to pollution to an appreciable but much smaller extent; (3) those from lakes in the Lake District, subject to a degree of pollution varying from very slight to moderate.

Samples from the Lake District were collected* by means of the Jenkin mud sampler (described by Mortimer, 1942). Samples referred to in Table 2 and those from rivers other than the Thames were taken from deposits exposed at low tide. For the collection of several of these samples a long iron tube consisting of a number of 2 ft. sections screwed together was used. A suitable length of this tube was pushed into the mud and then withdrawn. Portions could be removed from the resulting core after unscrewing the appropriate section. Some samples from a depth of up to 2 ft. were removed by means of a small shovel. Samples from the Thames, referred to in Table 1, were taken by a modified Van Veen grab operated from a motor launch in mid-stream.

Samples were analysed for their content of sulphide, organic carbon, and Kjeldahl nitrogen, and counts (Most Probable Number) were made of *Bacterium coli*, of *Streptococcus faecalis*, and of sulphate-reducing bacteria. Counts of *Bact. coli* were made in MacConkey medium (lactose-bile salts broth), subcultures being made from positive tubes in the preliminary incubation at 37° C. to fresh tubes of the same medium at 44° C. For counts of *Strep. faecalis* the glucose azide broth recommended by Hannay & Norton (1947) was used, but inoculated tubes were first incubated for 24–48 hr. at 37° C. and positive tubes were then subcultured (as soon as they were positive) to tubes of the same medium at 45° C., this procedure having been found to yield a much higher count than direct incubation at 45° C. Counts of sulphate-reducing bacteria were made in two media constituted as follows:

Modified Hotchkiss's medium (1923)	g./l.	Miller's medium (1949)	g./l.
K_2HPO_4	0·5	NH_4Cl	1·0
$(NH_4)_2SO_4$	2·0	$MgSO_4\ 7H_2O$	2·0
Sodium lactate	15·0	Na_2SO_4	18·25
†$FeSO_47H_2O$	0·2	K_2HPO_4	0·5
		$CaCl_22H_2O$	0·1
		Sodium lactate	21·0
		NaCl	10·0
		$CaCO_3$	1·0
		†$Fe(NH_4)_2(SO_4)_26H_2O$	Trace

Results of examination of sixteen samples of mud are shown in Table 1. From these and from results of further experiments certain broad conclusions may be drawn.

* The authors are indebted to Dr Margaret Thornley of the Freshwater Biological Association for arranging for the collection and despatch of these samples.

† A solution of the ferrous salt was sterilized separately by passing through a Berkefeld filter, and sufficient to provide the required concentration was added aseptically to each tube of medium just before use.

Content of organic matter

It is evident that the content of organic matter is not by itself a satisfactory index of pollution. Thus the mud from Esthwaite Water showed by far the highest figures for organic carbon and for nitrogen of all the samples examined, although the pollution in this lake is very much less than that of the Thames at Tilbury, for example, where the carbon and nitrogen contents of the mud were only about two-thirds of those of samples from the lake. The samples from the River Alde and the River Deben, which are known to be appreciably polluted, contained much less carbon and nitrogen than those from Thirlmere, known to be maintained in a condition of purity.

Differences in constitution of organic matter

Considered as a source of food for the growth of micro-organisms and other mud-dwelling flora and fauna the important characteristic of the organic matter is not its total quantity but the proportion of it which is available as a nutrient. Waksman (1929) has shown that in the decomposition of plant residues on land some of the organic constituents, including the sugars, cellulose, and certain hemi-celluloses, decompose rapidly; others, like the lignins, decompose slowly; the proteins, however, may actually increase in total concentration as a result of synthesis of cellular protein. Raymond & Stetson (1931) found that the surface layer of marine mud was rich in plant and animal residues undergoing decomposition by an active bacterial population and that it differed in composition from the muds at lower levels. These consisted of material which had already largely decomposed and which formed the true marine humus. Boysen-Jensen (1914), by digesting marine sediments with pancreatic enzymes, found that the surface layer contained appreciable quantities of digestible nitrogen, whereas material below a depth of 1 cm. usually contained none. Alsterberg (1927) and Kusnetzow (1935) explained the depletion of oxygen from the depths of a lake as the result of the activities of bacteria oxidizing organic matter in the bottom deposit. Anderson (1939) using the rate of consumption of oxygen from sea water as a measure of available organic matter, found that in about half the mud cores examined the organic matter in the surface layer was most easily destroyed, the rate of decomposition decreasing with depth. Some samples, however, of the middle or bottom layers were more easily oxidized. There was no correlation between the amount of oxygen consumed and content of organic carbon. Moore (1931) found that the nature of the mud changed with depth not only because of the activities of mud-dwelling forms but because annual deposition of diatoms in April tended to concentrate organic matter in narrow bands.

