THE BACTERIAL POPULATION AND THE PROCESSES OF HYDROGEN SULPHIDE OXIDATION IN THE BLACK SEA

By

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Methods are described to study the mechanism and rate of sulphide oxidation in water using ^{35}S. These methods were used in the Black Sea together with measurements of O_2, H_2S, *Eh*, bacterial biomass and production. Sulphide oxidation proceeds via two main steps: chemical oxidation of sulphide to sulphate and thiosulphate, and the biological oxidation of thiosulphate to sulphate. The rate of sulphide oxidation varied from 0·4–0·9 g H_2S/m^2 day. The maximum rate of oxidation took place at depths of 150–170 m at *Eh* – 10 to −20 mV, and at a maximum rate of chemosynthesis of 8 mg C/m^3/day. Data are given for the biomass of the microflora and its production in the water column. The maximum efficiency of chemosynthesis was 15%. The results are discussed in relation to the budget of the organic matter.

INTRODUCTION

The presence of the hydrogen sulphide zone in the Black Sea is a very important specific feature of the basin which influences its chemistry and biology (DANILTSCHENKO and TSCHIGIRIN, 1929; SCOPINTZEV, 1953; VODYANITSKI, 1954). Continuous study for several decades have shown the stability of the vertical depth position of the H_2S zone (SCOPINTSEV and SMIRNOV, 1962). In the presence of vertical turbulence (VODYANITSKI, 1948) this stability can be maintained only during the dynamic equilibrium between the oxidation of H_2S in the intermediate layer (layer of the mixing of aerobic and anaerobic water near the upper level of the H_2S zone), and its formation by sulphate-reducing bacteria (BOGDANOVA, 1959; DOBRZHANSKAYA, 1959; SCOPINTSEV, 1953; SOROKIN, 1964). When studying the magnitude of these conflicting processes in the sulphur cycle in the Black Sea it is very important to measure the possible rate of H_2S oxidation in the water column. Furthermore, it is necessary to evaluate the relative importance of chemical and bacterial oxidation of H_2S and to decide which is prevailing. This is an important problem because the two oxidations are not equivalent so far as the energetics are concerned. The energy of the H_2S oxidation is higher than that of the oxidation of an equivalent amount of organic matter. During chemical oxidation energy is lost as heat; during biological oxidation of H_2S by autotrophic bacteria up to 25% of the energy is utilised for chemosynthesis and is so included into the biological turnover and thus supports other biological

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and biochemical processes in the water basin. The bacterial biomass formed during chemosynthesis is partially used by the filtering zooplankton (DATSKO 1954; LEBEDEVA, 1959) and the remainder is carried by turbulence below the intermediate zone. There, in the conditions of low redox potential, bacteria are dying and their organic material became a source of energy for the additional production of hydrogen sulphide by sulphate-reduction. This latter process partially maintains the stability of H_2S zone (SCOPINTSEV, 1953; SOROKIN, 1964; SCOPINTSEV and GUBIN, 1955).

The problem of the rate and the mechanism of H_2S oxidation in the Black Sea have been studied and discussed by several investigators. According to the early hypothesis by EGUNOV (1895) the "bacterial plate" present in the intermediate layer of the Black Sea is analogous to that in some meromictic lakes. According to EGUNOV this "plate" (the layer of a dense population of thiobacilli) prevents the distribution of H_2S to the upper layers. The hypothesis was studied several times by microbiologists (ISSACHENKO and EGOROVA, 1939; KOPP, 1948; KRISS, 1963). They did not support it. Numerous analyses to find thiobacilli in the water from different depths of the Black Sea by the dilution method in Beijerinck liquid medium were as a rule negative. These authors were not able to find any detectable accumulation of thiobacilli even in the intermediate zone. These bacteria were rarely found. The hydrochemists who had discussed this problem (SCOPINTSEV, 1953; SCOPINTSEV *et al.*, 1959; BRUYEVICH, 1953) supported ISSACHENKO, who described the H_2S oxidation in the Black Sea as a purely chemical process. SCOPINTSEV *et al.* (1959) have drawn attention to other data as proving the latter opinion. They discussed the results of experiments by IVANOV (1957) in which the mechanism of H_2S-oxidation in mineral spring water was studied with the use of labelled sulphide (Na_2S^{35}). From these results it can be seen that the rate of oxidation is about equal in the presence and in the absence of antiseptic (formaldehyde).

The same result was obtained during an experiment on the estimation of the rate of H_2S oxidation in suspensions of the silt from the polluted area of the River Thames. The rate of H_2S disappearance in sterile and nonsterile suspensions was about the same (WHEATLAND, 1954). It was therefore concluded that H_2S oxidation was purely chemical.

But the subsequent study revealed that all these conclusions about the mechanism of H_2S oxidation in the Black Sea were not correct. With the aid of C^{14} it was shown that in the intermediate layer (150–300 m) there were active autotrophic microflora, which oxidized sulphur compounds. This microflora (thiobacilli) can be very easily discovered with the use of C^{14} in each of the samples taken in this layer. Samples introduced into a medium having a gradient of redox potential along the column of liquid in the test tube usually gives a positive result demonstrating the presence of active thiobacilli (SOROKIN, 1964). The unsuccessful attempts of earlier workers to discover the layer rich in thiobacilli in the Black Sea were a consequence of their use of the usual Beijernik mineral medium having too high a redox potential. But thiobacilli living in the Black Sea are adapted to a relatively low redox potential and in the layers with high redox potential they are absent.

The real significance of the experiments by IVANOV and WHEATLAND became clear later when the special studies were carried out using S^{35} (SOROKIN, 1968; 1968a). It was shown that thiosulphate is one of the main products of H_2S oxidation in natural waters in the presence or in the absence of thiobacilli.

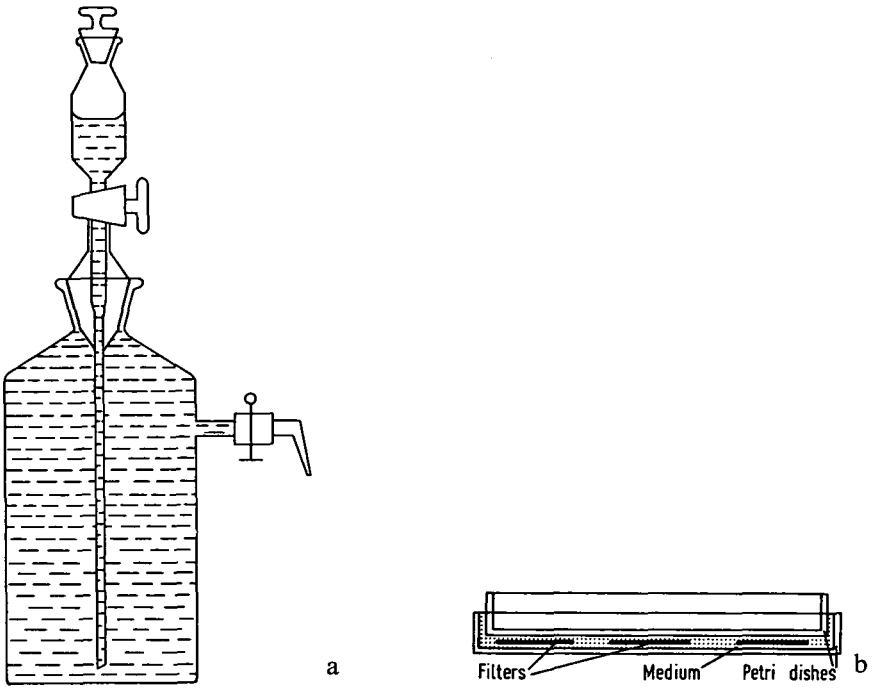

Figure 1. a. The bottle used for estimating O_2 in the presence of H_2S. b. Arrangements for making plate counts of sulphate reducing bacteria on millipore filters.

Earlier authors did not estimate thiosulphate at all. It was found that the role of bacteria is mainly in the final oxidation of this thiosulphate which is formed during the first stage of the chemical oxidation of H_2S to thiosulphate and sulphate. Estimates of H_2S disappearance, or of sulphate formation cannot give any information of the part played by the microflora in H_2S oxidation as the initial and final processes do not need bacterial participation.

During the cruises of R. V. "Lomonosov" and R. V. "Gonetz" in the autumn of 1964 and 1967 we have made further studies of microbiological processes and their role in the sulphur cycle in the Black Sea. The results are presented here.

METHODS

SAMPLING METHOD

Chemical and experimental samples were taken with a 3 or 7 l plastic water bottle. Samples for bacteriological analyses were taken with a special sterile bacteriological sampler (SOROKIN, 1964a). Sampling depths were selected by the following method. A series of preliminary samples were quickly taken and their H_2S content immediately estimated colorimetrically. So the depth of the beginning of H_2S zone was determined and the samples for the basic analyses and experiments were taken starting from the upper boundary of the H_2S zone.

In water samples in depths of 300–400 m the H_2S content was analysed by a colorimetric method (ZAWODNOV, 1962; SOROKIN, 1970) and in the lower layers by iodometry. In the intermediate layer O_2 and H_2S are both present. Oxygen in the presence of H_2S was estimated by the following method. Into the sample (150 ml bottle) 0·2 ml of a 3% solution of starch and 0·1 ml of 10% HCl were added.

As shown in Figure 1a, 0·1N I_2 solution was added to the bottle drop by drop through a funnel until a blue colour developed. Then the bottle was opened, the oxygen fixed and estimated by the Winkler method.

Redox potential was measured in balloons using a smooth platinum needle electrode (SOROKIN, 1970).

The total number of bacteria was counted by direct microscopy of stained cells on millipore filters (pore size 0·1–0·3 μm). A plastic film with a 1 × 1 cm square hole was placed below the filter to allow for a correction for filter contamination (SOROKIN, 1962). Some 20–30 ml of water were filtered through the 1 cm^2 of the filter.

The biomass of bacteria was calculated assuming that the average volume of one bacterial cell was 0·15 μm^3 in the 0–150 m layer, 0·3 μm^3 in the 150–300 m layer, and 0·2 μm^3 in the 300–2000 m layer. These values were found by measuring the cells of bacteria directly on the stained filters. The values of their average volumes were multiplied by 2·5, a correction coefficient for their contraction on the dry preparation. The carbon content was accepted as 10% of the raw biomass (TROITSKI and SOROKIN, 1967).

