

NEW ZEALAND
DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

INFORMATION SERIES—NO. 22

New Zealand Oceanographic Institute Memoir No. 3

CONTRIBUTIONS TO MARINE MICROBIOLOGY

Compiled by

T. M. SKERMAN

New Zealand Oceanographic Institute
Wellington

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Including papers read at a Symposium on Marine Microbiology held during
the 32nd Meeting of the Australian and New Zealand Association For
The Advancement of Science in January 1957 at Dunedin, New Zealand

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P R E F A C E

At the 32nd Meeting of the Australian and New Zealand Association for The Advancement Of Science, held at Dunedin, New Zealand, in January 1957, a Symposium on Marine Microbiology was included in the programme for the Section of Microbiology, Epidemiology and Preventive Medicine, and was also jointly attended by the Sections of Geology, Zoology, and Oceanography. Papers were presented by Professor C. E. ZoBell, Professor of Marine Microbiology at the Scripps Institution of Oceanography, University of California, U.S.A., Mr V. B. D. Skerman, Department of Bacteriology, University of Queensland, Brisbane, Australia, and by Messrs D. J. Rochford, E. J. Ferguson Wood, and I. R. Kaplan, all of the Division of Fisheries and Oceanography, Commonwealth Scientific and Industrial Research Organisation, Cronulla, Australia. A separate address, introducing the subject of marine microbiology, was given elsewhere in the programme by Professor ZoBell.

After the meeting, and with the approval of the ANZAAS Committee, all speakers were invited to prepare the scripts of their addresses for publication in a bulletin to be issued by the New Zealand Department of Scientific and Industrial Research. Coordination and compilation of the material were undertaken by the New Zealand Oceanographic Institute. Shortly after the conference, Mr I. R. Kaplan left for overseas study and the lack of time unfortunately prevented him from revising his paper. Professor L. G. M. Baas Becking, formerly of CSIRO, now of the Bureau of Mineral Resources, Canberra, Australia, was unable to attend the Dunedin meeting; nevertheless he kindly consented to prepare a paper for inclusion in this bulletin. The compilation was forwarded for publication after receipt of the last manuscript in June 1958.

It stands much to the credit of the organisers of the ANZAAS Section for Microbiology that time was allotted to a branch of this science that is currently making increasingly greater contributions to our knowledge both of the micro-organisms and the importance of their activities in the waters and sediments of the seas. The authors are thanked for their cooperation in preparing the papers included here. Acknowledgment is extended to the ANZAAS Committee for their consent to publication of those papers originally prepared for the symposium.

T. M. SKERMAN,
New Zealand Oceanographic Institute,
Wellington.

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INTRODUCTION TO MARINE MICROBIOLOGY*

By CLAUDE E. ZOBELL, Scripps Institution of Oceanography, University of California, La Jolla, Calif., U.S.A.

Bacteria, yeasts, fungi, microflagellates, blue-green algae, and allied microbes are widely distributed in the sea, where they have far-reaching effects on biological and geochemical conditions. The marine microbiologist is concerned with the occurrence, characteristics, and activities of such microbes in the sea. Besides being concerned with the deep sea and open ocean, he must also be cognisant of conditions of microbial life in the littoral zone, estuaries, inflowing rivers, and related lakes. Thus the domain of the marine microbiologist encompasses more than two-thirds of the earth's surface and 99 per cent of the hydrosphere.

The numbers and kinds of microbes occurring in the marine world are influenced by environmental conditions and the presence of higher organisms. A good many environmental conditions, in turn, and the well-being of many marine plants and animals are affected by the presence and biochemical activities of microbes. Obtaining quantitative information on these matters has required the development of new methods and techniques and world-wide surveys. Although bacteria have been recognised as regular components of the marine biota for nearly a century, the science of marine microbiology is still in its infancy, having received only relatively little attention. To illustrate – at the 1953 International Congress for Microbiology in Rome at which Marine Microbiology was accorded sectional status for the first time, less than 30 of the more than 5,000 registrants indicated an active interest in the sea.

EXTENT AND PROPERTIES OF THE MARINE ENVIRONMENT

Nearly 72 per cent of the earth's surface is covered with water (Sverdrup *et al.*, 1942). The area is approximately 143,000,000 square miles or about 3.7×10^{14} m². The average depth of the sea is approximately 3,800 m or about 12,000 ft. Several oceanic deeps exceeding 30,000 ft have been sounded. The greatest known depth is the Challenger Deep, 35,500 ft, in the Mariana Trench (Wüst, 1950). The total volume of the oceans of the world is estimated to be about 328,000,000 cubic miles or 14×10^{23} ml.

Exclusive of insects, few of which are marine, four-fifths of all known animal species live in the sea. Virtually all major animal phyla are represented by marine species, and part of the phyla are exclusively marine. The plant kingdom is represented by more than 8,000 species – mostly phytoplankton, although several hundred species of seaweeds and other sedentary plants are known. The known species of marine bacteria, yeasts, microflagellates, and mould fungi are numbered in hundreds only.

The depth at which photosynthetic organisms occur is limited primarily by the penetration of sunlight, a matter of 500 ft at the most. The vertical distribution of non-photosynthetic microbes and animals appears to be limited partly by the availability of food (produced primarily in the photosynthetic zone) and partly by hydrostatic pressure. The pressure-depth gradient in the sea is nearly a straight line function, pressure increasing with depth by approximately 0.1 atm/m or about 0.5 psi/ft.

Water temperatures range from -2° to 40° C. Surface water temperatures in the tropics reach 28° to 30° C or occasionally 40° C in localised areas of the littoral zone. In Arctic and Antarctic areas surface water temperatures may be as low as -2° C, the temperature at which sea water of high salinity freezes to form ice. Approximately one-half of the ocean's surface ranges from 15° to 30° C and one-fourth is colder than 10° C. Temperatures lower than 10° C characterise most water masses below the topmost strata affected by radiant energy from the sun. More than 90 per cent of the marine environment is colder than 5° C.

*Contribution from the Scripps Institution of Oceanography. New Series.

The freezing point and osmotic pressure of sea water are functions of its salinity, which is generally within the range 3.2 to 3.6 per cent. Lower salinities may occur in localised areas at the surface or near-shore where sea water may be diluted by rainfall, inflowing rivers, or melting ice. The salinity may be higher than 3.6 per cent in regions of rapid evaporation, as in tide pools, marine salterns, or in isolated basins such as the Dead Sea or Great Salt Lake, in which the salinity may exceed 25 per cent.

Sodium chloride makes up about 86 per cent of the dissolved solids in sea water. Magnesium sulphate is next in order of abundance, with much smaller quantities of calcium, potassium, strontium, bicarbonate, bromide, fluoride, and borate ions. More than 60 chemical elements have been detected in sea water and virtually all are believed to be present. The organic carbon content of sea water averages 5 mg/l. The total fixed nitrogen content, including organic nitrogen compounds, nitrate, nitrite, and ammonium, is generally less than 1 mg/l. The combined nitrogen as well as the organic carbon and dissolved oxygen content of sea water is influenced by the activities of bacteria and other microbes.

CHARACTERISTICS OF MARINE BACTERIA

Morphologically, marine bacteria, as well as yeasts, mould fungi, microflagellates, blue-green algae, and other marine microbes, are much like their better known terrestrial or freshwater counterparts. While undoubtedly most of the microbes found in the sea are specifically indigenous to the marine environment, many mechanisms make for an interchange of microbes between land and the sea. Some may become airborne (ZoBell, 1942) and others may be transported by birds or other animals. Countless numbers of bacteria from soil or sewage may be carried by rivers, sewage outfalls, or other terrestrial drainage into the sea where some may survive in brackish water or become acclimatised to water of higher and higher salinity.

The majority of the bacteria collected from the sea at places remote from possibilities of terrigenous contamination require sea water or its equivalent for optimal activity. Upon initial isolation such bacteria grow poorly or not at all in nutrient media having a salinity of less than 50 per cent that of normal sea water. Although sometimes characterised as halophilic, most marine bacteria tolerate solutions hypertonic to sea water no better than they tolerate hypotonicity. This is illustrated in table 1, which summarises comparative plate counts obtained from a large number of samples from various sources used to inoculate nutrient agar prepared with balanced mineral solutions of different salinities.

TABLE 1. Average Growth Index, Expressed as Per Cent, of Bacteria From Different Sources Which Formed Colonies in Nutrient Agar Having Different Salinities

Source of Bacteria	Salinity of Nutrient Agar (%)						
	0.5	1.0	2.0	3.5	7.0	10.0	25.0
Sea water	19.2	39.0	86.9	100.0	37.2	13.8	0.2
Marine mud	24.1	35.4	78.7	100.0	44.5	9.4	0.3
Marine saltern	4.8	7.1	10.3	18.8	51.2	60.3	100.0
Great Salt Lake	2.2	5.8	13.0	25.3	38.6	68.2	100.0
Garden soil	100.0	79.1	61.7	24.4	10.9	5.3	0.4
Sewage	100.0	69.2	43.5	13.8	6.7	2.8	0.0

In view of the coldness of the marine environment (mostly less than 5° C), one might expect a psychrophilic microflora to predominate. While the majority of the bacteria taken from the sea do grow slowly at refrigeration temperatures (0° to 5° C), we have yet to find an obligate psychrophile or one which will grow better at refrigeration temperatures or even at 12° to 15° C than at 18° to 20° C. This seems somewhat anomalous since the cultures come from habitats where low temperatures prevail. Even more anomalous is the occurrence of obligate thermophiles in deep ocean basins (Bartholomew and Rittenberg, 1949) and in Arctic ice (McBee and McBee, 1956). These thermophiles are probably passive mutants which, being unable to reproduce in the cold climate where formed and found, are preserved by the low temperature. Actually marine thermophiles are so rare that they are to be regarded as biological curiosities.

The maximum temperature tolerated by most marine bacteria is only a few degrees higher than the temperature at which they grow best. Some marine bacteria which grow readily at 20° to 22° c are killed in ten minutes at 25° c. The thermal death point of marine bacteria is, in general, much lower than for bacteria from soil or freshwater sources (see table 2). Many of the bacteria occurring in sea water or marine mud are killed by short exposure to the plating temperature of nutrient agar, i.e., 42° to 45° c.

TABLE 2. Effect of Temperature on Bacteria From Various Sources, Cultured on Nutrient Agar

All plates were inoculated with properly diluted samples, and were incubated at 20° c after heating for 10 minutes to the stated temperature.

The colony counts are expressed as percentages of the counts from samples heated to 20° c.

Sample or Inoculum	Colony Counts						
	Temperature to Which Sample was Heated						
	20° c	30° c	40° c	50° c	60° c	80° c	100° c
Sea water	100	81.3	21.9	6.8	3.0	0.2	0
Marine mud	100	68.5	18.3	10.3	5.2	0.7	0
Garden soil	100	87.8	74.2	62.4	21.0	8.6	0.3
Sewage	100	84.6	70.6	57.9	9.6	1.2	0.1

TABLE 3. Relative Numbers of Marine Bacteria Appearing on Nutrient Agar at Different Incubation Periods and Temperatures

The colony counts are expressed as percentages of the average counts after 18 days at 18° c.

Period of Incubation	Temperature of Incubation						
	4-5° c	10-12° c	17-18° c	21-22° c	25-26° c	30-31° c	37-38° c
2 days	0	18	30	36	41	44	8
4 days	0	28	41	60	65	61	12
7 days	4	46	67	82	78	69	12
10 days	9	67	91	96	84	71	13
14 days	17	90	98	97	85	70	10*
18 days	26	97	100	95	82	63	8
21 days	33	98	96	87	74	53	7
28 days	69	104	91	75	69	42	4
35 days	99	98	87	69	58	31	4

*Decrease in counts due to merging of colonies and desiccation of medium.

Colony counts are nearly always higher on plates of nutrient agar incubated at 18° to 22° c than at higher temperatures (ZoBell and Conn, 1940). As many colonies of marine bacteria may develop on nutrient agar at 5° or 10° c as at 20° c, but much longer periods of incubation are required for the formation of visible colonies at the lower temperatures (see table 3).

Bacteria taken from sea water or sediments at depths of less than 1,000 m are affected by hydrostatic pressure in much the same way as are common soil, sewage, and freshwater bacteria (ZoBell and Oppenheimer, 1950). Their growth is inhibited when cultures are compressed to 200 to 400 atm at optimum temperature and they slowly die off when held for a few days at pressures ranging from 300 to 600 atm (Oppenheimer and ZoBell, 1952). On the other hand, most of the bacteria taken from depths of 7,000 to 10,000 m grow only when incubated at pressures characteristic of their natural habitat, i.e., 700 to 1,000 atm (ZoBell and Morita, 1957a, b).

OCCURRENCE OF BACTERIA IN THE SEA

Throughout the photosynthetic zone the abundance of bacteria appears to be more closely related to the distribution of phytoplankton than to any other single ecological factor (Waksman *et al.*, 1933). The beneficial effect of plankton is attributed partly to the organic matter which they provide and partly to solid surfaces. Bacteria occur only to a limited extent free-floating in water; most of them are attached to suspended solids such as the bodies or remains of plankton

organisms. As pointed out by ZoBell (1943), solid surfaces tend to concentrate organic nutrients, retard the diffusion of exoenzymes away from bacteria, and provide a resting place for sessile forms. Waksman and Vartiomaara (1938) found most of the bacteria in marine bottom deposits adsorbed on or attached to particles of sediment.

In the open ocean the abundance of bacteria generally increases with depth from the surface down to a depth of 25 or 50 m, below which they decrease in numbers. Below 200 m living phytoplankton are only rarely encountered and the bacterial population is usually less than 10 per ml until the bottom is reached. Sea water is almost sterile in many parts of the ocean at depths below 1,000 m. Kriss (1952) obtained no growth in nutrient broth inoculated with 35 ml samples in 29.6 per cent of 270 deep-water samples from 50 stations in the Okhotsk Sea and northern Pacific Ocean.

At the mud-water interface, the zone of maximum bacterial activity, the bacterial population ranges from 10^2 to 10^7 per ml as compared with average counts of 10^2 to 10^4 per ml in the photosynthetic zone. Only in bottom sediments or in localised areas does the density of bacteria often exceed 10^4 per ml. Assuming an average bacterial population of 10 per ml in water throughout the oceans of the world, the standing crop of bacterial biomass would amount to about 10^6 tons. If these bacteria reproduce at an average rate of only once per day, a rate believed to be conservatively slow, the annual yield of bacterial biomass in the ocean would amount to 3.65×10^8 tons. The total primary productivity of sea has been estimated by various workers to range from 5×10^{10} to 9×10^{11} tons per year.