There is thus ample evidence that the organic matter in mud varies in composition. The methods used to demonstrate this by the authors, already referred to, did not seem entirely adequate for the present study. In less polluted waters decomposition of organic matter in surface layers of mud will no doubt be partially aerobic. It will certainly be partially anaerobic and in the subsurface layers of mud entirely so. Several workers (cf. ZoBell, 1946) have found that anaerobic decomposition of organic matter by aquatic bacteria results in the formation of methane, nitrogen, hydrogen sulphide, carbon dioxide and organic acids. It seemed likely,

Table 1. *Results of chemical and bacteriological examination of samples of mud from different sources*

Group of samples	Date of sampling	Source of samples	Approximate depth (in.)	Percentage dry weight: Sulphide (as H_2S)	Percentage dry weight: Organic carbon	Percentage dry weight: Kjeldahl nitrogen	Ratio, C : N	Most Probable No. of bacteria per g. dry weight: *Bact. coli*, Type I	*Strep. faecalis*	Sulphate-reducers: Hotchkiss's medium (modified)	Sulphate-reducers: Miller's medium
Lake District	15. xi. 51	Windermere	0–2	0·029	7·24	0·59	12·3	164	32	8	< 3
			2–10	—	—	—	—	1·6	< 1·5	4	< 1
		Thirlmere	0–2	0·011	7·31	0·57	12·9	3	2	< 1	15
			2–10	—	—	—	—	< 0·1	< 1·4	0·6	< 1
	14. i. 52	Coniston	0–2	Nil	8·53	0·67	12·7	40	28	20	7
			2–10	Nil	6·30	0·47	13·6	< 1	66	5·5	< 1
		Esthwaite	0–2	0·159	12·66	1·14	11·1	35	250	700	2
			2–10	0·136	12·67	0·96	13·2	1·7	84	8·4	< 0·6
Tidal rivers other than Thames	22. i. 52	River Alde at Orford	0–2	Nil	1·29	0·13	10·2	28	53	10·7	530
			2–10	0·05	1·59	0·13	11·9	0·86	3·2	1·0	318
		River Deben at Bawdsey	0–2	Nil	1·01	0·11	9·6	28	4·3	5·4	280
			2–10	0·05	1·16	0·08	14·7	< 0·5	1·4	0·77	251
	24. x. 51	River Crouch (Bridgemarsh Creek)	0–12 approx.	0·19	1·49	0·11	14·1	< 3	8	9	83
		River Crouch at Battlesbridge	0–12 approx.	0·15	2·37	0·23	10·4	1,810	3,100	155	130
		River Blackwater at Millbeach	0–12 approx.	0·09	2·25	0·18	12·3	81	42	81	81
Thames Estuary	22. vi. 51	Barking Power Station	0–12	—	—	—	—	—	—	2,840	19,900
	21. ix. 51	Gravesend	0–12	—	—	—	—	662	1,990	—	830
		Tilbury	0–12	—	—	—	—	1,110	199	—	2,210
		Ford's Jetty	0–12	—	—	—	—	1,900	660	—	380
		King George V Dock	0–12	—	—	—	—	1,900	1,210	—	1,350
		Barking Power Station	0–12	—	—	—	—	75,000	41,000	—	3,400
	20. xi. 51	Tilbury	0–12	0·372	7·59	0·87	8·7	83,000	63,000	6,300	3,900

therefore, that a true picture of the quantity of organic matter available for bacterial growth might be obtained by allowing a sample of mud to undergo anaerobic decomposition and measuring the volume of gases evolved. It was realized, however, that in some samples of mud, though organic matter might be available, the flora necessary to digest it might have died or become attenuated. For this reason it was considered advisable to add an inoculum of freshly digested sewage sludge to the test sample of mud.