Bacterial production was estimated by observations in isolated samples, kept in bottles under conditions close to those in situ. The samples from the

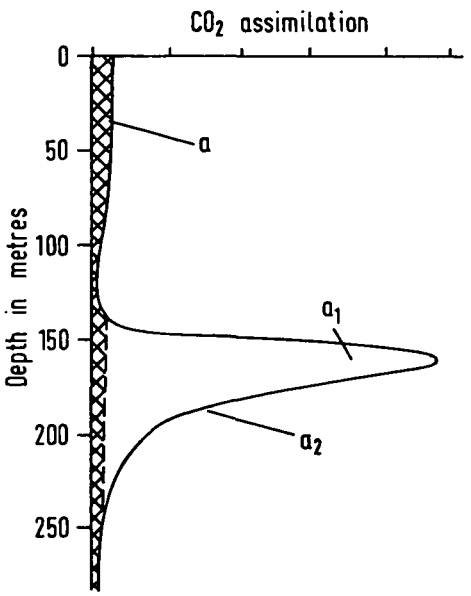

Figure 2. The relation between the heterotrophic assimilation of $CO_2(a)$; chemosynthesis (a_1); total dark assimilation of CO_2 by the aquatic microflora (a_2).

layer 0–150 m were previously filtered through the finest plankton net. The production of bacteria in the samples was evaluated by two different methods: by the estimation of the increase of the biomass and from CO_2 assimilation.

The increase of biomass was measured by direct count. The value of the total dark assimilation of CO_2 was estimated by the radio-carbon method (SOROKIN, 1964b). The assimilation of CO_2 in the layer 0–100 m was accepted as that used by heterotrophic microflora (a). The value of the autotrophic CO_2 assimilation – chemosynthesis – which took place below 100 m, was obtained by subtracting the average value of CO_2 assimilation found in the surface 0–100 m layer from the value of the total dark assimilation of CO_2 (a_2) in the samples (Fig. 2). This calculation is based on the assumption that the number of heterotrophic bacteria in the layers of active chemosynthesis (100–300 m) is about the same as that in the surface waters and is supported by data on the vertical distribution of these bacteria (Fig. 13).

Heterotrophic bacteria use about 3% of CO_2-carbon of the total carbon assimilated during biosynthesis (SOROKIN, 1961). So their production (P) can be calculated using values of heterotrophic carbon dioxide assimilation (ROMANENKO, 1964). It is equal to $P = 300\ a_1$ µg/l of raw biomass per day[1], if the value of a_1 is expressed as µg C/l per day. In the surface waters (0–100 m) the production of bacteria was calculated with the use of this formula. In the intermediate zone, where the chemosynthesis took place as well as heterotrophic CO_2 assimilation, the value of P_1 was calculated by the following way: $P_1 = a \cdot 300 + (a_2 - a) \cdot 10$ µg/l of raw biomass per day, where a is the average dark CO_2 assimilation in the layer 0–100 m, and a_2 total dark CO_2 assimilation, both as µg C/l.

The relative vertical distribution of the methane oxidizing bacteria and thiobacilli was studied by measuring the potential activity of their natural population in isolated samples (SOROKIN, 1964). Their activity was measured by chemosynthesis; $C^{14}O_2$ assimilation in samples enriched with a bubble of methane or 50 mg/l thiosulphate respectively and with a bubble of air or $NaNO_3$ solution as a source of oxygen.

The number of sulphate-reducing bacteria was estimated by counting their colonies grown on millipore filters. After filtration of a 100 ml sample the filters were placed in an agar medium, containing sulphate and ethanol as shown in the Figure 1(b). After a month the number of black colonies of sulphate-reducing bacteria were counted.

Heterotrophic bacteria was estimated by growing their colonies on the surface of millipore filters. After filtration of 10 to 100 ml of water the filter was placed on the surface of a fish-peptone agar medium or on the surface of a poor agar medium (seawater + 10 mg/l of peptone + 20 mg/l KH_2PO_4). Anaerobic heterotrophes were counted in the semi-solid fish peptone agar medium enriched with sodium sulphide.

THE RATE AND MECHANISM OF SULPHIDE OXIDATION

The rate and mechanism of the H_2S oxidation were studied with the use of labelled sodium sulphide Na_2S^{35}. The general principle of the method was suggested by IVANOV (1967). Labelled sulphide is added to the sample. After

1) In later studies the ratio $P:a$ was found to be 200 for natural microflora as against 300 found in cultures (SOROKIN, 1971).

a suitable incubation the radioactivity of the rest of the sulphide and of the products of its oxidation are measured.

The method has been revised (SOROKIN, 1970) and the following variant was used during this work in the Black Sea.

THE PREPARATION OF THE WORKING SOLUTION OF Na_2S^{35}

The method uses a scaled down version of the apparatus described by SOROKIN (1970) for the estimation of H_2S. Into the distillation test tube were added 3 ml 3% solution of Na_2CO_3, and some crystals of $Na_2S^{35} \cdot 9\ H_2O$ with the nominal radioactivity 5 millicuries, 20 mg of the non-labelled Na_2S, and 100 mg $CdCl_2$. Three ml 0·1N NaOH were added to the absorber of the apparatus. After acidification of the solution in the distillation test tube with 5% HCl the H_2S formed was swept out with a stream of hydrogen gas into the absorber. The solution of Na_2S so obtained in the absorber contained about 0·5 mg/ml S^{2-} with a radioactivity of about $70 \cdot 10^6$ c.p.m./ml. The solution was poured into the series of 0·1 ml glass capilliary ampoules which were then sealed.

Just before the use of the working solution the content of an ampoule was diluted in 1 ml of deep Black Sea water containing H_2S. Using a micropipette, 0·05 ml of this solution (containing about 0·002 mg S^{2-} with a radioactivity corresponding to about 10^5 c.p.m.) was added to a sea water sample of 300 ml, without leading to any significant change in the initial redox potential or the initial concentration of H_2S.

THE ESTIMATION OF THE RATE OF H_2S OXIDATION

The estimations were carried out with two subsamples in 300 ml balloons filled directly from a water bottle after pouring off 2–3 times their volume of water to avoid contamination with oxygen in the atmosphere. After adding the Na_2S^{35} solution the balloons were closed and kept at 5–7°C for 1–2 days. Then 20 ml samples were taken from the balloons and put into test tubes containing 0·5 ml of a "carrier" solution of nonlabelled sulphur compounds. The latter solution contained 8% $Na_2S \cdot 9\ H_2O$ and 15% $Na_2S_2O_3$. The test tubes were closed with rubber stoppers and their contents carefully mixed.

The solutions in the test tubes were analyzed to determine the relation between the radioactivity of the sulphate sulphur plus the sulphate sulphur present in the thiosulphate (r) and the radioactivity of all the sulphur compounds present (R). This is necessary because of the following considerations which followed from earlier experiments on the mechanism of sulphide oxidation in solutions (SOROKIN, 1970). Chemical oxidation of sodium sulphide results in the formation of sulphate and thiosulphate. In the presence of active thiobacilli the first stage in the oxidation is similar and there is a rapid oxidation to sulphate and thiosulphate. Molecular sulphur is formed only in insignificant amounts (0·5–1%) and the other possible products of sulphide oxidation (polythionates, sulphites) are not formed in detectable amounts if oxidation proceeds in neutral solution at a low initial concentration of sodium sulphide (less than 20–40 mg S^{2-}/l). So the rate of sulphide oxidation is not properly measured by its disappearance from solution or only by the accumulation of sulphate ions as attempted by others (IVANOFF, 1957; SCOPINTZEV *et al.*, 1969). The more appropriate measurement of the rate of sulphide oxidation is the rate of formation of sulphate sulphur taking into consideration also the

sulphate sulphur used in the formation of thiosulphate. This rate can be found if one knows the initial concentration of sulphide sulphur and relative changes in sulphate and sulphide over the period during which oxidation occurs. The latter is given by the ratio (r/R).

The practical details of the determinations are as follows. From the test tubes in which the content of balloons was fixed with the sulphide-thiosulphate 'carrier' solution, 4 ml subsamples were taken and transferred to the flasks containing 2 ml of 10% $AgNO_3$ solution. The Ag_2S formed as a precipitation of sulphide ions after the hydrolysis of thiosulphate ions and the precipitation of their sulphide sulphur. After the precipitation of Ag_2S only sulphates and the sulphate sulphur of the thiosulphate remained in solution. The sulphide sulphur was separated off from the sulphate sulphur by filtration on a millipore filter covered with a thin layer of $MnCO_3$ formed by the previous filtration of a suspension of $MnCO_3$. After heating and acidification the sulphates in the filtrate were sedimented as $BaSO_4$. The latter was filtered on a millipore filter and dried. Then the radioactivity (r) of the sulphate sulphur plus half of the thiosulphate sulphur was measured directly under the counter.

The analysis of the total radioactivity of the sulphur in the initial sample (R) was made by its total oxidation to sulphate. To do this a 2 ml sample was put into a flask to which was added 2 ml 0·2 N $KMnO_4$ and 20 ml of water, followed by 0·5 ml 10% HCl and 2 ml 10% $BaCl_2$. The mixture was boiled for 2–3 min, and then discoloured with hydrogen peroxide. The precipitate of $BaSO_4$ was filtered and its radioactivity was measured giving half of the total radioactivity of sulphur in the initial solution (R).

Radioactivity counts of the precipitates were made with an end window counter ($d = 25$ mm). Self-absorption correction coefficients (SOROKIN, 1970) were used in the final calculations of radioactivities. The absolute rate of the sulphide oxidation (V) was calculated according to the following formula:

$$V = (K \cdot (a - a_1) \cdot 24)/t \text{ mg/l per day},$$

where K = the absolute concentration of sulphide in the initial solution as mg/l; a = the final relation (r/R); a_1 = the initial relation (r/R) in the "working" solution of the labelled sulphide, and t = time in hours of the oxidation process.

THE MECHANISM AND INTERRELATION BETWEEN THE CHEMICAL AND BIOLOGICAL OXIDATION OF SULPHIDES AND THE EFFICIENCY OF THE USE OF ENERGY RELEASED DURING CHEMOSYNTHESIS BY BACTERIA

Estimates were made of the absolute oxidation rate of hydrogen sulphide, and of the rate of formation of all the main oxidation products to study the mechanism of the process and the role of biological factors. Experiments were made with and without an antiseptic and analyses were made of sulphur, sulphide, thiosulphate, and sulphate. The techniques have been described (SOROKIN, 1970) and the modification used in the Black Sea work is as follows.

The working solution of labelled sulphide was added to the subsamples drawn off into the balloons and pure chemical chloroform was added to one subsample. The balloons were kept at 5–7°C for 1–2 days, after which the distribution of S^{35} in the remaining sulphide and its oxidation products was determined. Separation was carried out after addition of the "carrier" solution as follows:

The separation of molecular sulphur

A 4 ml sample was filtered through a millipore filter (pore size 0·1–0·3 μm) and the filtrate collected in a flask with 1 ml 10% $CdCl_2$, and 0·2 ml 10% suspension of $MnCO_3$. The filter with the precipitated sulphur was washed several times with water whose total volume did not exceed 10 ml. The filter was dried, and the radioactivity of molecular sulphur on it was measured under the counter.