The storage of sea water in clean glass receptacles is usually accompanied by a fairly rapid increase in its bacterial population. Under storage conditions the bacteria may reproduce every hour or two. Their reproduction in stored water samples raises the question as to why there are so few bacteria in sea water, which is capable of supporting many more. Bacterial predators, which do not survive long under ordinary storage conditions, may help to keep counts low in the sea. The favourable effects of solid surfaces provided in storage conditions are believed to be chiefly responsible for the increased bacterial populations.

In sediments on the sea floor, where both solid surfaces and organic matter are usually more abundant than in overlying water, the bacterial population compares favourably in the diversity of species and in total numbers with fertile garden soil. Both the abundance and number of bacterial species decrease with core depth, the maxima occurring in the topmost few millimetres of mud. Within the topmost metre of mud the bacterial population generally falls off from 10^2 to 10^7 to less than 10 per ml. Viable bacteria, however, have been detected at the bottom of some of the longest cores examined, nearly 10 m, consisting of sediments as old as 10^5 years.

Most of the bacteria recovered from marine bottom deposits prove to be facultative or strict anaerobes. Bacteria are sparse in red clays and other pelagic sediments which are poor in organic matter (Morita and ZoBell, 1955). According to Kriss and Rukina (1952), Okhotsk Sea and northern Pacific Ocean sediments contain 10^8 bacteria per gram to give a bacterial biomass estimated to range from 0.2 to 1.4 g/m². If comparable bacterial densities occur in all ocean sediments, the standing crop of bacterial biomass would range from 7×10^7 to 5×10^8 tons.

Large numbers and many different species of bacteria are found associated with the integuments and digestive tracts of marine animals. Seaweeds – both living and dead – support the growth of an extensive microflora, including bacteria as well as mould fungi and yeasts.

OTHER MARINE MICROBES

Yeasts are only occasionally encountered in the sea except in association with seaweeds. Several varieties of *Torula*, *Mycoderma*, *Blastoderma*, *Oidium*, *Dematium*, *Endomyces*, and *Saccharomyces* have been isolated from marine materials. Yeasts are an important component of the microflora in the Black Sea where Kriss and Rukina (1952) found them associated with seaweeds, in superficial layers of water, and in deeper water masses.

Although mould spores and mycelium fragments are commonly detected in sea water throughout the photosynthetic zone and on the sea floor at the mud-water interface, the growth of fungi in the sea seems to be confined primarily to diseased or decomposing plant and animal tissues. Several genera of marine Fungi Imperfecti, Phycomycetes, and Myxomycetes have been described, mostly from near-shore materials. Among the many species of marine fungi described by Sparrow (1936) is an *Ectrogella* that infects certain diatoms. Seaweeds seem to be very susceptible to invasion by both saprophytic and parasitic fungi. Widespread mortality in oysters has been traced to a fungus disease caused by *Dermocystidium marinum* (Andrews and Hewatt, 1957).

Important constituents of nanoplankton or ultraplankton that escape most conventional plankton nets are the so-called microflagellates or naked flagellates which are mostly less than 5 microns in diameter. These inadequately investigated microbes are believed to contribute substantially to food cycles in the sea (Butcher, 1952; Knight-Jones and Walne, 1951).

Only a few varieties of filterable viruses have been reported in the sea, probably because these minute microbes have received only desultory attention. Certain marine aquarium fish have been shown to be infected by viruses and a few marine bacteriophages have been reported. A disease of the giant kelp, *Macrocystis pyrifera*, resembles virus infections of certain spermatophytes, but conclusive evidence for a virus infection of this or any other alga is still lacking. Marine diatoms and dinoflagellates appear to suffer from infections, but whether filterable viruses or other microbes are involved has not been determined.

Marine microbiology may include studies of diatoms, dinoflagellates, silicoflagellates, coccolithophores, ciliates, radiolaria, foraminifera, and other small organisms along with viruses, bacteria, yeasts, mould fungi, microflagellates, and blue-green algae. All are of great geological and biological significance in the sea and they have many properties in common. The former larger organisms are not treated here nor in my book entitled "Marine Microbiology" (1946a), because I believe the major domain of the marine microbiologist is the community of microbes that, owing to their size and behaviour, are studied primarily by cultural methods or with a microscope at a magnification of about 1,000 times.

HARMFUL EFFECTS OF MARINE MICROBES

Although their activities are preponderantly beneficial, there are ways in which marine microbes are deleterious to the economy of the sea or to the welfare of man. Their most obvious harmful effect is in causing infectious diseases of marine plants or animals. Salmon, lobsters, oysters, and numerous fish kept in marine aquaria are among the animals known to be adversely affected by marine microbes (ZoBell, 1946a). The wasting disease of eel grass, *Zostera marina*, is an outstanding example of a devastating microbial infection of marine plants (Renn, 1936). The sea, however, is inhospitable to most human pathogens. Organisms such as typhoid bacilli and cholera vibrio survive in the sea for only a few days. They may survive in waters or on beaches polluted with sewage long enough to be potentially dangerous to man. Likewise they may be transmitted to man in oysters or other contaminated shellfish.

Fish and certain other marine foods are extremely susceptible to bacterial spoilage. Considerable attention has been devoted to the enumeration, identification, and control of bacteria associated with fish, a subject which would require an extensive monograph to bring the voluminous literature together. Applications of the principles of modern sanitation, refrigeration, and chemical preservatives, including nitrite and antibiotics, have helped to lessen spoilage (Tarr, 1954). The spoilage of fish is caused primarily by bacteria that live in the digestive tracts or in the slime of the fish. Species of *Achromobacter*, *Pseudomonas*, *Flavobacterium*, and *Micrococcus* predominate in the spoilage of marine fish. These genera also predominate in the sea. Species of *Sarcina*, *Proteus*, and *Bacillus* occur less frequently. Many of the fish-spoilage bacteria are pigmented and may cause objectionable discolouration in refrigerated fish. Rapidly

cooling the fish as soon as they are caught and holding them at near 0° C will substantially lessen undesirable changes caused by bacteria, but temperatures lower than -7.5° C are required to stop the activity of facultative psychrophilic bacteria associated with marine fish.

Cellulose decomposers and other physiological groups of bacteria participate in the rapid rotting of wooden pilings, board boat bottoms, cordage, fish nets, lines, and ropes in the marine environment. Bacteria are also involved in the biofouling of ships' bottoms, water conduits, and other submerged surfaces. By producing acids in microspheres as well as by other mechanisms bacteria promote the corrosion or deterioration of man-made iron, steel, and concrete structures in the sea.

In localised areas, as in bays or stagnant basins, bacteria may vitiate water by depleting dissolved oxygen, by producing hydrogen sulphide, or by forming toxic amines. Taken as a whole, the marine bacteria probably consume more oxygen than all other marine organisms combined. When oxygen is consumed more rapidly than it can be replaced by solution from the atmosphere or by photosynthetic processes, conditions tend to become anaerobic and hydrogen sulphide may accumulate. Hydrogen sulphide is produced both by the anaerobic decomposition of proteinaceous compounds and the reduction of sulphate. In places like the Black Sea or stagnant fjords, bacteria may produce hydrogen sulphide in concentrations high enough to be incompatible with other forms of life. The catastrophic death of animals in the wake of "red tide" is believed to be partly attributable to the vitiation of water masses by bacteria.

Red tide is the name applied to waters discoloured by aggregations of algae, bacteria, ciliates, diatoms, flagellates, and other pigmented organisms. Extensive areas of water assume a red, brown, amber, or greenish-yellow hue. The dinoflagellates *Goniaulax monilata*, *Gymnodinium brevis*, and *Ceratium furca* and the bacterium *Flavobacterium piscicida* are among the many microbes associated with catastrophic mass mortality of fish and other marine organisms.

It was once thought (Brandt's hypothesis, 1929) that the denitrifying bacteria which liberate free nitrogen from nitrate limit the amount of nitrate available for the growth of phytoplankton in tropical seas. The activities of denitrifiers, which are known to be abundant in the sea, are unquestionably responsible for a leak in the nitrogen cycle. This effect is limited largely by a lack of organic matter which the denitrifiers require as an energy source.

MICROBIAL MODIFICATION OF ORGANIC MATTER

The most important activity of bacteria in the sea is the mineralisation or modification of organic matter (Waksman, 1934). In places this may be harmful to the economic interests of man or to the general economy of the sea, but it is mostly beneficial. It results in the formation of plant nutrients, notably ammonium and phosphate, and in the decomposition of the remains of plants and animals. Dissolved, colloidal, or waste organic matter unfit as food for other organisms may be transformed by bacteria into cell substance utilisable as nutritious food for animals.

Virtually all classes of organic compounds – carbohydrates, lipids, proteins, hydrocarbons – are attacked and almost quantitatively assimilated by bacteria. Except in localised areas the concentration of organic carbon in sea water is reduced to less than 5 mg/l. Most marine bottom deposits contain less than 5 per cent organic matter, the content decreasing with core depth, age, or the time that bacteria have had to act. Concentrations of organic carbon higher than 5 mg/l generally provide for the rapid reproduction of heterotrophic bacteria which oxidise organic compounds. Owing to the speed with which organic compounds are modified, the sea has been called the world's largest and most efficient septic tank.

Under aerobic conditions, the principal decomposition products resulting from the bacterial oxidation of organic compounds are water, carbon dioxide, sulphate, ammonium, and phosphate. The last two are plant nutrients which may limit primary productivity in the sea. Ammonium may be utilised directly by plants or it may be oxidised by bacteria to nitrite or nitrate. Carbon

dioxide, water, ammonium, phosphate, methane, molecular hydrogen, and hydrogen sulphide are liberated when organic matter is attacked under anaerobic conditions. Complex organic compounds are converted, in part, to marine humus (Waksman, 1933). Humus also consists partly of complex organic compounds making up bacterial cell substance.

From 10 to 40 per cent or more of the carbon content of organic compounds utilised by heterotrophic bacteria may be converted into bacterial protoplasm. Being nutritious and usually readily digestible, such bacterial cell substance constitutes an important part of the diet of many marine animals. Bacterial species that live normally in the gut of animals may aid them in the digestion of food. Bacterial enzymes catalyse the hydrolysis of cellulose, chitin, alginates, agar, hydrocarbons, and other complex organic compounds. Bacteria also produce vitamins essential in the nutrition of animals.

Chitin is a complex glucosamine occurring commonly in Mollusca, Coelenterata, Protozoa, fungi, and Crustacea in which it is the chief exoskeleton constituent. From data on the abundance of copepods and their chitin content it is estimated that just this one sub-class of Crustacea produces several million tons of chitin annually in the oceans of the world. Bacteria are believed to be the principal agents responsible for the decomposition or digestion of chitin. Hock (1940) isolated chitinoclastic bacteria from marine sediments, sea water, decomposing crabs, and from the intestinal contents of several animals which ingest copepods or other organisms containing chitin.

Agar, which normally occurs in a large variety of seaweeds, is a complex galactan which is digested by bacteria. Nearly fifty species of marine agar digesters have been described by Humm (1946). He detected from 2 to 150 agar digesters per ml of sea water and from 80,000 to 20,000,000 per gram of mud along the coast of North Carolina. All of Humm's agar digesters utilised cellobiose, 85 per cent attacked salicin, 70 per cent attacked alginic acid, 25 per cent attacked chitin, and 20 per cent attacked cellulose.

Cellulose-decomposing bacteria are generally present in sea water, being most plentiful in plankton tows and in bottom deposits. Most marine cellulose decomposers are aerobic. Anaerobic cellulose decomposers are only rarely detected in marine materials. The decomposition of cellulose fibre fishing nets was attributed primarily to aerobes by Kadota (1956). He studied several species of *Vibrio*, *Cytophaga*, and *Pseudomonas* from the sea that attacked cellulose but not cellulose acetate.

The paucity of amino acids and simple proteins in sea water and sediments is ascribable to the susceptibility of these nitrogenous compounds to microbial attack. As a class, marine bacteria are actively proteolytic; probably even more so than the microflora of garden soil or fresh water. Gelatin is liquefied so rapidly by so many marine bacteria that it is not a satisfactory substance to render nutrient media solid. Proteolytic bacteria are primarily responsible for the spoilage of fish, shellfish, crab meat, and other proteins decomposed with the liberation of ammonium, indole, trimethylamine, histamine, hydrogen sulphide, etc. (Beatty and Gibbons, 1937).

AUTOTROPHIC BACTERIA

Both chemosynthetic and photosynthetic autotrophs occur in the sea, where they contribute to the synthesis of organic matter. Photosynthetic bacteria are confined largely to shallow bottoms, brackish waters, and decomposing vegetation, where they may affect the sulphur cycle in the sea more than the carbon cycle. It has not been demonstrated whether they have any significant effect on the formation or transformation of carbon compounds. Below the photosynthetic zone chemosynthetic autotrophic bacteria appear to be the only primary producers of organic matter. Whether they produce enough to be of significance in the carbon economy of the sea is problematical. Rather widely distributed are bacteria which oxidise either hydrogen sulphide, methane, molecular hydrogen, or ammonium as an energy source while reducing carbon dioxide to build cell substance. There are no data to serve as a sound basis for estimating

how much organic carbon is produced in the ocean by these autotrophs whose activities must be limited largely by the availability of hydrogen sulphide, methane, molecular hydrogen, or ammonium respectively.

Hydrogen sulphide may be precipitated from solution as metallic sulphides, it may be abiotically oxidised to other products, or it may have other fates, but the more inert methane and hydrogen, which are produced in large quantities during the anaerobic decomposition of organic matter, must be oxidised by autotrophic bacteria or escape into the atmosphere. Since methane- and hydrogen-oxidising bacteria respectively are commonly found near places where these gases are being produced, and since little or no methane or molecular hydrogen occurs in water away from such places, it is tacitly assumed that these gases are oxidised by bacteria. Ammonium may be utilised directly by plants or bacteria as a source of nitrogen, and large quantities are probably oxidised to nitrite by nitrifying bacteria. The latter may fix or convert into bacterial protoplasm 1 mg of carbon dioxide carbon for each 5 or 6 mg of ammonium oxidised. The ammonium content of sea water ranges from 0.05 to 50 mg/m³.