The mud was made into a thin paste with distilled water, and 300–400 g. of the paste were mixed in a flask with a suitable quantity (for example 100 c.c.) of digested sludge from a sewage works. The flask was closed by a rubber bung carrying a leading tube to collect gas over saturated brine in a calibrated glass vessel (see Fig. 1). When the apparatus was incubated at 23° C., gas was slowly

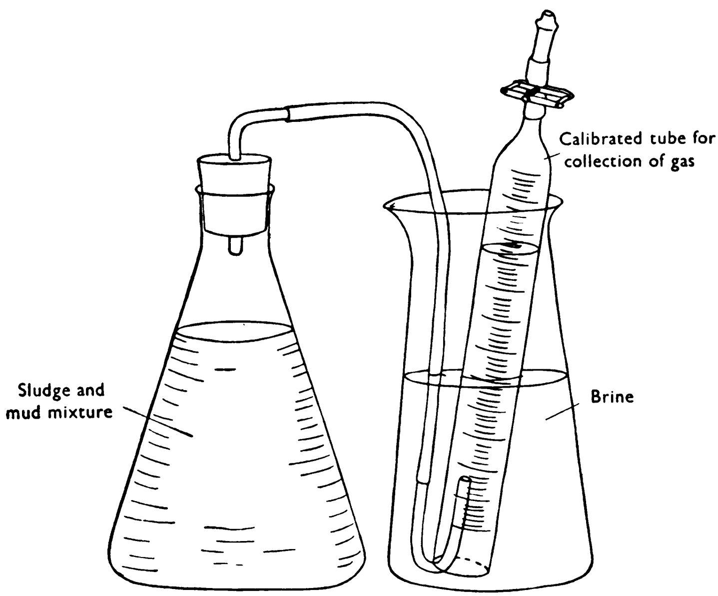

Fig. 1. Apparatus for the collection of gas evolved from the digestion of mud.

evolved and the volumes from duplicate flasks were recorded over an extended period of incubation*. A control flask showed the quantity of gas evolved from the sludge itself. From these records the quantity evolved from 100 g. of mud (dry weight) could be calculated. The results, shown graphically in Fig. 2, obtained with a sample of mud alone and with a similar sample to which sludge was added, show that the addition of sludge greatly increased the rate at which gas was evolved.

Usually the method yielded reasonably consistent duplicates when used for samples of mud taken from the same depth. Results from a number of samples taken from the Thames at Tilbury (Table 2) showed that differences between samples from different depths were far greater than the experimental error of the method. Evidently there were great differences in the quantity of organic matter available to micro-organisms at different depths; most of this in the samples

* When the evolved gas had nearly filled the calibrated tube, the clip at the top was opened, the liquid inside the tube drawn up to the top by suction, and the clip closed again. Readings were then continued.

tested was contained in the top 6 in. of mud, the content declining rather sharply with depth.

Even with the precaution of added sludge, samples are apt to vary considerably

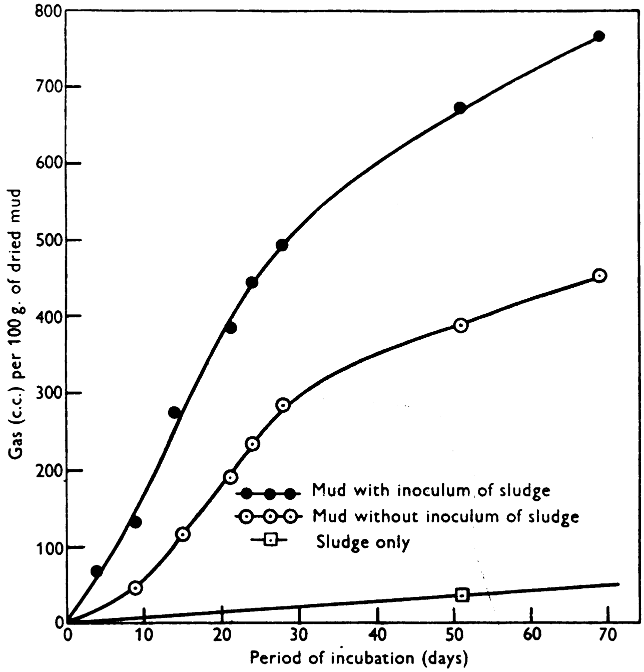

Fig. 2. Effect of addition of inoculum of digested sewage sludge on rate of evolution of gas from mud (from Thames estuary at Gravesend) during anaerobic digestion.