Separation of sulphide

The precipitate of CdS formed in the filtrate was filtered on the millipore filter covered with a layer of $MnCO_3$. The filter and precipitate was washed with 10 ml of water and then put into a small flask containing 20 ml water, 4 ml 0·1 N $KMnO_4$, 2 ml 10% $BaCl_2$, and 1 ml 20% HCl. The precipitate was washed out into this solution then boiled for 5 min, after which the hot solution was discoloured with H_2O_2. The precipitate of $BaSO_4$ formed was filtered on a millipore filter, dried, and counted.

Separation of sulphate

Following the separation of CdS, sulphate was separated from the filtrate by adding 1 ml 10% $BaCl_2$, phenolpthalein indicator, and several drops of 0·1 N KOH until a red colour developed. This procedure led to precipitation of $BaSO_4$ without heating in the presence of thiosulphate ions. After 2–3 min the solution was acidified with 2% acetic acid in the presence of methyl-red indicator. The precipitate of $BaSO_4$ was filtered, dried, and counted.

Estimation of thiosulphate sulphur

After the removal of sulphate the filtrate contained thiosulphate, and trace amounts of polythionate and sulphite. They were oxidized to sulphate and estimated as $BaSO_4$. To the filtrate were added 3 ml 0·1 N $KMnO_4$, 0·3 ml 15% HCl, and 2 ml 10% $BaCl_2$. The solution was boiled and discoloured with H_2O_2. The precipitate of $BaSO_4$ was filtered out and counted.

As a control the total radioactivity of all the sulphur in the solution was determined after oxidation to sulphate as has been described earlier (p. 429). The counts for the radioactivity of the separate sulphur compounds (r) were expressed as a percentage of R (the total count).

The separation of the chemical and biological processes of sulphide oxidation was made with chloroform, which inhibited bacterial activity in the water samples without any influence upon the chemical process of sulphide oxidation (SOROKIN, 1967). In the presence of chloroform only chemical oxidation of H_2S occurs (in the tables designated as "chem"). In the samples free from chloroform both chemical and biological oxidation of sulphide occur with the participation of thiobacilli (in the tables designated as "chem + biol"). A comparison between the sulphur compounds formed during sulphide oxidation in the presence and absence of chloroform allows an evaluation of the degree to which bacteria are involved in the process as "biol" = "chem + biol" − "chem". The amount of sulphide oxidized to sulphate was found from the values of the relative amount of the sulphate sulphur formed. The latter (n) = SO_4^{2-} −

$S + 0{\cdot}5\ S_2O_3^{2-} - S\%$. The absolute amount of S^{2-} oxidized to S^{6+} ($a-$) was found as described before (p. 429).

The efficiency (E) of the use of the energy of oxidation by bacteria for chemosynthesis was calculated accordingly from the formula:

$$E = \frac{122 \cdot m_1}{171 \cdot m_2},$$ where m_1 = chemosynthesis, in equivalents C/l/day;
m_2 = sulphide sulphur oxidized to sulphate, in equivalents S/l/day;
122 = calories for the biosynthesis of 12gC of organic matter from CO_2;
171 = calories released during the oxidation of 32g of sulphide sulphur to sulphate.

RESULTS OF ESTIMATION

NUMBER, BIOMASS, AND PRODUCTION OF BACTERIA IN THE UPPER AEROBIC ZONE

Station positions are shown in Figure 3, and relevant data are given in the Figures 4 and 5 and in Tables 1, 2, 3 and 4. They show that the total number of bacteria near the surface is equal to $6–15{\cdot}10^4$/ml, and their biomass to 10–30 mg/m³. These numbers are similar to those at the sea surface (SOROKIN, 1964a), and are close to the previously published data (LEBEDEVA, 1959; SOROKIN, 1964). Most of bacteria in the oxygen zone are short rod and coccoid forms of average volume $0{\cdot}15\ \mu^3$. So the biomass of bacterial-plankton in surface waters is 3–5 times lower than the biomass of phytoplankton which is about 100 mg/m³ (MOROZOVA-VODYANITSKAYA, 1954).

The ratio of production to biomass P/B (Table 1) in the layer 0–150 m averaged to 0·4–0·7. The bacterial biomass estimates from direct count and from CO_2 assimilation give similar values. This agreement was interesting as the level of heterotrophic CO_2 assimilation in this layer was so low that its precise estimation was difficult having regard to the sensitivity of the method. The latter rate of assimilation in the surface layer averaged 0·02–0·06 mgC/m³ per day.

The production of bacterial-plankton was about 2–10 mg of raw biomass/m³ or 0·4–2 mg/m³ dry organic matter per day. This is surely insufficient to serve

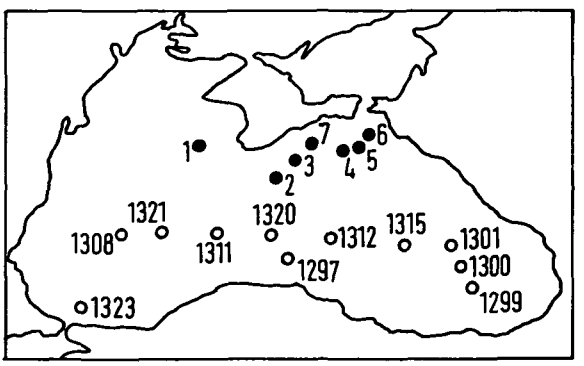

Figure 3. Station positions in the Black Sea
○ – 1964 cruise, R. V. "Lomonosov"
● – 1967 cruise, R. V. "Gonetz"

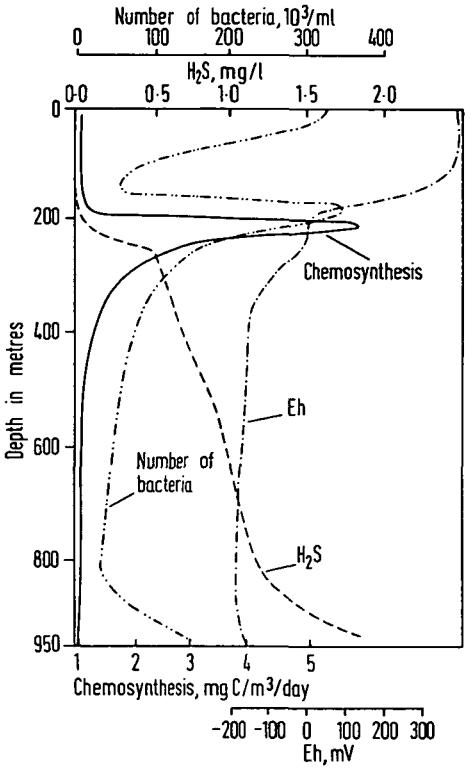

Figure 4. The vertical distribution of H_2S, *Eh*, chemosynthesis and total number of bacteria in the water column opposite the Bosporus (depth 1 000 m), Station 1323.

TABLE 1. The biomass and production of bacteria in the water column at Station 1297, open deep sea. For position see Figure 3.

Depth m	H_2S mg S^{2-}/l	*Eh* mV	Microscopic count of bacteria: Total number 10^3/ml	Microscopic count of bacteria: Biomass (*B*) mg/m^3 (raw weight)	CO_2 assimilation by microflora mgC/m^3/day: Hetero-trophic	CO_2 assimilation by microflora mgC/m^3/day: Autotrophic (chemo synthesis)	Production of bacteria as calculated from CO_2 assimilation (*P*) mg/m^3/day of raw weight	Ratio: *P*/*B*
2	0	+390	61	9	0·02	0	6	0·7
45	0	–	128	19	0·04	0	12	0·6
150	0	+290	94	14	0·03	0·07	9·7	0·7
156	0·01	+30	102	15	0·03	0·25	11·5	0·8
160	0·025	+10	136	35	0·03	1·30	22·0	0·6
161	0·045	−10	473	124	0·03	5·97	69	0·55
162	0·09	−10	246	64	0·03	4·80	57	0·9
172	0·15	−20	186	48	0·03	3·74	46	0·9
185	0·21	−30	221	57	0·03	2·51	34	0·6
200	0·41	−70	142	37	0·03	0·62	12	0·3
250	1·22	−105	113	29	0·03	0·13	7	0·3

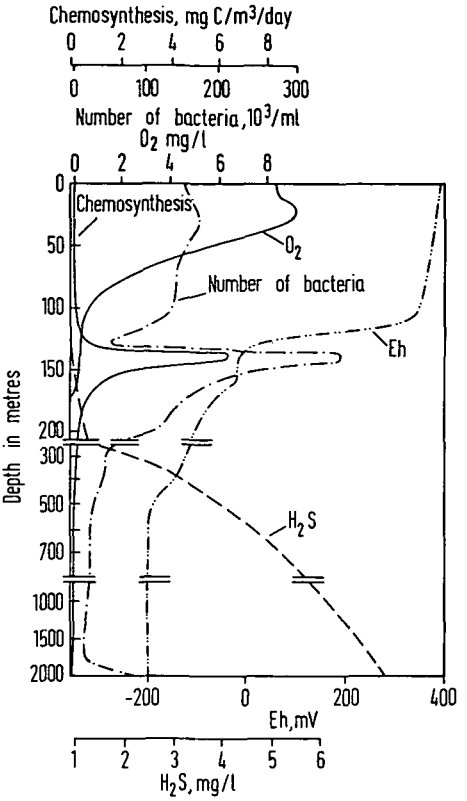

Figure 5. The vertical distribution of O_2, H_2S, *Eh*, chemosynthesis and total number of bacteria in the water column, Station 1321.

TABLE 2. The biomass and production of bacteria in the water column on the slope opposite Yalta, depth 750 m. (Close to Station 2 in Figure 3).

Depth m	H_2S mg S^{2-}/l	O_2 mg/l	*Eh* mV	Microscopical count of bacteria: Total number 10^3/ml	Microscopical count of bacteria: Raw biomass mg/m³ (*B*)	CO_2 assimilation by microflora mgC/m³/day: Hetero-trophic	CO_2 assimilation by microflora mgC/m³/day: Auto-trophic	Production of bacteria as calculated from CO_2 assimilation mg/m³/day (*P*)	*P/B*
0	0	8·6	+360	110	16	0·07	0	21	1·3
90	0	8·9	+300	94	14	0·02	0	20	1·4
120	0	1·6	+242	19	3	0·01	0	3	1·0
175	0	0·26	+120	56	8	0·01	0·1	4	0·5
180	0·04	0·12	−10	113	34	0·01	0·9	12	0·4
190	0·08	0·05	−20	264	79	0·01	1·45	17	0·2
200	0·14	0	−32	243	74	0·01	5·08	54	0·7
215	0·20	0	−40	170	51	0·01	2·90	32	0·6
250	0·45	–	−65	94	28	0·01	0·46	8	0·3
300	1·20	–	−75	42	12	0·01	0·11	1	0·1
500	2·80	–	−120	16	5	0·01	0·01	0·1	0·02
740	4·27	–	−145	75	16	0·01	0·03	0·3	0·02

TABLE 3. The estimation of the bacterial production in the water column at the open sea Station 1321 (see Fig. 3) by two different methods. Exposure time: 1 day at the temperature similar to that in situ.