NITROGEN FIXATION

Nitrogen-fixing *Azotobacter* and *Clostridium* species apparently do not occur commonly in the sea except near land. One would hardly expect to find *Rhizobium* species in the sea, since there are no marine legumes. Recent observations, however, suggest that biological nitrogen fixation is not confined to *Azotobacter*, *Clostridium*, and *Rhizobium* species. Certain hydrogenase-producing varieties of *Desulfovibrio*, which are widely distributed in the sea, have been shown to fix nitrogen (Sisler and ZoBell, 1951). According to Lindstrom *et al.* (1951), a good many other hydrogenase-producing bacteria, including species of *Rhodospirillum*, *Rhodopseudomonas*, and *Rhodomicrobium*, fix nitrogen. Pine and Barker (1954) have demonstrated nitrogen fixation by a *Methanomonas* species, and Rodina (1956) observed nitrogen fixation by a *Spirillum* species. Species of *Nostoc*, *Anabaena*, and certain other blue-green algae also fix nitrogen.

MICROBIAL TRANSFORMATION OF SULPHUR

From a geobiological point of view, the contribution of bacteria to the sulphur cycle in the sea is second in importance only to their essential role in the formation and transformation of carbon and nitrogen compounds. This cycle can be said to start with sulphate, of which sea water normally contains from 0.24 to 0.27 per cent. Plants as well as most bacteria can obtain the sulphur essential for the synthesis of their protoplasm from sulphate. In so doing sulphate is partly reduced and converted into organic sulphur compounds. The latter ultimately undergo decomposition, a process in which a large variety of bacteria participate.

Under anaerobic conditions sulphur is liberated from organic compounds primarily as hydrogen sulphide. Organic sulphates may be hydrolysed both aerobically and anaerobically with the liberation of sulphate. In aerobic environments hydrogen sulphide is also an important hydrolysis product of albuminous material but, depending upon the pH and Eh, an appreciable part of the hydrogen sulphide liberated in oxygenated waters may be abiotically oxidised to elemental sulphur or sulphate. In the presence of heavy metals part of the hydrogen sulphide will be precipitated from solution. Iron and other metallic sulphides may be buried in sediments, this being one of the chief causes of their blackness, or precipitated sulphides may be dissolved and returned to the cycle as a result of certain bacterial activities which tend to lower the pH.

Sulphate-reducing bacteria of the genus *Desulfovibrio* and possible other genera liberate large quantities of hydrogen sulphide from inorganic sulphates. Hydrogen sulphide from this source may accumulate in concentrations sufficiently high to render certain environments (e.g., the deeper parts of the Black Sea, stagnant water basins, wake of the "red tide") inhospitable to other forms of life.

In the presence of light at favourable hydrogen-ion concentrations purple sulphur bacteria oxidise hydrogen sulphide to elemental sulphur or to sulphate, even in the absence of free oxygen. Likewise in the absence of free oxygen where nitrate is present *Thiobacillus denitrificans* may oxidise hydrogen sulphide, elemental sulphur, or thiosulphate. This and other species of *Thiobacillus* catalyse similar oxidations in the presence of free oxygen.

Several genera of achromic sulphur bacteria that oxidise hydrogen sulphide, elemental sulphur, sulphite, or thiosulphate occur in the sea. Their activity, like that of other bacteria which enter the sulphur cycle in the sea, is influenced by oxygen tension, Eh, pH, light penetration, interrelations with other organisms, and organic content of the water or mud (van Niel, 1936; Baas Becking and Wood, 1955). Sulphate reducers are most abundant and active in bottom deposits (ZoBell and Rittenberg, 1948). Being strict anaerobes, sulphate reducers occur in water only in localised areas. They utilise a large variety of organic compounds. Some are autotrophs which can utilise molecular hydrogen as an energy source (Sisler and ZoBell, 1950). Their common association with petroleum and sulphur deposits coupled with other evidence suggests that sulphate-reducing bacteria play a part in the origin of oil (ZoBell, 1952) and the deposition of sulphur (Jones, 1955).

PHOSPHATE REGENERATION

A lack of phosphate in certain waters is believed to limit plant productivity. Phosphate may also limit the bacterial decomposition of certain kinds of organic matter. Phosphate is depleted from waters primarily by plants and bacteria which use it in their nutrition for the synthesis of phosphoproteins and phospholipids. The phosphorus requirements of animals are satisfied by the ingestion of plants, bacteria, or other animals. Large quantities of phosphorus become bound in the bodies of organisms or their remains. Part of this sinks to the sea floor and unless placed back in solution may be removed from the phosphorus cycle by burial. In alkaline environments phosphate may be precipitated from solution and ultimately buried in sediments.

Bacteria play an indispensable role in the phosphorus cycle by decomposing organic phosphorus compounds with the regeneration of phosphate and by producing acids which tend to dissolve phosphates (Renn, 1937). There is no evidence for a biological change in state of oxidation of phosphorus, although high-energy phosphates participate in many oxidations. The solubility of phosphate in sea water is largely a function of pH, a property which is influenced by bacterial activities.

EFFECTS OF BACTERIA ON PHYSICOCHEMICAL CONDITIONS

Sea water is normally well buffered to provide a reaction ranging from pH 7.5 to 8.5. In localised environments bacterial activities may cause the pH to be extended beyond this range. The pH may be increased by the (a) formation of ammonia from the decomposition of proteinaceous substances, (b) reduction of nitrate or nitrite, (c) reductions of sulphate, (d) oxidation or decarboxylation of organic acids, and (e) assimilation of carbon dioxide. During phytoplankton blooms, photosynthetic organisms may consume carbon dioxide rapidly enough to raise the pH of surrounding water to 8.8.

The pH of water or sedimentary materials may be decreased by bacteria which (a) oxidise ammonia to nitrite or nitrate, (b) oxidise hydrogen sulphide or sulphur to sulphate, (c) produce carbon dioxide or organic acids, or (d) liberate phosphate from organic compounds. Ordinarily under natural conditions both acidifying and alkalinising processes are taking place simultaneously so there is little or no change in pH, but in localised environments, as in sediments or microspheres, one bacterial process or another may predominate. For example, certain sulphur bacteria may produce enough sulphuric acid from the oxidation of hydrogen sulphide or sulphur to depress the pH of sea water to 4.5 or lower. On the other end of the scale the bacterial reduction (destruction) of nitrate has resulted in certain media becoming more alkaline than pH 9.5.

Although one might expect sulphate reduction to result in highly alkaline conditions, sulphate-reducing bacteria generally lower the pH because, in oxidising large quantities of organic matter or hydrogen as energy sources for the reaction, they produce acidic products in excess of the effect on pH of the sulphate reduced.

For many years the study of marine microbiology was motivated by the alleged importance of denitrifying bacteria in the precipitation of calcium carbonate in shallow tropical seas. The extensive observations of Drew, Lipman, Bavendamm (1932), Baier (1937), and others have established that calcium carbonate precipitation is influenced by microbial activities; mostly by their effects on the pH, which affects the solubility of calcium carbonate.

The solubility of iron and manganese is influenced by the pH. The solubility of these minerals as well as their state of oxidation is also influenced by the redox potential. The Eh and pH of water or sediments are probably much more important in the decomposition of iron and manganese in the sea than the activities of iron- or manganese-oxidizing bacteria. Bacteria appear to be the principal dynamic agents which lower the redox potential of marine sediments. This they do by consuming free oxygen and by destroying other oxidising materials or by producing hydrogen sulphide and other reducing agents. The redox potential of sediments affects their chemical composition, reactivity, diagenesis, colour, biota, and other properties (ZoBell, 1946b; Baas Becking and Wood, 1955). Reducing conditions favour the formation and preservation of petroleum hydrocarbons.

ORIGIN OF OIL

Crude oil or petroleum, which consists largely of gaseous, liquid, and solid hydrocarbons, is believed to be formed in shallow seas where the rate of sedimentation is rapid, organic productivity is high, and conditions are reducing. In such an environment, it is the general tendency of bacteria to attack organic matter with the selective removal of oxygen, nitrogen, sulphur, and phosphorus, thereby leaving carbon complexes that are relatively richer in hydrogen or more akin to petroleum in chemical composition than the original organic matter. In so doing, bacteria often produce appreciable quantities of methane, an important constituent of crude oils and natural gas. Large quantities of molecular hydrogen are also produced during the anaerobic fermentation of organic matter. Free hydrogen, however, is rarely detected in source sediments of petroleum, probably because it is used by bacteria either to reduce sulphate or to react with carbon compounds, including carbon dioxide, to form hydrocarbons or similar compounds (ZoBell, 1952).

The original organic matter, particularly that in oil-rich diatoms, may consist in part of hydrocarbons which bacteria liberate during the decomposition of associated proteins and carbohydrates. Some of the organic matter assimilated by bacteria is converted into bacterial protoplasm consisting of from 0.02 to 0.5 per cent of liquid and solid hydrocarbons. By dissolving carbonate or sulphate rocks on which oil is absorbed or held in capillary spaces, bacteria may promote its release, flow, and accumulation in pools.

Whether crude oil could be formed in the absence of bacteria or whether bacteria play more than a minor role in the origin of oil is yet to be determined. Unquestionably, though, bacteria do influence the modification of organic matter and physicochemical conditions in source sediments of petroleum.

BIOLUMINESCENCE IN MARINE MICROBES

Luminous microorganisms are preponderantly marine. Relatively only a few terrestrial forms are known. Fish as well as the flesh of most animals, both marine and freshwater or terrestrial, may become overgrown by luminous bacteria. Parasitic luminous bacteria infect freshwater shrimps, certain insects, amphipods, and millipedes, causing these terrestrial animals to glow brightly in the dark. Luminescence in dead vegetable matter such as wood, roots, potatoes, and fruit is usually due to luminous fungi generally of the class Basidiomycetes, none of which are marine.

It is in the sea, however, that most varieties and the largest populations of luminous bacteria occur. The organic remains of virtually all kinds of marine plants and animals may support the growth of such bacteria. Only a few animal species are known to be invaded by parasitic luminous bacteria. Beach fleas, certain crabs, and shrimps appear to be particularly susceptible to such infections. A good many other marine animals may be also, but getting incontrovertible evidence is difficult. Finding dead or moribund animals aglow with luminous bacteria fails to prove the pathogenicity of the latter. Luminous bacteria commonly found associated with apparently healthy fish, squid, or other animals may be either symbiotic or purely saprophytic species.

Luminescence in certain jelly-fish, ctenophores, pennatulids, euphausiids, squid, and fish has been attributed by various investigators to the activities of symbiotic luminous bacteria. Bacterial symbiosis, however, has been established in the light organs or glands of only a few species of fish and squid (Harvey, 1952; Haneda, 1955).

Most luminous bacteria are saprophytic, growing readily on a great variety of proteinaceous materials. They may grow in sufficient abundance on the dead and decomposing remains of plants and animals to create conspicuous areas of luminescence in the surf along beaches, in waves breaking over reefs, in the wake of vessels, etc. Luminous dinoflagellates, however, including species of *Goniaulax*, *Ceratium*, *Peridinium*, and *Prorocentrum*, are responsible for the most intense displays of luminescence.

Luminous bacteria from the sea require either sea water or a similar balanced mineral-salts solution containing from 1.5 to 3 per cent sodium chloride for their growth. From 3 to 4 per cent salt is required for maximum luminescence. Free oxygen is also required for luminescence. Light emission by luminous bacteria can occur at such low oxygen tensions that they have been employed to detect traces of oxygen.

METHODS IN MARINE MICROBIOLOGY

Various devices have been developed to provide for the aseptic collection of water samples from any desired depth in the sea (ZoBell, 1941). Most of these devices consist of tubes, bottles, flasks, or bulbs that can be sterilised and submerged sealed with a piece of capillary glass tubing. Provisions are made for breaking the glass tubing by a messenger-activated mechanism, thereby admitting water. Closing is not necessary to prevent contamination, because as the sample is hauled toward the surface the resultant diminishing pressure tends to force water or entrapped air slowly from the receptacle. Either tremendously sturdy receptacles or reversibly collapsible ones, like rubber or polyethylene bottles, are required to cope with high pressures encountered at great depths. Such sampling devices are usually designed to operate on a standard hydrographic wire in tandem with each other or with reversing thermometers, Nansen bottles, etc. In this way water samples or temperature data can be obtained from several different depths simultaneously by a single cast. Though useful, even the best bacteriological water samplers need improvement to make them perfect.

Samplers for collecting mud or other bottom deposits leave much to be desired. Various kinds of grabs, dredges, snappers, scoops, tubes, and corers that are used for this purpose generally provide only rather crude samples. It is rarely known with exactness the depth to which such samples penetrate bottom sediments, there may be some mixing of materials, and it is difficult to provide for aseptic operations. Despite these shortcomings bottom samples suitable for bacteriological analysis have been obtained from some of the deepest parts of the ocean. Results of quantitative significance have been obtained, because the bacterial population of bottom deposits is generally so much higher than that in the overlying water that contact with water makes little quantitative difference. Radially central sub-samples aseptically dissected from firm cores provide material for reliable results. Urgently needed are devices for obtaining microstrata at the mud-water interface, the zone of most intense bacteriological activity in the sea. Aqualung divers can obtain such samples in manually manipulated apparatus, but they are restricted to shallow depths.

Both water and sediment samples should be analysed as soon as possible following collection. Storage for only a few hours even under the most favourable conditions of refrigeration may result in appreciable changes in the total numbers and kinds of bacteria in samples of marine materials. This usually dictates the necessity of examining samples at sea, since it may require several hours or days to transport samples to the shore laboratory. Of cardinal importance is refrigeration of the samples owing to the thermosensitivity of so many marine microbes. Fast freezing followed by refrigeration near -10°C seems to be the best way to preserve samples with a minimum of change when field conditions make it impracticable to start examinations by cultural or direct microscopic procedures. Fast freezing, though, is not without its pitfalls.

The motions of a ship at sea coupled with a lack of facilities ordinarily found in laboratories ashore necessitates many modifications of routine techniques and equipment. Provisions must be made to prevent test tubes, bottles, pipettes, etc. from tipping over, rolling, or sliding away. Such a simple operation as pouring a plate of nutrient agar on a rolling ship is attended by many difficulties: unless the agar is cooled to near 42°C before pouring and rapidly cooled below 30°C immediately after pouring, many thermosensitive bacteria may be killed. Before congealing, the agar may slop out of plates unless stabilised on a platform hung on gimbals or mounted on a gyroscope. Flat-sided, six-ounce, screw-capped bottles are preferred to Petri plates for growing bacteria in nutrient agar at sea.