Table 2. *Volumes of gas evolved from duplicate samples of mud taken from different depths of a mud bank in the Thames estuary at Tilbury (mud inoculated with digested sewage sludge and incubated at 23° C.)*

Sample no.	Date of sampling	Period of incubation (days)	Depth of subsample below surface	Volume of gas evolved (c.c. per 100 g. dry mud) I	II	Average
1	26. ii. 51	40	0–1½ ft.	616	—	616
			5½–7 ft.	84	109	97
2	12. iii. 51	51	0–6 in.	613	639	626
			4½–5 ft.	39	35	37
3	2. iv. 51	79	0–½ in.	395	—	395
			3–6 in.	862	—	862
			1 ft.	450	—	450
			2 ft.	111	—	111
4	10. iv. 51	60	0–½ in.	523	533	528
			3–6 in.	429	432	431
			1 ft.	152	94	123
			2 ft.	10	−14	−2

in the extent of the lag-phase before maximum growth of gas-forming anaerobes occurs. It is therefore advisable to base comparisons on the results of prolonged periods of incubation.

That differences in the constitution of organic matter at different depths revealed by this method are not reflected in the figures for carbon content is shown in Table 3. Samples 3 and 4 were identical with the corresponding samples in Table 2.

Table 3. *Total content of organic carbon in samples from different depths of mud in the Thames Estuary (results as percentage of dry weight)*

Sample 3		Sample 4		Sample 5	
Depth below surface	Organic carbon	Depth below surface	Organic carbon	Depth below surface	Organic carbon
0–½ in.	5·64	0–½ in.	7·20	0–½ in.	2·27
3–6 in.	7·43	3–6 in.	3·18	1 ft.	2·43
1 ft.	5·35	1 ft.	3·90	2 ft.	6·91
2 ft.	6·15	2 ft.	3·08	4 ft.	6·79

Content of sulphide

The activity of sulphate-reducing bacteria depends on availability both of organic matter and of sulphate and also on the maintenance of anaerobic conditions, which in turn depends on temperature. The figures in Table 1 show that, although the sulphide content of mud may indicate the availability of organic matter for bacterial growth, it does not provide evidence of the presence of polluting material in the mud.

Counts of Bacterium coli

The count of *Bact. coli* in the surface samples of mud was roughly in accordance with the degree of faecal pollution to which the source of mud was known to be subject. For example, special precautions are taken to keep Thirlmere free from pollution since it is used as a source of drinking water, but the remaining three lakes from which samples were collected are known to be polluted. According to Taylor (1940), the North basin of Windermere receives the sewage effluent from Ambleside, which has a winter population of about 2500 and the South basin receives the effluents from Windermere and Bowness which have a combined winter population of about 6000. The same author states that pollution of Esthwaite Water is appreciable and is due mainly to drainage from farmyards and to sewage effluents and crude sewage from the village of Hawkshead with a population of about 800. Lake Coniston receives the sewage effluent from Coniston village. These differences in degree of pollution are reflected in the low count of *Bact. coli* in the mud from Thirlmere and in the much higher counts in samples from the other three lakes. The usefulness of this organism, however, as an index of pollution applies only to the surface layer of mud since the bacterial count of mud below a depth of about 2 in. was negligible in samples from all the lakes and from the rivers Alde and Deben.

Counts of Streptococcus faecalis

Counts of *Strep. faecalis* in general follow those of *Bact. coli*. They are, however, often less sharply affected by the depth at which the sample is taken. These organisms, moreover, differ from *Bact. coli* in their food requirements and in their sensitiveness to adverse conditions. Although counts of *Bact. coli* in sewage sludge are originally much higher than those of *Strep. faecalis* the streptococci survive in larger numbers and for longer periods in sludge which is deposited in dilute sea water. For these reasons it is very advisable to include counts of both *Strep. faecalis* and *Bact. coli* when testing muds for the degree to which they have been subject to faecal pollution. This supports the findings of Savage & Read (1916). As a result of examination of some 1300 samples of water they expressed the opinion that examination for streptococci was of decided value as evidence for or against excretal contamination, and thought that if the reliability of the method could be improved its diagnostic value would be much enhanced. The method used by these authors consisted in adding serial dilutions of the test sample to a suitable medium and, after incubation, examining the sediment under the microscope to see if streptococcal chains were present. No distinction between *Strep. faecalis* and other streptococci was made. In the present work the technique used was specific for *Strep. faecalis* (cf. Allen, Brooks & Williams, 1949).