		Production of raw biomass of bacteria (P), mg/m³/day						
		Calculation from the change in their number in the isolated samples				Calculation from the values of CO_2 assimilation		
Depth m	Eh mV	Initial number of bacteria 10^3/ml	Initial biomass mg/m³	Final number 10^3/ml	P_1	Heterotrophic CO_2 assimilation mgC/m³	Chemosynthesis mgC/m³	P_2
20	+420	142	21	188	6·9	0·04	0	12
125	+80	81	12	102	31	0·02	0	6
130	+10	56	8	64	1·2	0·01	0·25	5·5
135	−10	390	88	540	45	0·01	6·19	65
150	−25	265	56	320	19·5	0·01	1·86	21
175	−65	168	43	190	6·6	0·01	1·16	15
200	−100	114	29	130	4·8	0·01	0·33	6
300	−125	64	16	70	1·8	0	0·04	3·4
400	−160	56	14	43	−4	0	0·03	0·3
1000	−190	29	6	23	−1·7	0	0	0
2000	−195	47	10	55	+2·4	0	0·03	0·3

TABLE 4. The potential activity of the denitrifying thiobacilli populations in samples taken from different depths at the open sea Station 1312. Exposure time 3 days at 18°C.

		Unchanged samples		After adding 50 mg/l KNO_3 + 50 mg/l of $Na_2S_2O_3$	
Depth m	H_2S mgS²⁻/l	Total number of bacteria 10^3/ml	Chemosynthesis mgC/m³	Total number of bacteria 10^3/ml	Chemosynthesis mgC/m³
0	0	564	0·16	480	0·7
100	0	431	0·06	300	0·12
130	0	231	0·12	592	4·2
140	0	568	3·02	7 300	17
150	0·18	1 550	12·2	13 000	23
200	0·75	790	4·0	23 000	59
250	1·24	560	0·78	32 000	115
350	2·40	131	0·44	47 000	135
1 000	5·10	28	0·06	38	0·6

as a substantial source of food for the plankton filter feeders, for it is 5–10 times lower than needed to supply their normal requirements (JØRGENSEN, 1962). But their trophic role is probably greater in the layers of an accumulation of detritus and bacteria at density gradients, and in the regions of cyclonic upwellings where a mass of bacteria from the zone of active chemosynthesis is carried to the surface (LEBEDEVA, 1959; DATSKO, 1954). Regions with a relatively high biomass of bacteria have been found in the frontal zones of currents and counter-currents, for example, near the Bosporus (Fig. 4) and in the shallow north-western part of the sea (KRISS, 1963).

Near the shore the number of bacteria, their biomass and production sharply increased (Fig. 6). Close to the shore line the number of bacteria, their biomass and their production, was respectively 3–5, 8–10, and 20–25 times greater than that in the open sea. The reproductive rate in the littoral waters is very high and their number is probably stabilized by grazing of filter feeding molluscs and crustaceans. But only 3 km from the shore the number and production of bacteria fell close to those in the open sea. At a distance of 5–10 km it was equal to that in the surface waters of the deep sea regions (Table 5).

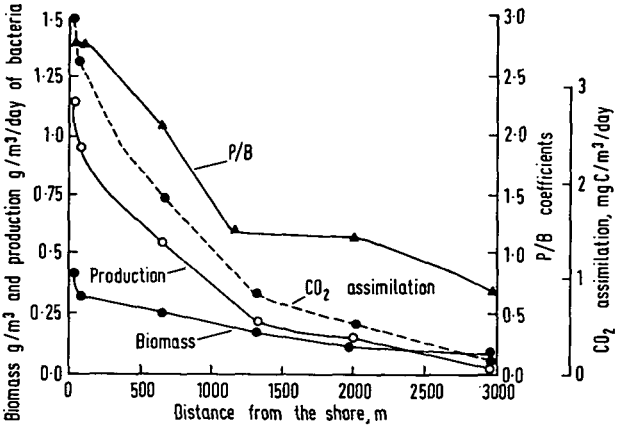

Figure 6. The changes in the number of bacterioplankton, biomass and production at the different distances from the shore opposite the Crimea.

TABLE 5. The total number of bacteria and the rate of CO_2 assimilation at the nearshore zone opposite Yalta (depth 165 m), close to Station 2 in Figure 3.

Depth, m	Total number of bacteria 10^3/ml	CO_2 assimilation by bacteria mgC/m³/day
0	122	0·10
170	56	0·05

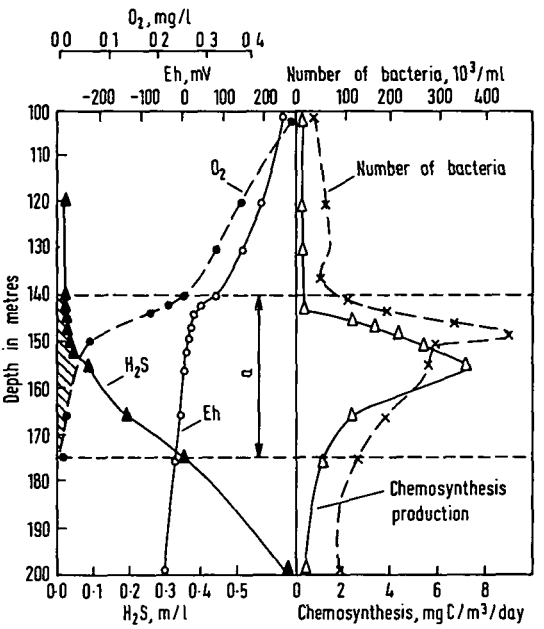

Figure 7. The vertical distribution of O_2, H_2S, *Eh*, chemosynthesis, and total number of bacteria in the intermediate zone, Station 1301. a, the layer of coexistence of H_2S and O_2.

THE NUMBER, BIOMASS, AND PRODUCTION OF BACTERIA IN THE INTERMEDIATE ZONE

Chemical characteristics, bacterial distribution and production were studied in the intermediate zone. Production was mainly by chemosynthesis. The results are given in Tables 1, 2, and 3 and Figures 4, 5, 7, 8, and 9. The data show that at the deep sea stations the H_2S zone begins at depths of 135–150 m. The lowest detectable levels of oxygen were found at depths of 170–180 m. So in the intermediate zone there is a 20–40 m layer where both these dissolved gasses coexist in very low concentrations (GOLOLOBOV, 1953; SCOPINTSEV *et al.*, 1966). Redox potential (*Eh*) in this layer has a low vertical gradient and only fell 5–10 mV. Below the intermediate zone the *Eh* and H_2S concentration gradients increased sharply. These features of the intermediate zone show that it is here that the most active oxidation of hydrogen sulphide occurs. This conclusion is supported by direct estimations (Figs. 16–19).

The number and activity of the microflora living in the intermediate zone showed the active participation of bacteria in the oxidation of H_2S and other reduced products that had accumulated in the anaerobic zone. Among the

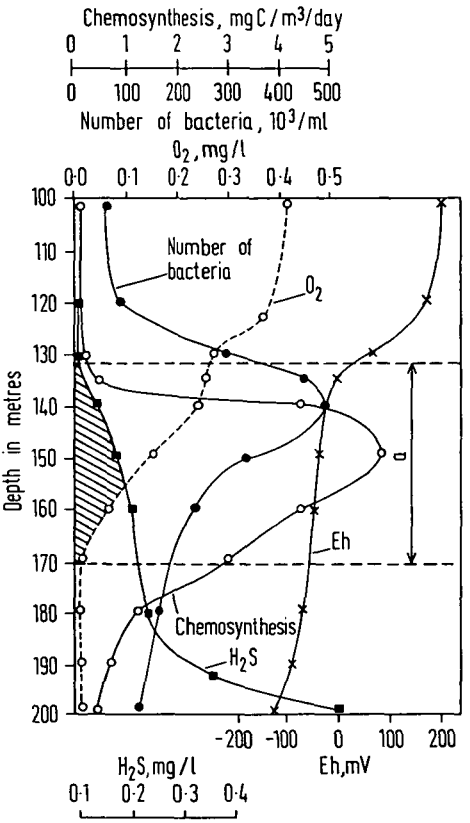

Figure 8. The vertical distribution of O_2, H_2S, *Eh*, chemosynthesis and the total number of bacteria in the intermediate layer at station 1308. a, the layer of coexistence of H_2S and O_2.

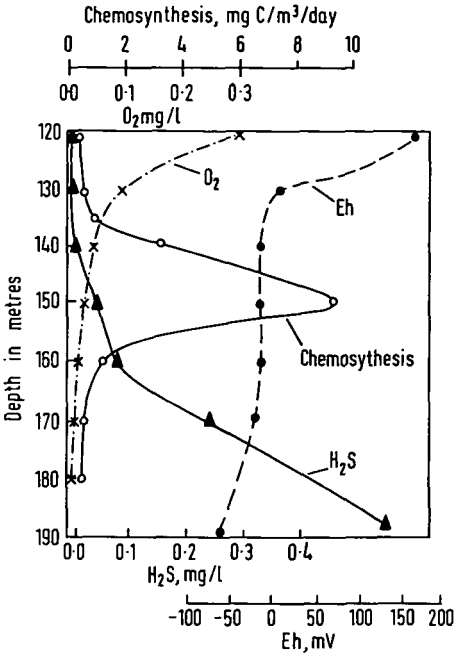

Figure 9. The vertical distribution of O_2, H_2S, *Eh*, and chemosynthesis at Station 1311.

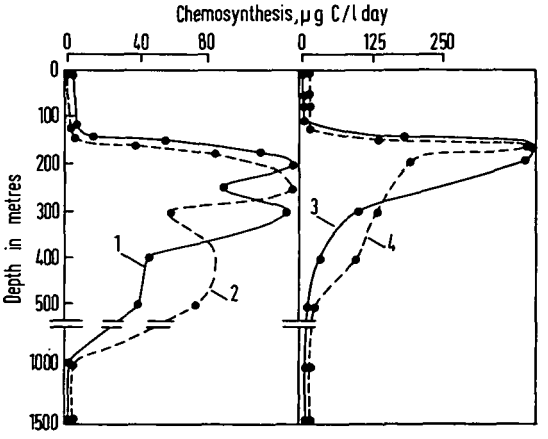

Figure 10. The potential activity of aerobic thiobacilli (1), denitrifying thiobacilli (2) and methane oxidizing bacteria in the presence of methane gas (3) or methyl alcohol (4); station 1315.