So diverse are the nutrient and cultural requirements of marine microbes from different materials or places that a nutrient medium is yet to be formulated to provide for the growth of more than a small percentage of the total number present. It is necessary to employ media and conditions tailored to satisfy the requirements of each particular kind or group of microbes.

The well known limitations of direct microscopic methods are multiplied by the motion of a ship at sea. Excessive vibrations may make it impossible to keep a compound microscope in focus at the high magnifications required to recognise bacteria. Only on calm seas and when the engines are idling or stopped is it possible to make direct microscopic counts of bacteria in sediment or other marine materials. The bacterial population of most water samples is so sparse that concentration by centrifugation, filtration, or flocculation may be necessary to make direct microscopic observations meaningful. The molecular or millipore filter is proving to be a useful tool for concentrating microorganisms for direct microscopic observation or for cultivating them as colonies.

SOME UNSOLVED PROBLEMS

An accurate assessment of the importance of bacteria and allied microorganisms in the sea requires many more observations on their numbers and kinds in water masses and bottom deposits in different parts of the world. There is a crying need for improved methods for collecting and analysing samples of marine materials. World-wide cooperation is desirable to provide for the standardisation of techniques and the exchange of ideas.

Microbiologists can contribute substantially to the science of the sea, to basic biology, and to the welfare of man by giving detailed attention to the solution of problems such as the following:

Microbial fixation of nitrogen in the sea.

Microbial infections of marine plants and animals. Are marine algae invaded by viruses?

Factors affecting the survival of bacteria of sanitary significance in the sea.

Microbial growth-promoting and growth-inhibiting properties of marine materials.

Microbial modification of marine food products and control measures.

Role of bacteria and microflagellates in nutrition of marine animals.

Microbial degradation of natural debris in the sea and on beaches.

Microbial concentration of trace elements such as vanadium, manganese, radioactive isotopes, etc.

Microbial characterisation of water masses by determining populations or using bioassay techniques.

Contributions of bacteria, microflagellates, blue-green algae, etc. to the biomass in the sea.

Phylogenetic position of microbes that are singularly affected by salinity, temperature, and/or hydrostatic pressure.

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ADDED IN PROOF

Among the many papers that have appeared since this article was prepared for the Symposium on Marine Microbiology are the following of outstanding significance:

- BRISOU, J., 1955: "La Microbiologie du Milieu Marin". Éditions Médicales Flammarion, Paris, 272 pp. (167 ref.).
- KRISS, A. E., 1959: "Marine Microbiology (Deep Water)". Academy of Science USSR, Moscow (In Russian), 452 pp. (450 references having Russian titles and 146 in other languages).
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APPENDIX: LIST OF MARINE MICROBIOLOGISTS AND THEIR MAJOR RESEARCH
INTERESTS IN VARIOUS COUNTRIES OF THE WORLD

	LOCATION OF LABORATORY	MAJOR RESEARCH INTERESTS
AUSTRALIA		
L. G. M. Baas Becking	CSIRO, Bureau of Mineral Resources, Canberra, Australia	Geobiology
A. B. Cribb	University of Queensland, Brisbane	Fungi and algae
E. J. Ferguson Wood	CSIRO, Division of Fisheries, Cronulla, N.S.W.	Bacteria and plankton
CANADA		
C. H. Castell	Fisheries Tech. Station, Halifax, N.S.	Microflora of fish spoilage
H. D. Dussault	Fisheries Tech. Station, Grand-Riviere, Quebec	Halophiles; microbes on salted fish
R. A. MacLeod	Pacific Fisheries Exp. Station, Vancouver, B.C.	Bacterial nutrition and metabolism
H. L. A. Tarr	Pacific Fisheries Exp. Station, Vancouver, B.C.	Fish microflora and spoilage
W. Yaphe	Maritime Regional Laboratory, Halifax, N.S.	Bacteria; agar liquefiers
DENMARK		
E. Fjerdingstad	Inst. Hygiene, Copenhagen Univ.	Pollution problems and microbial ecology
E. Steeman-Nielsen	Royal College of Pharmacy, Copenhagen	Organic production, antibiotics
EGYPT		
A. A. Aleem	University of Alexandria, Alexandria	Fungi and algae
ENGLAND		
R. W. Butcher	Ministry of Agriculture and Fisheries, Nottingham	Microplankton
K. R. Butlin	Chemical Research Lab., Teddington, Middlesex	Sulphate-reducing bacteria
Mary W. Parke	Marine Biological Lab., Plymouth	Microflagellates
J. R. Postgate	Humber Laboratory, Hull	Luminous bacteria; fish spoilage
R. Spencer	Microbiological Exp. Station, Porton, Wiltshire	Sulphate-reducing bacteria
P. C. Wood	Fisheries Laboratory, Burnham-on-Crouch, Essex	Pollution problems
FRANCE		
Jean Brisou	Institut de Biologie, Cannes	Systematic survey of bacteria
B. Callame	La Rochelle Marine Station, Charente-Maritime	Marine bacteria and fouling
Jacques Debyser	La Rochelle Marine Station, Charente-Maritime	Bacteria in sediments
J. Devèze	Endoume Marine Station, Marseille	Marine bacteria in general
Mlle C. Lalou	University of Paris, Paris	Calcium carbonate precipitation
A. R. Prévot	Institut Pasteur, Paris	Anaerobic bacteria
Jacques C. Senez	Centre de Recherches S.I.M., Marseille	Bacteria; sulphate-reducers
GERMANY		
Horst Engel	Staatsinstitut für Botanik, Hamburg	Aquatic chemo-autotrophs
Curt Hoffman	Institut für Meerskunde, Kiel	Phosphorus and sulphur cycles
Willy Höhnk	Institut für Meeresforschung, Bremerhaven	Aquatic fungi
Holger W. Jannasch	Hydrobiologische Anstalt, Plön, Holstein	Microbial ecology and physiology
R. Kreuzer	Bundesforschungsanstalt für Fischerei, Hamburg	Bacteriology of fish
V. Meyer	Institut für Meeresforschung, Bremerhaven	Marine bacteriology
Gertrud Müller	Hygienisches Institut, Hamburg	Water and sewage bacteria
Adelheid Müller	Institut für Microbiologie, Greifswald	Geomicrobiology
Wilhelm Schwartz	Institut für Microbiologie, Greifswald	Geomicrobiology
GREECE		
G. P. Alivistos	Institut d'Hygiène, Université Athènes	Water pollution problems
INDIA		
A. Sreenivasan	Fisheries Tech. Station, Kozhikode, S. India	Bacterial ecology and physiology
N. K. Velanker	Central Marine Fisheries Res. Station, Mandapam	Bacterial ecology
R. Venkataraman	Fisheries Tech. Station, Kozhikode, S. India	Bacterial ecology and physiology
ISRAEL		
M. Shelubsky	Laboratory for Fish Diseases, Jerusalem	Microplankton
ITALY		
Sebastiano Genovese	Instituto di Zoologia, Messina	Bacterial ecology and physiology
F. Petrilli	Instituto d'Igiene, Università di Genova	Pollution problems

JAPAN

Yoshihiko Hata	Shimonoseki College of Fisheries, Yoshimi	Bacterial physiology; sulphate reducers
Y. Hiyama	Tokyo University, Tokyo	Microplankton
Hajime Kadota	Kyoto University, Maiduru	Bacterial biochemistry
Masao Kimata	Kyoto University, Maiduru	Sulphate reduction
Yosio Kobayashi	National Science Museum, Tokyo	Marine fungi
Nobuo Taga	Institute of Fisheries, Tokyo University, Tokyo	Fish microflora
Eiichi Tanikawa	College of Fisheries, Univ. of Hokkaido, Hakodate	Microflora of fish spoilage
Y. Tomiyasu	Dept. Fisheries, Kyushu Univ., Kyushu	Microflora of fish spoilage
B. Zemitani	Dept. Fisheries, Nagasaki Univ., Nagasaki	Marine yeasts; microflora of fish

JUGOSLAVIA

Vlaho Cviic	Institute of Oceanography, Split	Bacterial ecology and physiology
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NEW ZEALAND

T. Martin Skerman	New Zealand Oceanographic Institute, Wellington	Biofouling and bacterial ecology
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NORWAY

Sverre Hjorth-Hansen	Fisheries Tech. Station, Bergen	Halophiles; fish microbiology
Olaug M. Sømme	Biologisk Laboratorium, Oslo	Fish microflora

RUSSIA (U.S.S.R.)

V. J. Birusova	Institute of Microbiology, Moscow	Bacterial biomass
M. D. Chumace	Institute of Microbiology, Moscow	High pressure
V. O. Kalinenko	Institute of Oceanography, Moscow	Nitrification; bacterial fouling
S. A. Kolesnik	All Union Research Institute, Leningrad	Geological microbiology
A. E. Kriss	Institute of Microbiology, Moscow	Bacterial biomass and activities
S. Kusnetzov	Institute of Microbiology, Moscow	Geomicrobiology
M. N. Lebedeva	Biological Station, Sebastopol	Ecology of marine microbes
E. L. Limberg-Ruban		Bacterial ecology
M. A. Litvinov	Botanical Institute, Leningrad	Marine fungi
I. E. Mischustina	Institute of Microbiology, Moscow	High latitude microbiology
M. J. Nowozhilova	Institute of Microbiology, Alma-Ata	Marine yeasts
Prof. Perfilov	Institute of Microbiology, Leningrad	Microbiology of sea water and sediments
L. A. Rosenberg	Institute of Oceanography, Moscow	Nitrogen fixation in the sea
Mrs E. A. Rukina	Institute of Microbiology, Moscow	Bacterial biomass and physiology
L. D. Shturm	Institute of Microbiology, Moscow	Geomicrobiology; sulphate reducers
T. L. Simakova	All Union Research Institute, Leningrad	Geomicrobiology of petroleum
A. S. Tichonenko	Institute of Microbiology, Moscow	Bacterial physiology
A. J. Zhukova	Institute of Marine Fisheries, Moscow	Bacterial nutrition of marine animals

SCOTLAND

M. R. Droop	Marine Station, Millport	Microflagellates
D. G. Floodgate	Torry Research Station, Aberdeen	Taxonomy of marine bacteria
C. E. Lucas	Marine Laboratory, Aberdeen	Microbial metabolites
James E. Shewan	Torry Research Station, Aberdeen	Fish microflora and spoilage

SWEDEN

L. E. Ericson	Royal Inst. Technology, Stockholm	Bacteria and algae
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WALES

Mabyn Hudleston	University College of Wales, Aberystwyth	Bacterial ecology
Nial Reynolds	Fisheries Experiment Station, Conway	Bacterial populations of inshore waters
C. P. Spencer	Marine Biological Station, Anglesey	Bacterial physiology
Peter R. Walne	Fisheries Experiment Station, Conway	Microflagellates
Irene Wilson	University College of Wales, Aberystwyth	Marine fungi

U.S.A.

Galen E. Jones	University of California, La Jolla, California	Microbial nutrition and metabolism
Bostwick H. Ketchum	Woods Hole Oceanographic Institution, Woods Hole, Mass.	Ecology and pollution
C. S. McCleskey	Coastal Studies Institute, Baton Rouge, La.	Bacteria in sediments and shellfish
T. W. Johnson	Duke University, Durham, North Carolina	Marine fungi
John Liston	University of Washington, Seattle, Washington	Fish microflora and spoilage
J. R. Merkel	Fort Johnson Mar. Biol. Lab., Charleston, South Carolina	Microbial biochemistry

S. P. Meyers	The Marine Laboratory, Miami, Florida	Marine fungi; toxic bacteria
Richard Y. Morita	Houston University, Houston, Texas	Bacterial ecology and physiology
Carl H. Oppenheimer	Inst. Marine Science, Port Aransas, Texas	Bacterial ecology and metabolism
E. J. Ordal	University of Washington, Seattle, Washington	Diseases of marine animals; pollution
David Pramer	Rutgers University, New Brunswick, New Jersey	Antagonistic effects of sea water
Luigi Provasoli	Haskins Laboratories, New York City, New York	Microbial nutrition
Sydney C. Rittenberg	Hancock Foundation, Los Angeles, California	Geochemistry and pollution
Frederick D. Sisler	Navy Defense Lab., Panama City, Florida	Ecology, biofouling, geochemistry
Robert L. Starkey	Rutgers University, New Brunswick, New Jersey	Sulphate reduction; corrosion
Theodore J. Starr	Fish and Wildlife Service, Galveston, Texas	Bacterial nutrition and metabolism
R. F. Vaccaro	Woods Hole Oceanographic Institution, Woods Hole, Massachusetts	Nitrification; bacterial ecology
Helen Vishniac	Yale University, New Haven, Conn.	Marine fungi
B. E. Volcani	Scripps Institution of Oceanography, La Jolla, California	Halophilic bacteria, biochemistry
S. W. Watson	Woods Hole Oceanographic Institution, Woods Hole, Massachusetts	Marine fungi and virus diseases
O. B. Williams	University of Texas, Austin, Texas	Sulphate-reducing and other bacteria
Claude E. ZoBell	University of California, La Jolla, California	General survey of marine microbes

HYDROLOGY AND MICROBIOLOGY

By D. J. ROCHFORD, CSIRO, Division of Fisheries and Oceanography, Cronulla, Australia

THE SCOPE OF HYDROLOGY IN MARINE SCIENCE

Marine science has many disciplines, with physicists, chemists, biologists, geologists and others contributing specialised knowledge to the general picture. In this sense the hydrologist's approach is largely physico-chemical, but in its wider sense hydrology has to take account of certain ecological factors as well. Thus whilst the hydrologist must use the techniques of physics and chemistry to establish a descriptive picture of the ocean waters, and their complex movements, he must always be aware of the living nature of the sea and of the part that biological processes can play in the various cycles that occur. Moreover he must often make use of biological data to supplement and in some cases direct his use of physico-chemical variables in the identification of water masses, or to establish particular structural features.

In relation to microbiology, therefore, the hydrologist has to supply descriptive data about the origin, properties, and circulation of the water masses of a region, and of the seasonal and longer term changes in their circulation paths. The surface and deep circulations must both be considered so that a full history of each water mass can be established.