Counts of sulphate-reducing bacteria

The counts of sulphate-reducing bacteria suggest that there were at least two different types of these organisms. In mud from fresh-water lakes much higher counts were usually obtained in modified Hotchkiss's than in Miller's medium; in samples from estuaries, where saline conditions obtain, the reverse was usually found. As with the sulphide content, the count of these organisms probably reflects the amount of organic matter available for their growth rather than the degree of pollution obtaining. Depletion of available organic matter through the activities of other bacteria would thus tend to limit their growth to the upper layers of the mud. Whether sufficient is known about conditions affecting growth of these organisms to permit of counts obtained in laboratory media being regarded as an accurate index of the numbers present in the sample examined is doubtful.

That the bacteria in bottom sediments are largely concentrated in surface layers has been agreed by several workers (cf. Lloyd, 1931; ZoBell & Anderson, 1936; Rittenberg, 1940; ZoBell & Feltham, 1942). The numbers of organisms per gramme found by different investigators, however, vary enormously as do the rates of decline with depth. ZoBell & Anderson, for example, found the greatest diminution in the first few centimetres, aerobes declining more rapidly than anaerobes, whereas Lloyd recorded a much more gentle gradient and an appreciable count at a depth of 30 cm. The distribution of bacteria with depth will vary with the type of organism; those finding suitable nutriment in the constituents of mud would be expected to increase considerably in numbers and to be associated particularly with the upper layers where supplies of available organic matter are greater. The usefulness of *Bact. coli* and *Strep. faecalis* as indicators of faecal pollution will depend not only on the fact that they are consistently present in very large numbers in faeces, but on their virtual absence from other likely sources of pollution and on their inability to grow in mud. *Bact. coli* may occasionally find natural conditions outside the animal body which are suitable for proliferation—for example, in liquid in which flax is retting (cf. Allen, 1946)—but generally, in bodies of water subject to pollution the numbers of this organism derived from such sources will probably be negligible in comparison with those derived directly from faeces.

In the course of the varying conditions they meet from the time they are discharged to a body of water until they are deposited in the sediment these faecal bacteria may possibly find conditions suitable for growth. There is little or no evidence on this point. Once they have been deposited, however, the numbers found will depend less on the ability of the organisms to grow than on their capacity to survive under the conditions existing in the mud. On this view, counts would be expected to be larger in the more recently deposited surface layers. They would, however, be affected by several conditions such as pH value, temperature, degree of oxygenation, and concentration of organic matter (cf. Allen, Pasley & Pierce, 1952).

It is of interest to recall that Savage (1905) concluded that samples of mud yielded more reliable bacteriological evidence of the degree of faecal pollution of a tidal river than samples of either water or oysters, which tended to reflect only pollution actually present at the time of sampling. He considered that mud samples showed evidence of past pollution for at least several weeks. Nevertheless, it should be borne in mind that materials polluting a river and potentially capable of injuring fish, destroying other aquatic life, or causing embarrassment to water undertakings, are not necessarily of faecal origin. *Bact. coli* and *Strep. faecalis* are indicators only of faecal pollution.

SUMMARY AND CONCLUSIONS

Chemical and bacteriological examination of muds from sources differing widely in the degree of pollution to which they were subject showed great differences in the contents of carbon, nitrogen and sulphide. These differences were not correlated with differences in the severity of faecal pollution. The amount of organic matter available for growth of micro-organisms in the mud of different depths was not reflected in the figures for organic carbon. A convenient index of this factor was obtained by measuring the volume of gas evolved during anaerobic digestion over a prolonged period of incubation. The rate of evolution was increased by the addition of an inoculum of digested sludge from a sewage works.

Sulphate-reducing bacteria appeared to be of two different types. In samples of mud from fresh-water lakes much higher counts were usually obtained in a medium containing comparatively low concentrations of inorganic salts and of lactate than in a medium containing much higher concentrations of these constituents. In samples from locations where conditions were more saline the reverse was usually true.

Counts of *Bact. coli* and of *Strep. faecalis* together probably constitute the best index of faecal pollution in the examination of samples of mud. These organisms are, however, largely confined to the surface layers.

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