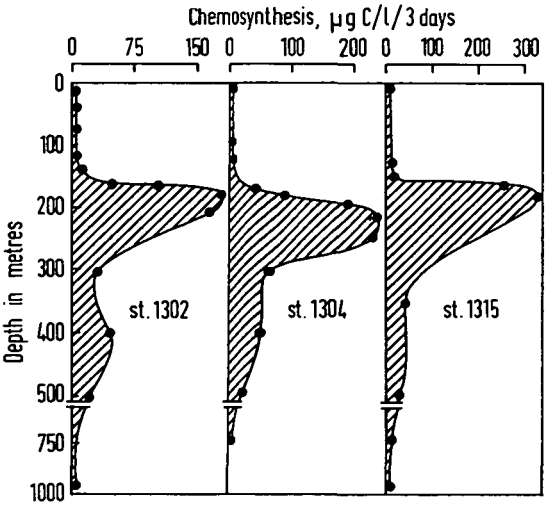

Figure 11. The potential activity of thiobacilli in the water column.

latter the most important is methane. This is shown by the very high potential activity of the methane-oxidizing bacteria, which are very specific forms (Fig. 10).

The total number of bacteria has a maximum in the intermediate zone and is equal to 20–40·10⁴/ml, that is 2–4 times higher than in the surface waters.

The biomass of bacteria increases in the intermediate zone 4–6 times, for the volume of the cells here is about twice that in the surface waters.

The bacterial plankton is represented here by long rods and chain forms, which have been described as "filamentous" bacteria (KRISS, 1962). The daily production of bacteria is accelerated by chemosynthesis and approximates to 40–60 mg/m³/day and the ratio P/B is also high (0·5). The layer of active chemosynthesis is very thin, only 10–15 m. The layer of the most active chemosynthesis was usually found at the depth where the *Eh* value was about −10 to −20 mV, H_2S concentration 0·1 to 0·3 mg/l, and the O_2 content 0·10 to 0·15 mg/l.

Measurements show that the greatest potential activity of thiobacilli occurs between 150–300 m (Figs. 10, 11). Within this layer thiobacilli are spread up and down by vertical turbulence and carried on the surface of sedimenting detritus. So the layer within which the active thiobacilli come to be distributed is about 10 times thicker than the layer of their actual production, the layer of intensive chemosynthesis. Denitrifying thiobacilli are also very active in this layer (Table 4, Figs. 10, 11). The addition of nitrate into water from this layer leads to a rapid increase in the rate of chemosynthesis.

In the intermediate zone and in the layers just below it there was a surprising high potential activity of methane-oxidizing bacteria. Introduction of a bubble of methane (or methyl alcohol) into samples taken from depths of 150–400 m inevitably caused a rapid increase in the number of these bacteria, the rate of the chemosynthesis and the number of bacteria increasing several hundred

times (Fig. 10). One would expect this phenomenon to be associated with samples from gas or oil deposits or marshy lakes, rather than from samples from an oligotrophic basin such as the Black Sea. These data suggest the inflow of significant amounts of methane into the deep water of the Black Sea. If this is so, the most possible source of the gas might be geological rather than the methane formation in the bottom sediments. Previous data (SOROKIN, 1964) have showed that the microbiological processes at the bottom of the Black Sea are very weak and proceed only in a thin layer (5–10 cm). So the inflow of methane gas into the Black Sea from the surrounding sedimentary rocks formed in an ancient shallow basin rich in organic material appears to be possible. Geologists have also draw attention to the presence of gas carrying rocks in the Black Sea area. So it is of some interest to have measurements of the methane dissolved in the deep waters of the Black Sea. First analyses confirmed the presence of methane (SOROKIN, 1963; ATKINSON and RICHARDS, 1967) and gave similar results indicating concentrations of 0·1 cm^3/l (at NTP) methane in deep waters.

At the stations up the slopes opposite Yalta (Station 1, Table 2) and opposite the Bosporus (Station 1323, Table 4) the upper boundary of the H_2S zone was deeper at 180–200 m. The intermediate zone is much thicker here than in the open sea and the layer of active chemosynthesis is also correspondingly much larger.

THE NUMBER AND PRODUCTION OF BACTERIA IN THE DEEP ANAEROBIC WATERS

The metabolic activity and the number of bacteria fall rapidly in the deeper water (Tables 2 and 3, Figs. 4 and 5), owing to the lack of available hydrogen acceptors and assimilable organic matter. The redox potential is stabilized at a level of −160 to −190 mV at depth 400–500 m. At so low a potential the life of anaerobic microflora is possible only in the presence of a relatively high concentration of assimilable organic matter or in the presence of nitrates, for the efficiency of biosynthesis in anaerobes (excluding denitrification) is about 5 to 7 times less than that of aerobes.

The total number of bacteria here is 5–10 times lower and is equal to $1–2 \cdot 10^3$; per ml. The greater number of the cells are represented by the hardly staining "shadows" of bacteria which form chains.

Attempts to estimate the production of bacteria in deep waters by direct microscopy gave mostly negative values. From this result, and that of the

TABLE 6. The total number of bacteria in the near bottom water layer at the open sea Station 1300 (depth 2 000 m)

Distance of sampling above the bottom, m	Total number of bacteria 10^3/ml	Predominate form of bacterial cells found by microscopic examination
50	25	small rods and coccoid
6*	112	small rods and coccoid
3*	138	small rods and coccoid
1*	392	chains of rods ("filamentous") forms

* The samples were taken with water bottles attached to a special frame lowered to the bottom

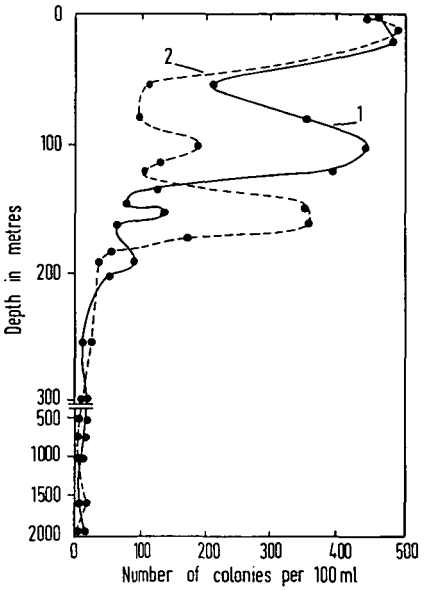

Figure 12. The distribution of the heterotrophic bacteria (plate count) at Stations 1308 (1), and 1320 (2).

zero values of CO_2 assimilation and low potential activity of bacteria, one would conclude that microflora which is detected here by microscopy on filters include mainly starving and dying cells. They could be carried into the

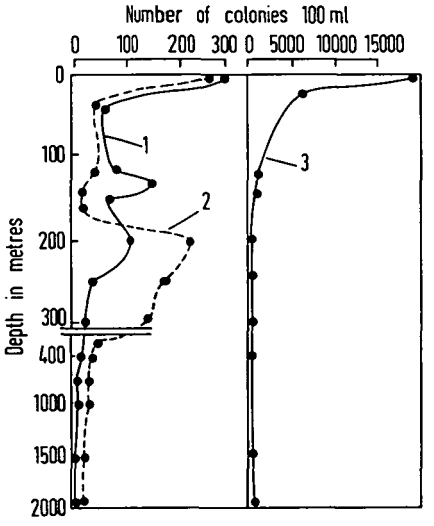

Figure 13. The distribution of the heterotrophic aerobic (1) and anaerobic (2) bacteria, and bacteria which were grown in the "poor" agar medium (3) at Station 1321.

deep water passively on the surface of the suspended material or by turbulence, and are preserved here in a state of low metabolic activity by the inhibition of sulphide ion and low *Eh*. It is only very near the bottom that bacteria are relatively abundant (Table 6). Chain forms are usually found here.

VERTICAL DISTRIBUTION OF HETEROTROPHIC BACTERIA AND OF SULPHATE-REDUCING BACTERIA

Plate counts of heterotrophic bacteria showed that their first maximum (up to 500 per 100 ml) was near the surface and that there was a second but lesser maximum in the intermediate layer (Fig. 12).

The number of the anaerobic heterotrophes in the surface layer was about 50–300/100 ml. So the number of heterotrophic bacteria which grow in fish-peptone media is very low in the open sea, and is close to that found in the poor oceanic water (SOROKIN, 1964a). The ratio "total number": "number of plate counted heterotrophes" varies in the Black Sea from 30000 to 500000. It is much higher than that in the oligotrophic tropical waters of the Pacific Ocean (10000 to 20000). The value of this relation reflects firstly the low content of the assimilable organic matter of protein nature in the Black Sea waters, and secondly the important role of autotrophic production of bacteria cells which is not directly connected with the disintegration of the organic matter.

Bacterial counts obtained by growing colonies on the poor agar medium are about 100 times greater than those obtained on fish-peptone agar medium (Fig. 13). The growing colonies are very small and can only be seen after staining and examining the filters under low magnification. The numbers

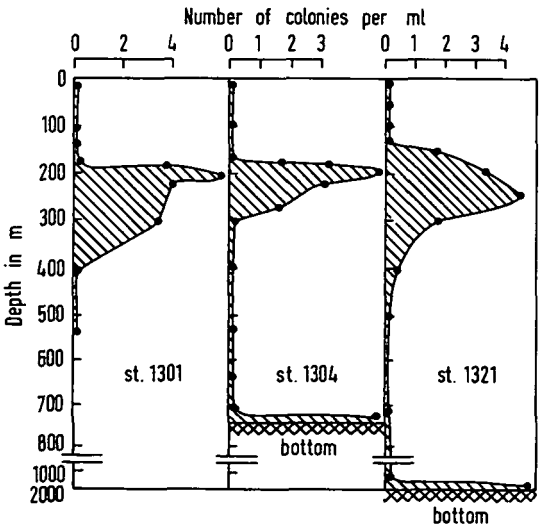

Figure 14. The vertical distribution of sulphate-reducing bacteria.