The origin, utilisation, and redistribution of the various major and minor trace elements in the sea must also be studied by the hydrologist, who must recognise the probable role of microbiological effects in some at least of these processes.

The microbiologist is anxious to know how quickly carbon dioxide and oxygen can be exchanged across the air-water boundary at the surface, and how rapidly these gases can be transported downward by linear movement or eddy mixing. In general terms the hydrologist can supply such information.

In the deeper basins of the sea the hydrologist can afford to neglect the possible role of bottom sediments in the trophic cycles. In proximity to continental slopes and submarine rises, however, elements such as molybdenum, copper, iron, manganese, that are in minute supply in the upper euphotic zone, are often carried up from the relatively enriched bottom sediments to the surface layers, where they can be transported (Jerlov, 1953) for considerable distances by ocean currents.

SOME GENERAL FEATURES OF THE OCEAN

In general the temperature of the ocean decreases with increasing latitude and depth. The range of surface temperature from the Tasman Sea to the Antarctic continent is almost the same as the vertical temperature profile in the mid-Tasman Sea, from surface to depth. The salinity of the ocean has a maximum value in a sub-tropical belt where evaporation is high and precipitation low, and minimum values near the equator and towards the polar regions.

The vertical profile of salinity therefore does not parallel the meridional distribution at the surface, but can be related to the same subsurface circulation as temperature.

The variation in dissolved oxygen content with depth follows a complex pattern. For example, extensive sub-surface regional maxima and minima are found in the South Pacific. Biological processes are involved. Oxidation of organic matter produced in surface layers reduces the oxygen content of these layers and to some degree that of the sub-surface layers. The content of major inorganic nutrients, such as phosphates and various nitrogen compounds, increases with depth since their regeneration cycles are not complete in surface layers and some fraction is always lost to the deeper regions. The light which is utilised in photosynthesis in the sea, suffers considerable reduction in quantity with depth. At about 50 m

below the surface in waters of average turbidity, only about 1 per cent of the surface illumination is found; in very clear waters this value is not reached until the depth is 200 m; in very turbid water the 1 per cent value may be reached at only 10 to 20 m depth.

The surface layers (down to 100 m) of the ocean circulate at speeds of up to 3 knots. Some of the deep ocean waters however may take hundreds of years to recirculate.

WATER MASSES AND THEIR IDENTIFICATION

In the oceans certain combinations of meteorological and circulatory factors form, either seasonally or continually, bodies of water which possess characteristic ranges of salinity, temperature and nutrient levels. From their source region these bodies of water, which are called water masses, move along particular circulatory paths until their identity is destroyed by mixing or until they sink and recirculate at subsurface levels with only small changes in properties.

The general physical features of source regions, together with nutrient levels and light intensities, could determine the development of a characteristic microbiological population in these water masses. The species composition of this microbiological population is often just as characteristic of the water mass as are the physico-chemical variables.

TYPES OF CIRCULATION IN THE OCEAN

When we say that a water mass is following a particular path in the ocean, we refer to its average resultant displacement. Within this average movement, however, there are significant minor circulatory systems, which, along the boundary regions particularly, involve complex convergence, divergence, and eddy movements.

In a convergence the two water bodies move together and one may sink to recirculate at deeper levels. Often these convergence zones act as surface traps for floating and suspended material such as oil slicks, particulate matter, and plankton.

In a divergence the two water bodies are moving apart at the surface, and part of their motion is a vertical movement from deeper levels. In a divergence zone, therefore, deeper waters, rich in organic nutrients, may be brought into the euphotic zone. Often bodies of water break off from an ocean current and circulate independently as eddies.

Ocean circulation thus generally consists of the paths followed by the major water masses from source to disintegration or disappearance, with associated complex minor systems of circulation waxing and waning in intensity. It can be postulated for example that individual water particles in the south-flowing East Australian current probably move in spiral fashion from right to left at the surface and from left to right in the lower 100 to 200 m stratum.

In studying the microbiology of any one region of the ocean therefore, the microbiologist must allow for the varying degree of stability of the circulation pattern and for the degree of advective influence upon the microbiotic picture.

NUTRIENT CYCLES IN THE SEA

Early workers in marine science found seasonal changes in the quantities of phosphates and nitrates in sea water, which were the inverse of changes in the numbers of diatoms. From this it was concluded that phosphates and nitrates are essential nutrients of the sea, whose concentration influences the amount of organic matter that can be synthesised by diatoms and marine algae in general.

Essentially, this is still held to be true, although it is now realised that high fertility can prevail in some regions of low phosphate concentration because the turnover rate is very high, and it is only because there is no time for phosphates to accumulate in the water that their concentration is low. Recent work suggests that nitrogen compounds other than nitrates can be utilised by diatoms.

The amount of silicates also varies seasonally in some parts of the ocean as does that of the minor trace elements such as zinc, manganese, molybdenum, copper, and iron. The seasonal cycle of nutrients is studied by sampling at a fixed location over a period of time. From the seasonal decrease in phosphates one can determine the amount of organic matter that has been formed. Similarly from the changes in pH the hydrologist can obtain indications of the amount of carbon dioxide involved in photosynthesis. Both phenomena assist in attempts to estimate the productivity of ocean waters.

However, because the sea is a dynamic medium, the seasonal picture at any one location is being continually changed by advective processes, as different water masses pass through the region of observation. Before the microbiologist can relate the seasonal changes in microbiota to local environmental conditions, it will be necessary for the hydrologist to furnish him with a description of the seasonal changes in water mass composition and thus provide an estimate of the stability of the local environment.

Along the east Australian coast the local seasonal cycle for any coastal region is very difficult to determine because of advective changes. In summer the bottom coastal waters off Port Hacking can be changed by advection in a matter of days to have phosphate values some 500 per cent higher than normal, and temperatures below winter values.

With short-term fluctuations of this order it is very difficult to separate biological and advective effects in the seasonal cycle of nutrients in east Australian coastal waters.

HYDROLOGICAL FEATURES OF THE TASMAN SEA

Various features of Tasman Sea circulation have been investigated, and examples from this work are given to demonstrate the complexity of the hydrological environment of micro-organisms.

The General Circulation of the Tasman Sea

Along the east Australian coast there occurs a southerly flow of tropical water during the spring and summer months. There has been much conjecture about the origin and characteristics of this current since Halligan's report to the 4th Pacific Science Congress in Batavia in 1929.

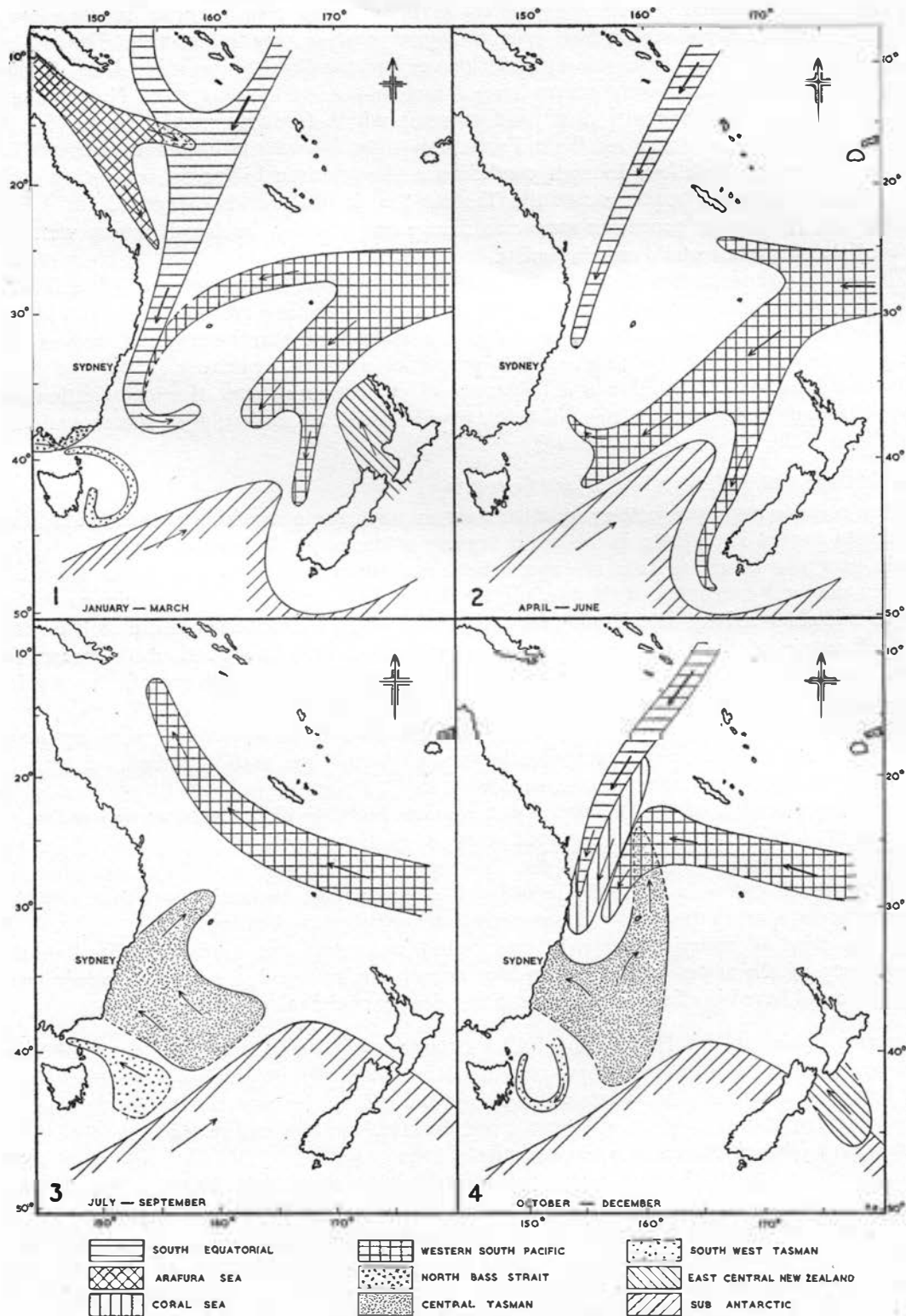
Recent investigations by the Division of Fisheries and Oceanography suggest a complex but most interesting origin (Rochford, 1957).

In the mid-summer period the current is largely composed of waters originating in the south equatorial current, which flows south through the Coral Sea (fig. 1). During the autumn the proportion of this water decreases (fig. 2). This decline is associated with a slowing down of the southerly flow, and by mid-winter the central Tasman water mass is dominant and the southerly flow is very slight or reversed to the north (fig. 3).

In winter, however, there is a flow of waters from the south-west Pacific into the Coral Sea. When the new flow of south equatorial waters commences in the period October–November (fig. 4), entrainment of this south-west Pacific water mass occurs in the Coral Sea, and the east Australian current off Sydney in November and December contains a larger percentage of this water mass (western south Pacific of fig. 1 to 4).

This picture represents only an average condition, however, since at various regions within the current, vertical movements are bringing deeper water to the surface, creating differing micro-environments within the general flow.

In the summer period, waters from the south-west Pacific and the central Tasman lie at subsurface and intermediate depths down to about 500 m, and various amounts of these are brought to the surface by vertical movements. In the winter the central Tasman water is at the surface and the Antarctic intermediate water lies beneath it. Vertical movements in this period bring some of the latter to the surface.



Figs. 1-4. Seasonal trends in the general surface circulation in the Tasman Sea

Certain of the water masses encountered in the Tasman Sea originate at considerable distances, and to some extent their microbiological content may reflect this. The western south Pacific water mass, for instance, which moves into the Coral Sea in mid-winter probably originates in a large-scale eddy system several thousand miles to the east of New Zealand. This is a region of minimum cloud and constant winds from the south-east so that the evaporational losses of water are high. Presumably light intensity is high and nutrients low because of vertical isolation. In such conditions a characteristic biological population could arise. When this water mass reaches the Tasman Sea, it has probably travelled for 3 to 6 months, but its physical properties appear to change only slightly. Its flow is largely westward so that light conditions would remain similar.

The south equatorial flow into the Coral and Tasman Seas brings water which originates in a region of high precipitation and cloudiness and is therefore probably more turbid, but has a moderately high nutrient content. Again a specialised microbiota could develop and remain a characteristic of that water until its properties were lost by mixing.

In studies of the distribution and behaviour of circulation systems, the identification and general biological study of the microbiota may well provide an indication of the geographical derivations of the water masses concerned.

The Transport of Sediment by Surface Currents

Deep ocean waters and bottom sediments act as traps for many trace elements which are of microbiological importance in the upper regions of the ocean. Because of the low solubility of the generally occurring salts of iron, cobalt, manganese, and molybdenum in sea water, reserves cannot be maintained in the surface layers and depletion of them occurs during intense microbial activity. By complex formation with organic compounds in the chelate form, more of certain of these salts can be held at the surface. But, in general, a mechanism for upward movement of these trace substances from the deeper layers must exist for production to be maintained in the surface layers.

Solution of some of these trace substances however can only take place in a reducing environment. If this environment is available in the sediments then vertical turbulence must be invoked to transport the nutrients to the surface.

From recent studies of the Tasman Sea it appears probable that phosphate-rich sediments are brought to the surface and transported seawards to distances of 50 to 100 miles from the slope region of eastern Australia. In the Tasman and Coral Seas, phosphate anomalies are found in surface waters in proximity to submarine banks: these anomalies have been assumed to represent transport to the surface of constituents of the bottom sediments.

The quantity of materials derived from bottom sediments has a marked effect on the transparency of the water and, by providing growth surfaces and a supply of minor trace elements, could have big effects on the growth and density of the micro-organisms.

Long-term Cycles in the Hydrological Characteristics of East Australian Coastal Waters

In any one location the circulation pattern varies from year to year, and the hydrologist must know the extent of this variation throughout the region: he must further decide whether these variations are part of a long-term trend in circulation or merely the result of local effects upon a fairly consistent general circulation.

Studies of the coastal hydrology off Sydney carried out since 1942 illustrate how difficult such a decision can be. As fig. 5 shows, there has been a rise in the level of total phosphorus in this area from 1942 to 1949 with maximum changes between 1947-49, and near-stationary conditions since 1950. These changes have been paralleled by changes in the inorganic-phosphate level but not in nitrate nitrogen levels. Slight temperature variations and considerable chlorinity and rainfall variations occurred during the same period (fig. 6).