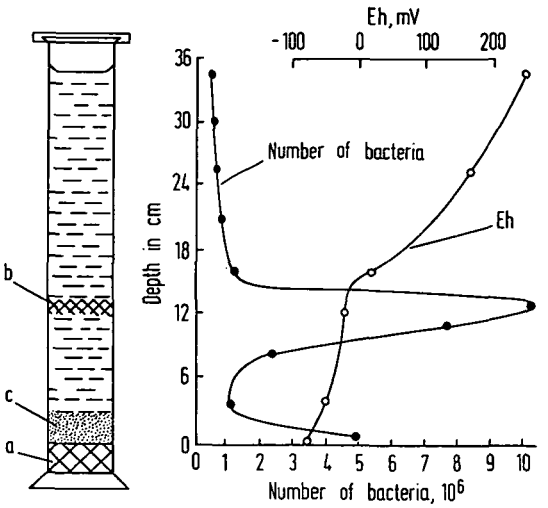

Figure 15. Details of the experimental model in a 2 l cylinder and the distribution of the total number of bacteria and *Eh*. a, agar with Na_2S; c, zone of the secondary development of sulphate-reduction; b, layer of growth of thiobacilli.

of oligocarbophylic bacteria decrease in deeper water and fall by several orders of magnitude below 150 m, suggesting that only a small part of the planktonic organic matter reaches the deeper layers.

The distribution of the sulphate-reducing bacteria counted in nutrient medium (Fig. 14) supports previous data (SOROKIN 1964), obtained by a radio-sulphur method. In the Black Sea the layer of the sulphate reduction in the water column lies just below the intermediate layer of active chemosynthesis at 200–300 m.

AN EXPERIMENTAL MODEL OF THE PROCESSES OF SULPHUR TURNOVER IN THE BLACK SEA

The processes of sulphide oxidation and the related autotrophic bacterial biosynthesis were reproduced in an experimental model similar to that described by EGUNOV (1895) (Fig. 15). Agar medium (2·5% of agar, 3% of thiosulphate; 0·5% of Na_2S. 9 H_2O; 5% NaCl; 0·1% $(NH_4)_2SO_4$; 0·2% KH_2PO_4; $FeSO_4$-traces) was poured into a 2 l glass cylinder. When the agar had set the cylinder was filled with H_2S containing water taken from 200 m (Station 1308). Two weeks later at the distance 15 cm above the agar a layer showing the strong development of thiobacilli was observed. Below this layer there was the slightly muddy anaerobic water containing H_2S and above it it a layer of clean water containing oxygen. Stratification was maintained by the higher concentration of salts – dissolved from the agar – in the lower layer of water. In the intermediate layer thiobacilli formed a film from which strings began to hang down to the bottom. Some of these strings broke off and fell to the bottom. After several days sulphate-reduction began at the surface of agar at the expense of the organic matter in the sedimented film

of thiobacilli, other organic matter being absent. Sulphate-reduction was detected by the appearance of a black ring of small colonies of sulphate-reducing bacteria on the walls of the cylinder.

The vertical distribution of *Eh* and the distribution of bacteria in the cylinder were quite similar to those in the Black Sea, as can be seen by comparing Figures 4 and 5 with Figure 15.

SULPHIDE OXIDATION AND THE EFFICIENCY OF CHEMOSYNTHESIS
R. V. LOMONOSOV, AUTUMN 1964

Table 7 gives the results of several replicate estimations of the relation between the various sulphur compounds formed in the same sample, after the oxidation of labelled sulphide. The deviations of ratios r/R (see p. 429) do not exceed 1·3% of the mean which shows that the method is reliable.

First attempts to determine the rate of H_2S oxidation were made in 1964. In these experiments the "working" solution of Na_2S^{35} was used which contained a significant amount of the unlabelled sulphur compounds (see p. 428), leading to detectable changes in *Eh* and H_2S content. So the results must be considered relative rather than absolute.

Typical results are shown in Tables 8 and 9. At the Station 1315 the H_2S zone began at 140 m. At the slope station opposite the Bosporus this zone began much deeper (190 m) and the intermediate zone of mixing between aerobic and anaerobic waters was more extensive, as a consequence of intensive vertical mixing in this region.

TABLE 7. The results of the several estimastions of the ratio of sulphur compounds in an experiment on the oxidation of labelled sulphide, Station 7; exposure 2 days; depth of sampling 230 m

No. of replicates	Radioactivity of sulphur compounds, in c.p.m. $SO_4-S + SO_4-S$ of thiosulphate (r)	Total S (R)
1	541	5 042
2	584	5 086
3	525	5 121
4	557	5 091
5	530	5 056
6	546	5 010
Mean	547	5 067

In samples taken 20–40 m up in the anaerobic zone where H_2S is absent oxidation of the added labelled sulphide proceeds very rapidly with the formation of sulphate and thiosulphate in nearly equal proportion but with the latter predominating. The addition of chloroform did not change this relation and had no influence on the rate of oxidation. So the oxidation of sulphide in these samples must be mainly chemical, the numbers and activity of the thiobacilli being low in a layer removed from the zone of active chemosynthesis. The actual values for the chemosynthesis and potential activity of thiobacilli were close to zero here (Figs. 4, 7, 8).

Quite a different picture was found with samples from the intermediate layer where the *Eh* values ranged from 0 to −30 mV. Here the activity of thiobacilli

TABLE 8. The rate of H_2S oxidation and the efficiency of the use of its energy for chemosynthesis at the eastern halistatic region, Station 1315. Exposure time 36 h at 6°–8°C

Depth m	*Eh*, mV	H_2S, mg/l (after addition Na_2S^{35})	Conditions of oxidation of S^{2-}	The final ratio of radioactivity of various forms of sulphur to that of total sulphur (*r*/*R*), %					S^{2-} oxidized to S^{6+}				
				S^0	S^{2-}	$S_2O_3^{2-}$	SO_4^{2-}	$^1/_2\ S_2O_3^{2-} + SO_4^{2-}$	Totally during the experiment, mg/l	per day µ mole/l	During the experiment only by microflora, mg/l	Chemo-synthesis µ moles C per day	Efficiency of chemo-synthesis %
120	+210	0·35	Chem	2·0	29	35	34	51·5	0·180	3·74	–	–	–
			Chem + biol	2·5	34	31	33	48·5	0·170	3·55	0	0·017	0·33
150	−15	0·41	Chem	2·0	81·5	8	8	12·0	0·050	1·04	–	–	–
			Chem + biol	2·5	77	3	17·5	20·5	0·084	1·75	0·034	0·351	14·1
180	−90	1·5	Chem	0·5	98·2	0·8	0·6	1·0	0·010	0·21	–	–	–
			Chem + biol	1·5	96·2	0·7	1·6	1·95	0·020	0·42	0·010	0·022	3·0
260	−120	1·61	Chem	0·6	99·58	0·25	0·17	0·3	0·005	0·09	–	–	–
			Chem + biol	0·3	99·15	0·25	0·25	0·37	0·006	0.12	0·001	0·0015	0·35

TABLE 9. The rate of H_2S oxidation and the efficiency of the chemosynthesis at the nearshore region opposite the Bosporus, depth 900 m. Exposure time 40 h at 6–8°C, Station 1323.

Depth m	*Eh* mV	H_2S, mg/l (after addition Na_2S^{35})	Conditions of oxidations	The final ratio of radioactivity of various forms of sulphur to that of total sulphur (*r*/*R*), in %					S^{2-} oxidized to S^{6+} after 40 hours, mg/l		Chemo-synthesis µ moles C/l per day	Efficiency of chemo-synthesis %
				S^0	S^{2-}	$S_2O_3^{2-}$	SO_4^{2-}	$^1/_2\ S_2O_3^{2-} + SO_4^{2-}$	Totally	Only by biological way		
140	+200	0·35	Chem	< 1	33	38	29	48	0·169	–	–	–
			Chem + biol	< 1	31	38	31	50	0·175	0·006	0·007	0.15
185	+10	0·42	Chem	< 1	82	10	7	12	0·050	–	–	–
			Chem + biol	< 1	81	6	15	18	0·076	0·026	0·109	5·37
200	−20	0·56	Chem	< 1	91	5	4	6·5	0·036	–	–	–
			Chem + biol	< 1	82	2	16	17	0·095	0·059	0·47	18·5
250	−65	1·15	Chem	< 1	94	2·5	2	3	0·035	–	–	–
			Chem + biol	0	95	2·0	4	5·5	0·063	0·028	0·158	9·4
500	−185	1·63	Chem	0	98·5	1	0·5	1	0·016	–	–	–
			Chem + biol	< 1	99	0·6	0·4	0·7	0·009	0	0·002	0·83

and chemosynthesis were at their greatest. The rate of oxidation of labelled sulphide was lower here than in the samples from the upper levels as there was a lack of hydrogen acceptors. In the samples from the intermediate layer less than one fifth of the total initial H_2S was oxidized per day. But there is no doubt that here thiobacilli took part in the process. Their participation can be detected, not from the rate of disappearance of sulphide as it had been supposed by previous investigators, but from the increased rate of the disappearance of thiosulphate formed during the first stage of the chemical oxidation of sulphide. For example, in a subsample from depth 165 m (Station 1315) during the chemical oxidation of sulphide in the presence of chloroform the ratio "thioshulphate : sulphate" was close to 1·0, but in the replicate subsample without the chloroform the ratio was only 0·17.

In the samples taken below the intermediate zone, where *Eh* values dropped below −60 to −80 mV, the rate of H_2S oxidation fell sharply because of the lack of hydrogen acceptors. At the lower boundary of intermediate zone the amount of the sulphide oxidized per day did not exceed 1–2% of that presented in the initial sample. Nevertheless, even in such anaerobic water where oxidation is strongly supressed, the participation of thiobacilli was quite detectable.

R. V. Gonetz, autumn 1967

In September 1967 an improved technique with S^{35} was used. The amount of the unlabelled sulphide introduced into the subsamples was reduced to a minimum. So the results give a measure of the absolute rate of oxidation.

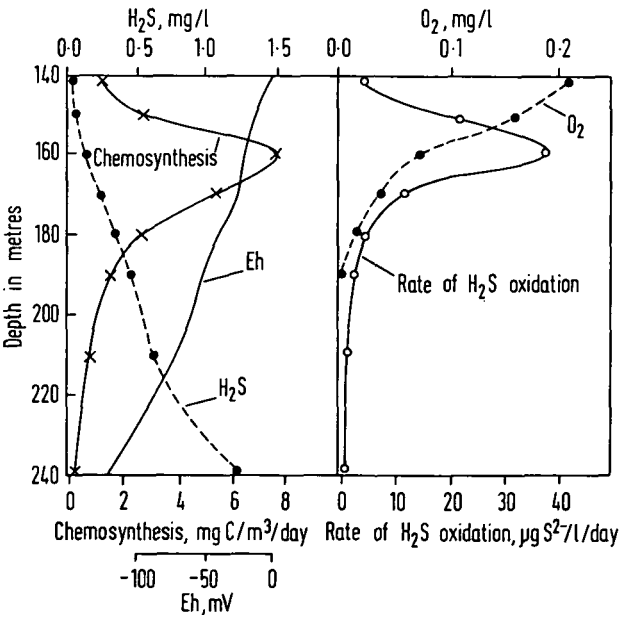

Figure 16. The vertical distribution of *Eh*, chemosynthesis rate (Ch), mg C/m³/day; and rate of H_2S oxidation, µg/l/day in the water column, Station 3.