These changes appear to be due to a change in oceanic circulation off Sydney such that in the period 1942-47 more central Tasman cold waters entered the area while after 1949 more Coral Sea or warm waters became involved in the circulation.

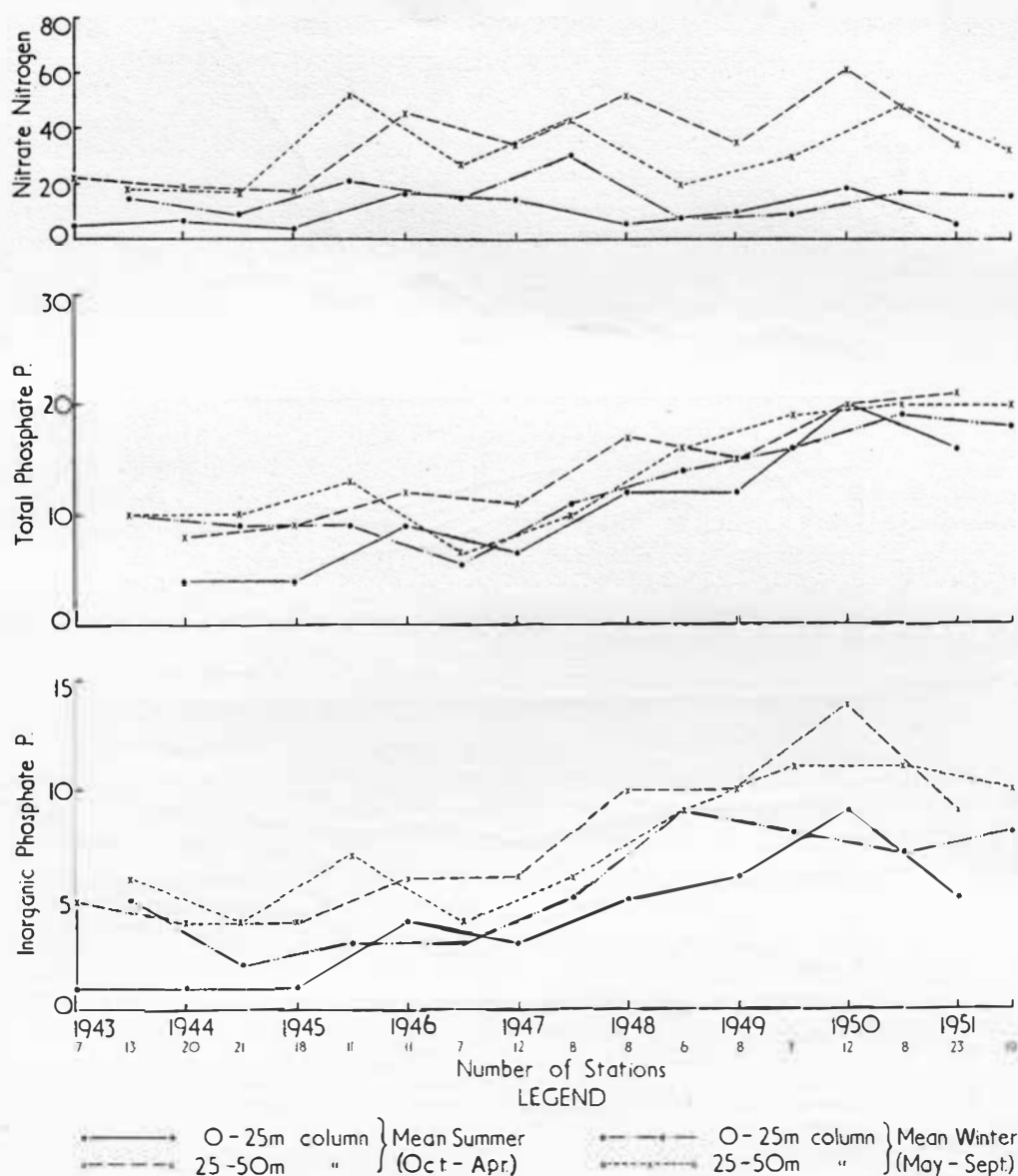


Fig. 5. Variations in nitrate nitrogen, inorganic and total phosphorus, in coastal waters off Port Hacking, 1943-51, (expressed in $\mu\text{g/l.}$)

This change in circulation was part of a change in climatic conditions in that dry westerly winds dominated to 1949 while moist easterly winds dominated after 1950. In the period 1947-49 the westerly winds caused lateral movements and some upwelling of phosphate-rich slope waters off Sydney.

However, after 1949 the phosphate values remained high despite a change in water-mass structure. Could this lag mean that phosphates have been fixed in the benthic community, which continues to retain them despite subsequent changes in water-mass composition?

The clue to this phosphate retention could lie in the microbiological activity on the surface of the bottom sediments where phosphates are solubilised, absorbed, and desorbed under pH and redox conditions largely under biological control.

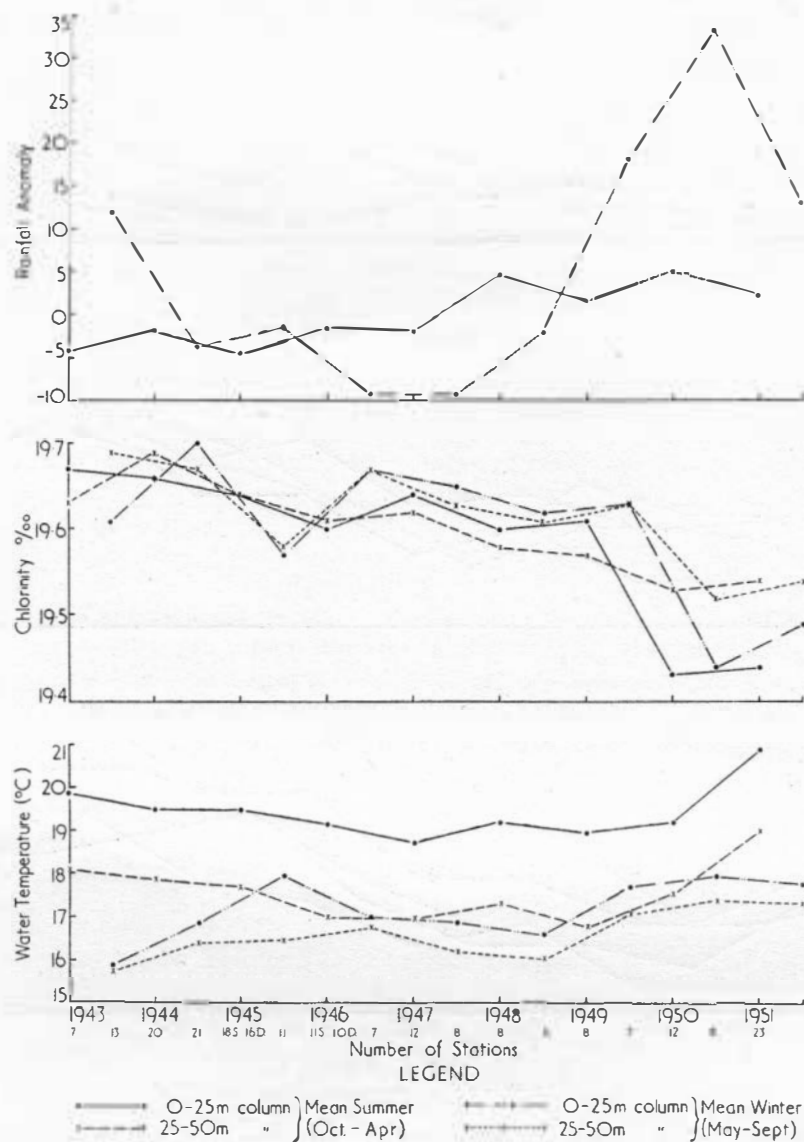


Fig. 6. Variations in mean summer and winter temperature and chlorinity in coastal waters off Port Hacking, 1943-51. Yearly rainfall anomalies (in inches departure from 50-year average) compiled by the N.S.W. Meteorological Bureau are also shown.

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MARINE PRODUCTIVITY

By E. J. FERGUSON WOOD AND P. S. DAVIS, CSIRO, Division of Fisheries and Oceanography, Cronulla, Australia

In oceanography, we are interested in several concepts regarding the utilisation of solar energy by marine plants: firstly, the gross gain in energy by biological means. This may be defined as "gross productivity." Chemoautotrophs are not included in this definition because they utilise energy already stored in the earth, either by previous biological activity such as sulphate reduction, or from igneous sources. Because, in their metabolism, they reduce the energy levels of the substances they use, they probably do not represent a net gain in energy even to the oceans. Gross productivity is a purely theoretical concept, of use to geobiologists and others interested in the energy relationships of biological and abiological processes.

A concept of more practical significance is the net productivity, or the net gain in energy taking into consideration photosynthesis and carboxylation on the positive side and respiration on the negative. The term gross production represents the total mass of material synthesised (which can, in theory, be measured by the gross amount of carbon dioxide consumed or of oxygen released in photosynthesis), and net production represents the amount of plant material actually produced.

The methods usually used to measure production are measures of the biological activity of the photosynthetic organisms, and in these methods little account is taken of the rates of reproduction of the members of the plant associations or of the rate at which the plants are being grazed by the zooplankton, or other marine animals.

Macroscopic seaweeds are limited to some 2 per cent of the ocean surface, and the major role in plant production in the sea is played by the widely distributed microscopic organisms. Of these, diatoms, blue-green algae, flagellates, including dinoflagellates, coccolithophores, chrysomonads, cryptomonads, and silicoflagellates, constitute the potential producers, while colourless flagellates, ciliates, rhizopods, and bacteria are consumers. The autotrophic bacteria (apart from photosynthetic forms) are producers, but derive their energy from the substrate. The term "potential producers" is used because many of the chlorophyll-bearing forms can also live by heterotrophic or phagotrophic nutrition, and so cannot be regarded as wholly productive. The quantitative relations in such cases between photosynthesis on the one hand and phagotrophy and heterotrophy on the other are not known.

BASIC FACTORS IN PRODUCTIVITY STUDIES

Light Penetration

Sea water absorbs various wavelengths differentially. In clear ocean waters, the blue and blue-green penetrate deepest, but in shallow and turbid waters, scattering due to particulate matter reduces the penetration of the blue light so that red light has a greater penetration. Because of this and because the carotenoid pigments of algae can transfer excitation energy to chlorophyll *a*, which alone appears to be capable of actual photosynthesis (except in the purple and green sulphur bacteria), there are difficulties in assessing the depth to which photosynthesis remains effective. The depth at which respiration balances photosynthesis is known as the "compensation depth" and is important as giving us a third dimension for productivity. The compensation depth may vary from a few metres in turbid, inshore waters to 100 m or more in clear tropical waters when the sun is directly overhead at midday.

Temperature

The effect of temperature on the reproduction of micro-organisms is complex. Some phytoplankton organisms are cosmopolitan but others are restricted to cooler or warmer waters, being confined to narrow temperature ranges. The general picture is that in cooler waters, large aggregations of phytoplankton occur during spring and fall but contain relatively

few species, while in tropical waters there is a large number of species which occur in fewer numbers throughout a large portion of the year. However, in certain localised areas, chiefly neritic, large concentrations of phytoplankton occur in warmer waters and constitute the so-called "red tides" in which the organisms may be so numerous as to deoxygenate the water and to cause mass mortalities of fish. Little attention appears to have been paid to the oceanic red tides consisting of *Oscillatoria* (*Trichodesmium*) which are known to occur commonly in the tropical Pacific and Indian Oceans.

Nutrients

The nutrients chiefly studied are nitrate and phosphate, and the conclusions drawn regarding the biological significance of these do not appear to be adequate. Chemists have ignored or minimised the fact that many phytoplankton organisms utilise ammonia, while others can make use of either nitrate, nitrite, or ammonia. Phosphate has been estimated without regard to the minimal amounts required by micro-organisms such as the diatoms. In fact, plankton swarms can occur with quantities of phosphate too low to be estimated by the usual chemical means, according to figures given by Goldberg (Goldberg *et al.*, 1951) and by Mackereth (1953) for species of the diatom *Asterionella*. In neritic and estuarine environments, dissolved nitrogen may be fixed by blue-green algae such as *Anabaena* and *Nostoc* spp., and in muds by anaerobic bacteria such as *Desulphovibrio*, *Clostridium*, *Chlorobium*, and *Chromatium*. Waksman *et al.* (1933) reported *Azotobacter* in the sea, but this has not been confirmed, and despite numerous attempts the authors have not yet isolated a strain of *Azotobacter* which could be regarded as marine. Baas Becking and Mackay (1956), have shown how the activities of sulphate-reducing bacteria may contribute to the liberation of phosphate, and how diatoms may concentrate phosphate in their mucilaginous sheaths.

Sulphur is important to phytoplankton for several reasons, in addition to the release of phosphate. The presence of sulphhydryl ion appears to be necessary for the deposition of silica in the frustules of diatoms and also assists the growth and reproduction of many species. Recently we isolated *Desulphovibrio*, *Chlorobium*, and *Chromatium* from phytoplankton tows taken aseptically some 10 miles east of Cronulla. It is not known whether these bacteria were autochthonous or were derived from the continental shelf mud. The effect of trace elements on phytoplankton is being investigated at the Haskins Laboratory in New York, but a great deal more remains to be done before this work can be assessed. An interesting finding was made by Fogg (1957) that certain organic excretory products of algae are able to chelate metals, thus concentrating them from sea water or possibly reducing their activity if present in quantities toxic to the algae concerned.

Ionic Balances and Salinity

The sea contains a biologically well balanced mixture of salts, the most important ions being sodium, potassium, calcium, magnesium, chloride, sulphate, carbonate, bicarbonate, and borate. The effect of salinity is usually measured by the estimation of chloride ion. Certain organisms are limited in occurrence by chlorinity, but most oceanic phytoplankters can tolerate wider chlorinity ranges than can be found in the open sea. However, the variation of monovalent-bivalent cation ratios and the precipitation of calcium carbonate from sea water at pH 9.4 are important in the biology of marine plants. Plankton swarms have been regarded as essentially aerobic, though anaerobic conditions may be produced at night by the respiration of phytoplankton in high concentration, e.g. in red tides. Active photosynthesis can produce supersaturation of the water in oxygen during the early part of the afternoon. At the same time, the carbonate-bicarbonate ratio is increased and the pH can rise to 9.4 when calcium carbonate tends to precipitate, while the rate of photosynthesis is reduced. Studies on the pH/Eh relationships of phytoplankton swarms could well be of value, but little information is available. The authors have, however, grown phytoplankton at low Eh values (to -240 mV) and have observed reproduction (with the formation of long chains of diatoms) at these low redox potentials.

Hydrostatic Pressure

Hydrostatic pressure in the sea increases 1 atmosphere for each 10 metres of depth. Experiments have suggested a slight increase of net photosynthesis at pressures of 25 atmospheres as against 1 atmosphere, with a gradual diminution from 25 to 100 atmospheres. This work is new, and has not been evaluated, but could mean that the compensation depth calculated from light-dark bottle experiments is too shallow. Even a few metres increase in compensation depth could mean a vast increase in total photosynthate in the oceans.

Vitamins, Growth Factors, External Metabolites, Antibiotics

Study of these is only now attracting marine microbiologists. Work by Provasoli, Hutner and Pintner (see Hutner and Provasoli, 1951) at the Haskins Laboratory and by Droop (1954) at Millport suggests that cobalamin and thiamin in particular may be selective in deciding the species of phytoplankton which will be dominant. Starr and co-workers (1956, 1957) at Galveston have also investigated the occurrence of vitamin B₁₂ in sea water. Microbiological assay methods for the B vitamins in marine environments are being studied by Hutner, and his co-workers (Hutner *et al.* 1956). The presence of external metabolites is regarded as important by Fogg (1952, 1957), Lucas (1955), and others, and this introduces a very important field of inquiry.

Bacterial antibiotics have been recorded by Rosenfeld and ZoBell (1947), and algal antibiotics by Rice (1954), Steemann Nielsen (1955), and others, but the importance of these is difficult to assess, especially as many phytoplankton organisms will grow in the presence of bacteria but not in their absence possibly due to bacterial synthesis of growth factors. Hence the effect of excretory products, auxotrophy, and antibiotics should be an important feature of productivity studies. The failure of phytoplankters, during certain physiological states, to multiply in spite of apparently favourable conditions, as mentioned by Harvey (1955), may be in part due to such external metabolites.

Reproduction Rates

Reproduction rates have not been adequately studied. Estimations made by Braarud (1945) and others in northern Europe suggest generation times of diatoms and flagellates as of the order of 1 to 2 days, but cultures and field observations made at our laboratory suggest much faster times ($\frac{1}{2}$ to 4 hours) for a number of species taken in the vicinity of Cronulla. This may be a function of temperature, but the work is still far from complete. We certainly get the usual "bacterial type" of growth curve, and discrepancies in figures may be due to lack of recognition by planktologists of the significance of logarithmic and stationary phases in the cultures. There do seem to be some intrinsic factors, possibly natural rhythms governing reproduction, as well as the extrinsic ones already discussed.

Grazing

Grazing rates have not received the attention they deserve. It stands to reason that if zooplankton is present, the total standing crop of phytoplankton will be the residue after grazing by phytophagous animals. Therefore, the rates of reproduction and of grazing are very important in dynamic estimations of productivity as shown by the work of Clarke and co-workers (Clarke and Gellis, 1935), of Lucas (1955), and of Marshall and Orr (1955).

Interrelationships of Micro-organisms

In estuaries it has been found that the most active environments biologically are those in which oxidation-reduction reactions predominate. This, it is believed, is because of the rapid energy changes associated with these reactions. In oceanic plankton swarms, it would be expected that many of the gradient organisms would not occur, e.g., the iron bacteria, *Thiothrix*, *Beggiatoa*, *Thiovulum*, and these have not been found outside the estuaries. However, the existence in the phytoplankton of reduced sulphur compounds such as methionine, cysteine,

and cystine, the production of dimethyl sulphide and mercaptans by certain marine algae and bacteria, and the ability of many marine bacteria to produce H_2S all suggest the possibility of sulphur-oxidising organisms since the transformation S to SO_4 is essentially biological. It is therefore not impossible that the sulphate reducers and purple and green sulphur bacteria found in phytoplankton may be autochthonous. Kriss and Rukina (1953) have found them at 2500 m in the Black Sea muds. Moreover, we have found that certain blue-green algae, diatoms, and coccolithophores can photoreduce, thus confirming the work of Nakamura (1937-8) and of Frenkel, Gaffron, and Battley (1949). It is interesting to note that the photoreduction of carbon dioxide using H_2S as the source of hydrogen is thermodynamically about eight times as efficient as photosynthesis using H_2O (15,000 kcal against 115,000 kcal). If reducing conditions are produced in large phytoplankton swarms, the photoreduction of CO_2 by plankton micro-organisms could increase the compensation depth.

The effect of heterotrophy and phagotrophy has already been mentioned, and the decomposition of decadent plankton swarms by saprophytic bacteria is well documented. It should be pointed out that this bacterial decomposition short-circuits the food chain, thus becoming quantitatively important in the biologist's concept of net production, since the phytoplankters thus destroyed are not available for grazing. Of course, the bacteria may be grazed on by zooplankton or they may die in turn and release nutrient material to the sea.

MEASUREMENT OF PRODUCTIVITY

While the interpretation of productivity measurements is fraught with many pitfalls, the actual measurement is also difficult. Firstly, we have an unavoidably large sampling error owing to the fact that phytoplankton swarms are not randomly distributed, either horizontally, vertically, or temporary (for plankton studies are essentially four-dimensional). Usually, collections are made on fixed traverses at certain regular intervals or on round-the-world expeditions at very irregular ones. The movements of water masses are generally variable, and even in the comparatively regular southern Californian waters W. E. Allen (1941) concluded that over 22 years of phytoplankton observation, no corresponding month, week, or day in any two years gave the same picture. In sampling, we are merely making a grab at plankton populations as they pass by and can then only take a minute sample, hoping that we can get a story. The wonder is, not that our knowledge of productivity is so meagre but that our information does seem to have significance.

The methods used for productivity estimations fall under two heads:

Standing Crop Measurements—

- Plant pigment analyses
- Organic phosphate
- Particulate nitrogen
- Particulate carbon
- Phytoplankton counts
- Estimates of total suspended matter

Estimates of the Rate of Production of Organic Matter—

- Uptake of CO_2 based on ^{14}C measurements
- Rate of photosynthesis by light-dark bottle methods
- Harvey's phosphate method
- Uptake of CO_2 by pH variation
- Measurement of O_2 consumption.

Most attention is paid to plant pigment analyses (Harvey 1934; Richards and Thompson 1952; Ryther 1956), light-dark bottle experiments (Gaarder and Gran 1927; Gran 1927; Riley 1941), and ^{14}C (Steemann Nielsen, 1952; Ryther and Vaccaro, 1954; Ryther, 1956), the other methods being more or less of historical interest.

There is considerable controversy at the present time over the validity of all three methods. Ryther claims that the measurement of chlorophyll *a* can be shown to have a linear relationship with photosynthesis at a given (adequate) light intensity. He neglects chlorophylls *b* and *c* and the carotenoids. This may well be valid since it is agreed that chlorophyll *a* is the active agent in photosynthesis and that the accessory pigments merely transfer excitation energy to

chlorophyll *a*. Pigment analysis, however, measures only standing crop. Ryther has attempted to establish a gross productivity equation by measuring certain variables such as daily surface radiation, the extinction coefficient of visible light, and the chlorophyll content of the water at a number of depths. The chlorophyll content per cell varies over a wide range, depending on the physiological state of the organisms, quality and intensity of light, and presence of organic substances, so it is not easy to relate chlorophyll to total plant material.

Steemann Nielsen has criticised the light-dark bottle method because he considers that there will be a relative increase in the bacterial content and therefore of respiration in the dark bottles owing to the bactericidal effect of sunlight penetrating the light bottles. This claim is not supported by adequate evidence. He also considers that long-term experiments (24 to 48 h) allow of too many biological changes, and are therefore not reliable.

Criticisms of the ^{14}C method of Steemann Nielsen are voiced by Ryther, who suggests that the method yields net and not gross productivity. Care must be taken that experiments to measure the rate of production are not standing-crop methods in disguise. Although bottle experiments give a time factor, this factor is valid only if the populations in the bottle and in the sea remain parallel and react in the same manner throughout the experiment.

CONCLUSIONS

The future development of a comprehensive attack on productivity should, we believe, take the following trend:

1. Further work on the various methods is required in various parts of the world so that results can be correlated and adequate conclusions drawn.
2. Studies on nutritional requirements of, and the effect of physical and chemical factors on, phytoplankton organisms should be intensified in order to get an understanding of plankton blooms, successions, and the absence of phytoplankton blooms in certain areas and at certain times.
3. Laboratory studies are essential, as continuous field work is impossible and introduces too many variables. However, results obtained in the laboratory can and must be checked in the field.
4. As field observations are occasional, and therefore tend to give pictures of standing crop, relations of standing crop to the dynamic picture of phytoplankton must be determined.
5. Finally, a coordinated effort between several disciplines is needed at each laboratory and a free exchange of ideas, results, and programmes should be encouraged between institutions.

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TAXONOMIC CONSIDERATIONS

By V. B. D. SKERMAN, Department of Bacteriology, University of Queensland, Brisbane, Australia

As an introduction to the general subject tribute should be paid to the work done in the field of taxonomic microbiology by the late Professor R. S. Breed, who as Chief Editor of Bergey's Manual of Determinative Bacteriology has done more towards clearing up the chaos in taxonomy than any other single person of his time.

After a period of twenty-five years since its establishment in 1930, the interest of the International Committee on Nomenclature of Bacteria is turning from the problem of determination of validity of names to the more pressing problem of determining the basis upon which these names rest, viz, the criteria and methods for description of bacterial species. This is being done in an indirect way by the setting up of specialist groups to deal with different genera. Whilst this action has undoubtedly led to a marked clarification of ideas within genera such as *Salmonella*, *Shigella*, *Escherichia*, and *Aerobacter*, and has to some degree produced uniformity in the procedures to be adopted, the author is of the opinion that this selective treatment creates a new danger to taxonomic studies. It presupposes a clear knowledge of the species to be dealt with and a lack of affinity between them which validates their being studied by different procedures. This may be true enough for practical purposes in clinical and non-clinical diagnostic laboratories where work is essentially of an applied nature. It does not hold in purely taxonomic studies.

One of the major factors contributing to our present taxonomic difficulties is the totally inadequate descriptions of most species and lack of uniformity of procedure with respect to those apparently well described. Expert treatment of different groups by different methods will not resolve this difficulty. It is most important that the criteria and procedures be standardised and that all species be examined on a common footing. This would inevitably give a lot of negative data, which are, however, equally as important as positive data and equally as relative to the technique used.

Take, for example, the intracellular deposition of sulphur. It is commonly supposed that this property is restricted to a very limited group of organisms. Perusal of the literature shows that the bulk of these organisms has not been isolated and in many instances has been described only once or twice from crude materials. The classical study of the sulphur inclusions in *Beggiatoa* by Winogradsky appears in most instances to have been accepted by many of his contemporaries and more recent workers who have made the identification of the globules by purely optical means. The sulphur bacteria tend to be considered by those not immediately interested in them as a microbiological curiosity. They consequently presuppose, without testing, that H_2S oxidation would not occur.

Made curious by the apparent morphological resemblance of *Sphaerotilus natans* to *Thiothrix* sp. the author was led to test the ability of the former to deposit sulphur intracellularly when exposed to H_2S . The test proved positive. As a result of this and the fact that the globules appeared to form in close proximity to granules appearing in the unexposed cells under phase contrast, work has proceeded on the hypothesis that such intracellular deposition is possibly a function of any large cell and that the granules were possibly mitochondria as defined by Mudd (1953).

Quite rapid intracellular deposition occurs in some yeasts and in the only fungus so far examined. Within the yeasts the granules which are associated with the globules give positive tests attributed to mitochondria. The process is improved by aeration of the cells during growth and inhibited by flushing with N_2 .

A single test carried out with *Bacillus subtilis* has proved negative. This needs to be repeated. It is possible that the failure was due to the smallness of the cell. The opinion has been put forward that the failure of the green sulphur bacteria to deposit the sulphur intracellularly is due to the small size of the cell.

A similar situation exists in relation to *Sphaerotilus* and *Leptothrix*. Pringsheim (1949) has shown very clearly that *Sphaerotilus* grown in an iron-containing medium is indistinguishable from certain species of *Leptothrix*. A similar situation possibly exists between numerous other iron bacteria and more commonly recognised species.

There is some suggestion that the genus *Naumanniella* may also be only an environmental form of *Sphaerotilus* since the latter organism when grown in a peptone base with an iron nail produces many single and short chains of unsheathed cells having a very striking optical resemblance to *Naumanniella*.

Of immediate interest to the marine microbiologist is the relationship of the halophilic to the non-halophilic organisms in which characters other than that of halophilism are essentially the same. Unless some definable genetic factor can be determined which is responsible for this difference there would appear to be no sound taxonomic ground for their separation at a species or generic level, but this same argument can be so aptly applied to almost all the proposed bacterial species that it warrants little further comment. For practical purposes, halophilism is an easily determined character and serves equally well for the separation of one culture from another as does, for example, acid production from glucose. There is no good reason why it should not be used as a basis of separation at a species or generic level.

The safest course to be followed at the present time would seem to be to treat all isolates on a common basis, examine them in a truly comparative sense over a wide series of specified procedures, record all data to the strain level and build up a comprehensive artificial system of identification whereby individual strains can be identified and variations more accurately tabbed, leaving the development of a *natural classification* until a much clearer picture of the grounds upon which it can be based has been obtained.

Bacterial taxonomy appears to be a scientific discipline in which conclusions have been drawn before sufficient experimental details have been obtained.

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MICROBIOLOGY OF OIL*

By CLAUDE E. ZOBELL, Scripps Institution of Oceanography, University of California, La Jolla, U.S.A.

Virtually all commercial deposits of oil occur in ancient marine sediments, where microbes appear to affect its origin, chemical composition, preservation, and migration. Probably most pronounced are the effects of microbial activity upon source sediments of petroleum; particularly the organic fraction generally believed to be the mother substance of oil. Under certain conditions crude oils or products refined therefrom are attacked by bacteria and allied microorganisms. Such microbes may play a part in the recovery of oil from natural formations, and microbial methods may be applicable to the discovery of oil. Sadly lacking, though, are quantitative data to assist the geologist or chemist in assessing the overall importance of bacteria.