The results obtained with samples taken at Stations 2, 3, 7 in the open sea opposite Yalta are summarised in Tables 10 and 11 and in Figure 16. The hydrology of this region is similar to that of the halistatic regions. The anaerobic water layer is relatively shallow and has its upper level at 135–140 m. The intermediate layer where both O_2 and H_2S are present is 40–60 m thick. Within this intermediate layer the redox potential falls by 40–60 mV, and the H_2S content varies from 0 to 50 µg/l. The layer of active H_2S oxidation was found within the intermediate zone. The maximum rate of oxidation was observed where *Eh* values ranged from −10 to −20 mV, and the H_2S concentration from 1·0–1·5 mg/l. Chemosynthesis was also greatest at this depth. At *Eh* values of about −80 mV, H_2S oxidation was completely inhibited by the lack of hydrogen acceptors. The maximum absolute rate of sulphide oxidation was about 15–20 mg S^{2-}/l per day and the intensity of chemosynthesis was about 4–5 mg C/m^3/day. The efficiency with which the energy of sulphide oxidation was used for chemosynthesis by the natural population of bacteria, calculated on the basis of these data, varied from 20% to 40%. This is a very high efficiency and suggests that other bacteria, in addition to autotrophic hiobacilli, are involved in chemosynthesis in this layer.

TABLE 10. The rate of H_2S oxidation and the efficiency of chemosynthesis at the deep sea Station 1. Exposure time 27 h at 5–7°C.

Depth m	H_2S mg/l	O_2 mg/l	*Eh* mV	Final ratio of radioactivity of sulphur $SO_4^{2-} + {}^1/_2 S_2O_3^{2-}$ to that of total sulphur (r/R), %	Rate of oxidation of S^{2-} to S^{6+} µg S/l per day	Chemosynthesis µg C/l per day	Efficiency of chemosynthesis %
135	0·01	0·17	0	33·2	3·2	0·2	12
140	0·02	–	−10	25·5	5·1	0·6	23
145	0·05	0·12	−10	23·5	10·3	2·1	36
150	0·10	–	−10	20·2	20·7	2·4	22
155	0·15	0·08	−15	18·6	25·4	4·4	33
166	0·20	0·06	−20	11·0	19·8	4·3	41
170	0·32	–	−32	3·5	10·2	2·1	38
180	0·43	0·02	−36	3·8	7·0	1.5	39
210	0·86	0	−100	0·3	2·3	0·3	25
240	1·36	0	−120	0	0	0·02	–
350	2·30	0	−148	0	0	0	–
500	3·20	–	−170	0	0	0	–

TABLE 11. The rate of H_2S oxidation in the water column at Station 7. Exposure time 40 h, at 5–7°C.

Depth m	H_2S mg/l	O_2 mg/l	Radioactivity of sulphur at the end of exposure c.p.m./4 ml r ($SO_4 + {}^1/_2 S_2O_3$)	R (total S)	r/R %	r/R (subtracting the initial ratio at the beginning of experiment)	Rate of oxidation S^{2-} to S^{6+} µg S/l per day
160	0·01	0·21	1130	3 510	32·1	29·0	1·7
180	0·03	0·12	1040	3 980	26·2	23·1	4·1
200	0·07	0·06	824	4 610	17·9	14·8	6·2
200	0·30	0·02	412	3 940	10·4	7·3	13·2
240	0·55	0	272	3 380	8·1	5·0	16·8
270	1·06	–	128	3 860	3·3	0·3	1·9
320	1·25	–	114	3 750	3·1	0	0
406	3·10	–	80	2 410	3·0	0	0
500	4·8	–	85	2 710	3·1	0	0

The total rate of H_2S oxidation in the water column was found equal to 400–500 mg S^{2-}/m^2 per day (Table 12). In the regions influenced by the cyclonic currents (Stations 1, 4, 5, 6) the H_2S zone lies deeper (more than 180 m) and the intermediate zone is much thicker than those in the halistatic (about 80–100 m). Here the rate of H_2S oxidation reaches levels up to 700–1000 mg/m^2,

TABLE 12. Total rate of oxidation of H_2S in water column under 1 m^2. (Cruise in September 1967)

Station no.	The depth of the upper boundary of H_2S zone, m	Rate of H_2S oxidation mg S^{2-}/m^2/day
1	135	530
3	140	390
7	150	395
5	170	670
4	180	740
6	190	970
2	200	1100

TABLE 13. The rate of oxidation of H_2S in the water column at the edge of the western halistatic, Station 4

Depth m	H_2S mg/l	*Eh* mV	O_2 mg/l	Exposure, hours	Rate of oxidation S^{2-} to S^{6+} µgS/l per day
180	0·01	0	0·22	42	3·2
190	0·02	−10	0·18	42	7·5
200	0·15	−10	0·12	40	28·0
220	0·32	−20	0·05	43	17·4
240	0·55	−45	0·02	40	7·7
300	1·15	−60	0	46	1·8
400	2·70	−120	0	46	0

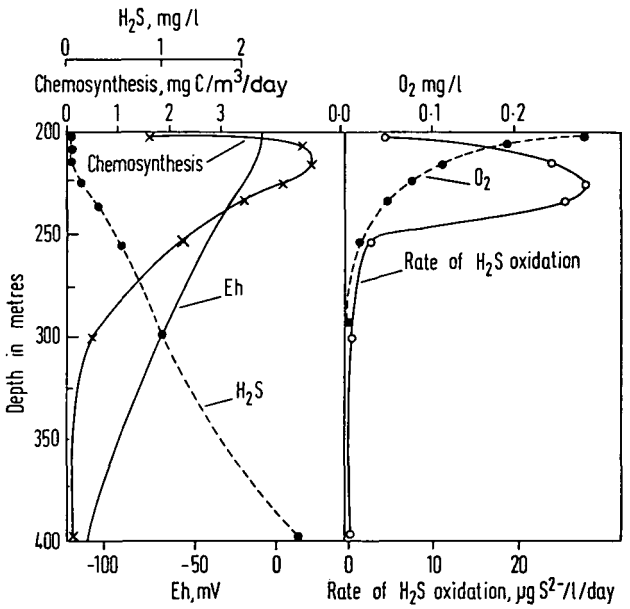

Figure 17. The vertical distribution of *Eh*, and rate of chemosynthesis in mg C/m^3/day; and rate of H_2S oxidation (A), µgS^{2-}/l/day in the water column, Station 2.

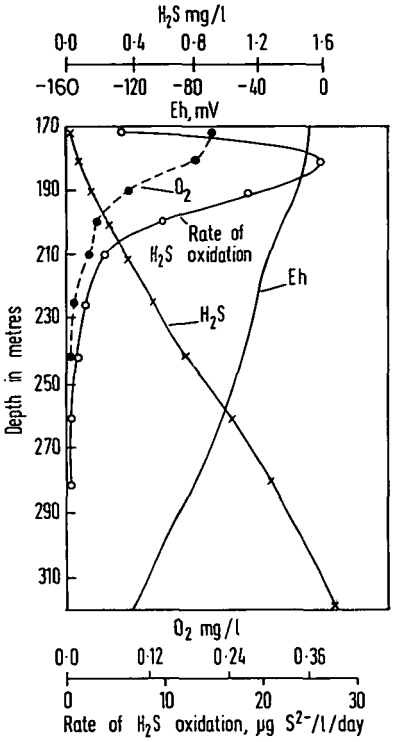

Figure 18. The rate of H_2S oxidation, µg/l/day, Station 5.

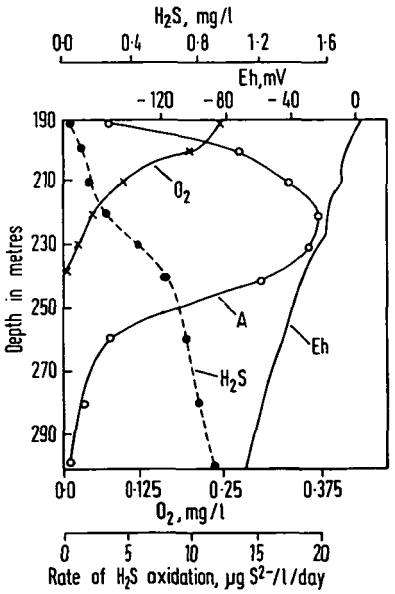

Figure 19. The rate of H_2S oxidation, µg/l/day, Station 6.

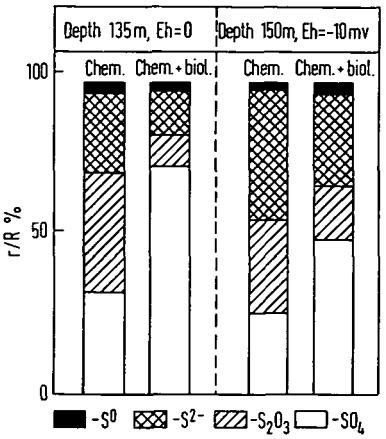

Figure 20. The relative content of sulphur compounds (r/R) formed during sulphur oxidation in the samples from different depths. Exposure time, 42 h; chem, in the presence of chloroform; chem + biol, in natural samples (without antiseptic).

as a consequence of greater vertical turbulence (Tables 12, 13, Figs. 17, 18, 19).

Analysis of the interrelation between the sulphur compounds during sulphide oxidation showed that at the top of the intermediate layer most sulphide is oxidized to sulphate. At the bottom of the intermediate layer, where there is a lack of hydrogen acceptors, the main end products are sulphate and thiosulphate. If the biological oxidation is stopped by chloroform, hydrogen sulphide is oxidized to sulphate and thiosulphate in a proportion close to 1:1 (Figure 20). These data support our previous assumption that in the presence

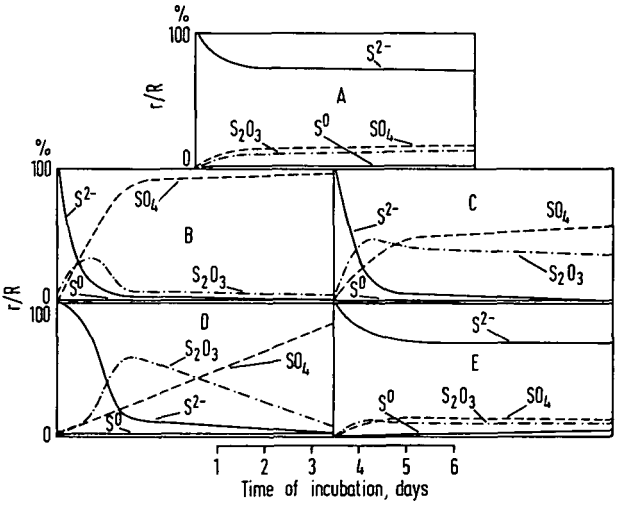

Figure 21. The ratio of sulphur compounds (r/R) formed during sulphide oxidation in samples from 220 m (Station 7). The samples were enriched with Na_2S (see text) and treated as follows: A, + antiseptic; B, + air bubble; C, + antiseptic and air bubble; D, + 50 mg/l $NaNO_3$; E, + 50 mg $NaNO_3$ and antiseptic.

of free oxygen thiobacilli do not oxidize sulphide at all, but only the sulphide part of thiosulphate, which is formed during the process chemical oxidation. They may not be able to compete with the process of quick chemical oxidation of sulphide to sulphate and thiosulphate.

To study the interaction between chemical and the biological processes of the sulphide oxidation in Black Sea water a series of experiments were carried out with samples from the intermediate layer. These samples were enriched with S^{2-} ions by the addition of Na_2S, 0·5 mg S^{2-}/l. Observations were made on the dynamics of the sulphide oxidation and the formation of oxidation products when the natural water was enriched with O_2 or NO_3^-, as additional hydrogen acceptors.

The experiments showed that sulphide oxidation by participation of nitrate oxygen was possible if microflora was involved. Indeed the addition of nitrate did not influence the rate of chemical oxidation of sulphide in the presence of chloroform but it had a strong influence upon the rate of oxidation and its end products in the presence of living microflora (Fig. 21, A and D).

During the initial stages of the experiment oxidation proceeds at the expense of oxygen introduced into the bottles when they are filled and at this stage there is no difference between samples with or without chloroform. Biological oxidation of sulphide to sulphate and thiosulphate, and the further oxidation of thiosulphate to sulphate at the expense of NO_3^--oxygen then proceeds. But in the presence of chloroform the latter two stages are absent (Fig. 21, E).

If the subsample is enriched with oxygen gas (bubble of air) the addition of chloroform does not cause a significant change during the first stage of the oxidation process, that is the rapid chemical oxidation of sulphide to sulphate and thiosulphate. This stage usually lasts 1–1·5 days and about 90% of sulphide is oxidized in the presence or absence of chloroform. Later on thiosulphate is oxidized in the presence of living microflora to sulphate. But this oxidation process is stopped after the addition of chloroform and the chemical oxidation proceeds very slowly (Fig. 21 B, C).

DISCUSSION

These data on the number, production and activity of bacterial-plankton in the Black Sea are generally in agreement with our earlier results (SOROKIN, 1964). In the intermediate layer near the upper boundary of the anaerobic water there is an active oxidation of hydrogen sulphide and, possibly, of the products of the anaerobic decomposition of organic matter, particularly of methane.

Attempts were made to calculate the amount of the reduced material oxidized from the actual values of the intensity of chemosynthesis. In making these calculations it was taken into account firstly, that a substantial part of the potential energy of hydrogen sulphide is lost as heat during its chemical oxidation. Experiments with labelled sulphide showed that less than half of the initial potential energy is used in bacterial production as bacteria oxidized not only sulphide, but mostly the thiosulphate forming during its chemical oxidation. Secondly, during the estimation of the chemosynthesis – for example, that of methane oxidizing bacteria using C^{14} carbonate – its value is under-

estimated by about a factor of two, for these bacteria use for their biosynthesis only about 30–40% of CO_2 carbon. Nevertheless, the production of chemosynthesis in the water column under 1 m^2 was about 100 mgC per day and with an average chemosynthesis efficiency of 15% needs 0·3 g/m^2 of reduced compounds (methane and hydrogen sulphide have to be oxidized). The direct estimation of the rate of sulphide oxidation using S^{35} gives values of the same order as expected, particularly in the halistatic regions (Table 12).

Accordingly to the calculations by BOGDANOVA (1959) the rate of vertical transport of sulphides (and consequently the rate of their oxidation) is equal to 550 mg H_2S/m^2 per day. If we accept the time of the total elevation of the deep water to the surface in the Black Sea as being equal to 130 years (SCOPINTSEV, 1953; VODYANITSKY, 1958; SCOPINTSEV *et al.*, 1959) we can calculate the amount of sulphide oxidized each day by dividing the total of sulphide in the Black Sea under 1 m^2 (12·9 kg) by the time for the total turnover in days ($4{\cdot}7 \cdot 10^4$). It turns out about 270 mg/m^2 per day. But actually it could be higher, for within the intermediate layer there is an additional production of sulphide.

Numerous observations over several decades have shown that the H_2S zone is stable although there must be a significant amount of vertical turbulence in the absence of any appreciable density gradient for more than a 100 m below the zone. Let us accept that the stability of H_2S zone in the Black Sea is based on the dynamic equilibrium between the processes of hydrogen sulphide oxidation and of its formation as a result of the sulphate reduction by bacteria.

Let us suppose that the average rate of sulphide oxidation in the water column is 300 mg/m^2 per day, and that the rate of the opposite process of sulphate reduction is similar. But sulphate reduction requires easily utilisable organic matter or molecular hydrogen (SOROKIN, 1966a). Where is the source of the organic matter required for the production of this sulphide? Now this is quite an enigma, for the Black Sea is an oligotrophic basin. The average annual primary production in the open water of the Black Sea is about 300 mg/m^2 of organic matter (SOROKIN, 1964), and not more than 5–10% of it reaches the bottom. But the amount of sulphide oxidized requires 1 000 mg/m^2 of organic matter to be available for sulphate reduction. Although rich with organic matter, the bottom sediments of the Black Sea may not be able to meet this demand and perhaps cannot be able to release such amounts of organic substance or sulphide ions. In the bottom sediments sulphide ions are usually present as stable iron sulphides. The formation of hydrogen sulphide during sulphate reduction proceeds in a thin surface layer (2–3 cm) and results in production of not more than 5 mg S^{2-}/m^2/day (SOROKIN, 1964).

It seems clear that the process of sulphate reduction in the bottom zone cannot compensate for the oxidation of sulphide in the intermediate zone. But can we find some other source of hydrogen sulphide, or other reduced substances where oxidation could provide the energy for the intensive chemosynthesis in the intermediate layer? One possibility might be the intensive sulphate reduction taking place in the slope sediments between 150–1 000 m. The concentration of free H_2S and the rate of its formation are much higher here than in the bottom sediments of the halistatic regions (VOLKOV, 1961; SOROKIN, 1964). Here the reduction of sulphates proceeds using the organic material sedimenting from the more productive coastal waters. Carried with

the near bottom muddy currents they quickly reach the anaerobic zone of sulphate reduction, which begins below 150–200 m.

I have studied an analogous process at Lake Gek Gel (Caucasus, 30 km south of Kirowodad), which is an excellent model of the Black Sea (SOROKIN, 1970). This oligotrophic mountain meromictic lake has a stable H_2S zone. The vertical characteristics of its chemistry, hydrology and biological stratification are quite similar to that of the Black Sea (Fig. 22). Estimations of the rates of the sulphate reduction and the H_2S oxidation using S^{35} have shown that the initial source of energy supporting the dynamic equilibrium between these processes is mainly the allochtonous organic material reaching the lake with the river inflow from the surrounding forests and meadows. So in this lake equilibrium is supported by an external source of energy.

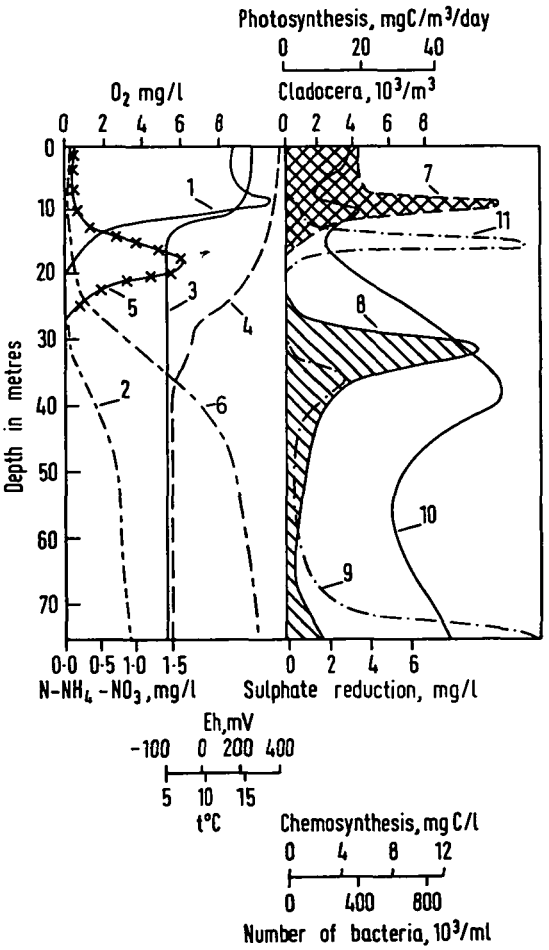

Figure 22. The vertical distribution of O_2 (1); H_2S (2); t°(3); *Eh* (4); NO_3^- (5); NH^+_4 (6); photosynthesis (7); chemosynthesis (8); sulphate-reduction rate (9); total number of bacteria (10), and crustaceans *Cladocera* (11) in the lake Gek Gel.

In the Black Sea the role of allochtonous matter is not significant. But there are reasons for considering that a similar role could be played by methane, and possibly other gaseous hydrocarbons, passing into the water from the sedimentary rocks. For example, as has been pointed out earlier, the very high activity of methane-oxidizing bacteria cannot be explained by the recent formation of methane gas in the bottom sediments.

The Black Sea was formed where there was previously an ancient shallow eutrophic basin. It is therefore quite plausible to expect the sedimentary rocks to be enriched with hydrocarbon gases. These gases could enter the basin and being oxidized in the intermediate zone, could support its stability and high bacterial production.

During the discussion of the causes of the stability of the H_2S zone the possible role of the secondary sulphate reduction at the lower boundary of the intermediate zone must be taken into account (Fig. 14). This process proceeds using organic matter formed during chemosynthesis (SOROKIN, 1964). It provided additional H_2S and so increases the efficiency of the reduction process in the sulphur cycle. Indeed during the oxidation process part of the sulphide energy is bound up in the organic biomass of autotrophic thiobacilli. When they die it is used by sulphate-reducing bacteria for additional H_2S production as demonstrated in the model experiment (Fig. 15). The same phenomenon has been found also in meromictic lakes (SOROKIN, 1970).

Further experimental studies of the balance of organic matter, the sulphur cycle, the processes of hydrocarbon formation and oxidation, may discover the nature of the sources of the reducing power supporting the stability of H_2S zone in the Black Sea, and so will permit us to solve one of the most difficult enigmas of this unique basin.

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