COMPOSITION OF CRUDE OILS

Crude oil or petroleum consists of complex mixtures of gaseous, liquid, and solid hydrocarbons with lesser amounts of non-hydrocarbon constituents containing sulphur, oxygen, nitrogen, phosphorus, and at least 15 metallic elements. Petroleums from different geological provinces, fields, pools, wells, or depths may differ in density, colour, and chemical composition (Gruse and Stevens, 1942).

The average range in the chemical composition of crude oils from different parts of the world is as follows:

	Per cent by Weight		Per cent by Weight
Carbon	82.2–87.1	Oxygen	0.1–4.9
Hydrogen	11.7–14.7	Nitrogen	0.05–1.2
Sulphur	0.1–5.5	Metallic elements	0.01–0.08

Among the metallic elements found in the ash of many oils are calcium, magnesium, iron, copper, nickel, and vanadium (Bonham, 1956). Vanadium is believed to occur as a porphyrin complex of biological origin (Hodgson, 1954). Almost invariably present in crude oils are optically active compounds – a class of compounds produced only by living organisms as far as is known.

Aliphatic hydrocarbons of the alkane or paraffin series range in complexity from methane (CH_4) to molecules having up to 94 carbon atoms ($\text{C}_{94}\text{H}_{188}$). Naphthenes of the polymethylene series range from cyclopropane (C_3H_6) to cycloparaffins having up to 30 carbon atoms in their rings ($\text{C}_{30}\text{H}_{60}$). From 10 to 40 per cent by weight of oils consists of aromatic hydrocarbons or the benzene series. Commonly occurring are benzene (C_6H_6), methylbenzene or toluene ($\text{C}_6\text{H}_5\text{CH}_3$), dimethylbenzenes or xylenes ($\text{C}_6\text{H}_4\text{CH}_3\text{CH}_3$), naphthalene (C_{10}H_8), anthracene ($\text{C}_{14}\text{H}_{10}$), phenanthrene ($\text{C}_{14}\text{H}_{10}$), and an indeterminate number of polycyclic alkanes.

Sulphur in crude oils may be present as free sulphur, hydrogen sulphide, and in organic compounds, including thiols or mercaptans, alkyl disulphides, thiophenes, thiophanes, sulphones, and other complex cyclic sulphides.

Oxygen occurs in phenols, cresols, fatty acids or their derivatives, naphthenic acids, resinous compounds, and asphaltic substances.

Nearly all oils contain small quantities of nitrogen. It occurs in pyridines, quinolines, carbazoles, pyrroles, indoles, and other complex forms. The absence of simple nitrogenous compounds commonly occurring in plant and animal remains from which oil is believed to be derived suggests microbial degradation processes. Natural gases associated with crude oils contain appreciable quantities of gaseous nitrogen (N_2) and from a trace to 0.2 per cent ammonia. Pyrobituminous material in oil shales contains up to 2 per cent nitrogen.

OCCURRENCE OF OIL

Oil pools have been discovered in formations of all geological ages from the Cambrian through the Pliocene (Levorson, 1954). Some oil has been found in Pleistocene strata, but

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whether it is indigenous or has migrated there from older formations is uncertain. Oil pools occur in rocks ranging in age from nearly a million to about 500 million years. The occurrence of little oil in rocks younger than 1 million years suggests that oil formation is a slow process.

The oil and/or gas content of a single deposit or reservoir is called a pool. An oil field is made up of one or more pools closely related geologically or geographically. A basic geological requirement for oil pools is a porous or permeable body of rock, called the reservoir rock. It must be overlain by an impervious layer of roof rock and deformed or obstructed in such a manner as to trap the oil. There are many kinds of structural or stratigraphic traps.

Oil reservoirs consist of sedimentary rocks; commonly marine sandstones, limestones, and dolomite. Reservoir rocks may contain oil in amounts ranging from only a few parts per billion to a few barrels per ton. Reservoir fluids are made up of various admixtures of oil, gas, and water. These fluids occur in pores of reservoir rocks having radii averaging 20 to 200 microns in sandstones. Some pores are much larger in limestone and dolomite reservoirs. The pores are much smaller in oil shales, which consist of varying mixtures of complex organic matter in shale and clay.

Oil shales contain little or no free oil and not much water. Oil and gas are liberated from the pyrobituminous substances in oil shale upon heating to about 350° C. Significantly there is no evidence of bacteria indigenous to, or able to live exclusively on, oil shale, unlike findings for oil reservoir fluids. Many constituents of crude oil are thermolabile; being unstable at temperatures higher than 150° to 200° C suggests a relatively low temperature for the origin of oil.

RESERVOIR FLUIDS

Most oil and gas pools or reservoirs occur in aquifers, i.e., water-saturated rocks. Water fills the smaller interstitial or pore spaces and forms a film on sedimentary particles making up the reservoir rock. Interstitial water in oil reservoirs is seldom less than 10 per cent and may fill more than 50 per cent of the pore space. Besides the water held by capillary attraction or adhering to solid surfaces, there may be much water emulsified in oil. The main body of water, however, is under the oil pool or at its boundaries, the so-called bottom or edge water respectively. Having a density greater than most oils, water may percolate down through the reservoir.

Water associated with oil pools may be meteoric, connate, or a mixture of the two. Meteoric water from rain may permeate permeable rocks through bedding planes, fractures, or permeable layers. Most connate water is fossil sea water, often having a salinity considerably greater than sea water (Case *et al.*, 1942). The mineral content of oil-field waters ranges from less than 1 to 25 per cent, as compared with an average of 3.4 per cent for modern sea water. Sea water and all reservoir fluids that have been examined contain all the minerals required for the growth of microorganisms. The reaction is within the biological range, i.e., pH 6.8 to 8.4. The sulphate-chloride ratio of oil-field waters or brines is nearly always less than in sea water, a condition that could be caused by the activities of sulphate-reducing bacteria commonly found in oil-well brines.

THEORIES ON THE ORIGIN OF OIL

Many theories have been advanced to explain the origin of oil (Stevens, 1956). None accounts for all the facts and, indeed, it is unlikely that all oils have had the same mode of formation. There is little evidence to support the belief of Berthelot that free alkali in primordial earth reacted with carbonic acid to yield simple hydrocarbons, some of which combined under the influence of heat, pressure, and catalysts to form oil. Conceivably, small quantities of oil could have resulted from the polymerisation of acetylenes resulting from the reaction of metallic carbides with water, as proposed by Mendeleeff, but like most inorganic origin theories this one fits few of the facts. Similarly, cosmic origin theories, championed by Oparin (1943), are not tenable with the conditions of occurrence and characteristics of oil.

Hydrogen from organic sources plays an essential part in the origin of oil. Whether hydrogen from inorganic sources also contributes importantly to the formation of oil is less certain. Free

hydrogen commonly occurs in minute amounts in igneous rocks. It may have been present in larger concentrations or at great depths in primordial earth. Hydrogen in appreciable quantities is emitted from volcanoes and it occurs in fumarole gases (Rankama and Sahama, 1950). Deep in the earth's crust, iron or its oxides might react with superheated steam to form hydrogen. The alpha particle bombardment of water frees hydrogen, and oil-bearing sediments are generally radioactive (Sheppard, 1944).

Regardless of its source, hydrogen is chemically rather reactive and physically extremely mobile. These properties, plus the ability of bacteria to oxidise hydrogen, may explain why free hydrogen is rarely found in natural gas, oil pools, or sedimentary materials (ZoBell, 1947). From 11.7 to 14.7 per cent by weight of oil consists of hydrogen as compared with only 7 to 10 per cent in marine organic matter from which oil is believed to be formed.

There seems to be general agreement among modern petroleum chemists and geologists that most, if not all, oil is primarily of organic origin (Cox, 1946). The organic remains of both animals and plants, probably mostly marine but maybe partly freshwater or terrestrial, are believed to be the main mother substance of oil. The organic matter may have been modified by bacteria, contact catalysts, surface-active substances, pressure, temperature, radioactivity, and/or other agencies by processes that appear to require considerable time. Although complex hydrocarbons in small amounts are common constituents of recent marine sediments (Stevens *et al.*, 1956), petroleum pools are mostly a million or more years old.

Modifications of the organic matter seem to have taken place and oil occurs primarily in marine sediments. Although there may have been some migration of oil, gas, or protopetroleum, the main bulk of oil appears to have been formed in or relatively near the places where the pools are found. Migration could be no more than a few thousand feet vertically or no more than a few miles (mostly less than 100) horizontally.

The hydrostatic pressure of the environments where oil is found or was formed is believed to be no more than 20,000 psi and mostly less than 10,000 psi. The temperature to which oil has been subjected is probably less than 150° c and the temperature of most oil pools is considerably less than 100° c. The temperature gradient of sedimentary rocks approximates 0.9° c per 100 ft; pressure increases with depth by about 50 psi per 100 ft. These conditions seem to rule out the possibility of high temperatures or pressures being essential for the conversion of organic matter into oil. They do not rule out the possibility of bacterial activities contributing to the process, as will be elaborated below.

Accepting the premise that oil originates from organic matter, it is almost axiomatic that bacteria contribute to the process, because virtually all kinds of organic matter are susceptible to microbial modification in marine sediments. Less certain is the extent to which bacteria affect the origin of oil and whether their action is essential or merely incidental to the process. Besides modifying organic matter deposited in source sediments of petroleum, bacteria produce certain hydrocarbons, oxidise others (ZoBell, 1950), create reducing conditions which may favour the formation and preservation of hydrocarbons (ZoBell, 1946b), and affect other properties of sediments (ZoBell, 1942).

BACTERIA IN MARINE SEDIMENTS

An abundant microflora representing many species occurs in marine bottom deposits (ZoBell, 1946a). Bacteria occur in greatest numbers at the mud-water interface in recently deposited sediments rich in organic matter. In such sediments the bacterial population ranges from 10^5 to 10^8 per gram of wet material. The number and varieties of viable bacteria and also the organic content of the sediment decrease with core depth or with increasing age of the deposits (ZoBell and Anderson, 1936; Anderson, 1940). Such findings in the field, including changes in the chemical composition of organic matter with core depth or age, coupled with observations on recent marine sediments incubated in the laboratory, strongly suggest that bacteria are responsible for changes in the organic content.

From 10^1 to 10^4 bacteria per gram have been detected in the recent sediments taken from core depths of 3 to 7.5 m representing material several hundred thousand years old (Rittenberg, 1940; Morita and ZoBell, 1955). In more rapidly deposited near-shore sediments only a few thousand years old, bacteria were detected at a depth of 30 m below the mud-water interface. How much deeper or in how much older sediments indigenous bacteria may occur is yet to be determined. Bacteria have been recovered from oil pools of great antiquity, but it is difficult to prove their indigeneity. In the first place it is difficult to obviate contamination from extraneous sources and, secondly, there are possibilities of microbial migration and the movement of reservoir fluids.

Bastin (1926), Gahl and Anderson (1928), Ginter (1930), Ginsburg-Karagitscheva (1933), Müller and Schwartz (1949), and several other investigators have reported finding bacteria in reservoir fluids taken from depths down to several thousand feet. Under the auspices of the American Petroleum Institute we collected with utmost precautions samples of reservoir fluids from 66 different formations 2,680 to 10,470 ft below the earth's surface. Sulphate-reducing bacteria were detected in 29 of the samples. Many of the bacteria would grow only when subjected to higher pressures and temperatures characteristic of the formations from which the samples were taken. This suggests, but does not prove, that the bacteria were indigenous to the oil-bearing formation, because they may have been introduced during drilling operations and become acclimatised to the higher pressures and temperatures. There is also nearly always the possibility of bacteria being carried into the formation by the movement of ground water. The numbers and kinds of bacteria detected in some of the samples establishes that bacteria have been reproducing *in situ* in the reservoir fluids. Finding no bacteria in non-bactericidal reservoir fluids shows that it is possible to collect samples aseptically.

Müller (1944) found bacteria in 13 out of 38 aseptically collected side-hole samples of oil sands and oil-bearing limestones. We have found bacteria in a good many, but not all, cores drilled from sulphur-limestone-anhydrite and oil-bearing rocks from depths to nearly 10,000 ft representing sediments from 10^6 to 10^8 years old.

In 1950 we obtained through the cooperation of the Shell Oil Company several core samples from an oil well being drilled in the Ventura, California, Field. The oil company engineers were eager to learn whether living bacteria were indigenous to this formation where there was evidence (Doig and Wachter, 1951) of bacterial corrosion of oil well casings at depths of several thousand feet. In order to minimise possibilities of contamination, a new well was drilled with alkaline (pH > 11.5) drilling mud. At the well head, cores of oil-bearing sand about 3 in. in diameter and 8 to 18 in. long were dipped in boiling paraffin wax to destroy surface contaminants, to exclude atmospheric oxygen, and to retain the fluid content of the samples. Thus protected the core material was brought to the laboratory where radially central sub-samples were removed aseptically with heat-sterilised instruments. Viable sulphate-reducing bacteria were detected in most 0.1 and 0.01 g sub-samples of oil-bearing rock taken from strata 4,337 to 4,412 ft deep. No such bacteria could be detected in the alkaline drilling mud, and the bacteria differed from those ordinarily occurring in soil or associated with surface materials. The absence of heterotrophic aerobes indicated freedom from surface contamination. Unquestionably the sulphate-reducing bacteria were indigenous to the oil-bearing formations where found. Extensive tests demonstrated that the oil-bearing formations possessed all of the prerequisites for the growth of sulphate-reducing bacteria; including water, minerals, nutrients, energy sources, favourable pH, tolerable temperature, and other conditions. There was no way, however, to determine how long the bacteria had been present in the formation nor the extent of their activity *in situ* prior to disturbance by drilling operations.

These and similar observations establish the presence of viable bacteria—mostly non-sporulating anaerobes—in many oil pools. Not enough field work has been done to warrant generalisations regarding how commonly bacteria occur in oil pools or whether there is any correlation between the occurrence of bacteria and the properties of oil-bearing formations. In certain formations no living bacteria could be detected by most meticulous examinations.

Such negative findings demonstrate the feasibility of aseptically collecting samples under difficult conditions. Whether bacteria were present or active earlier in the geological history of these bacteria-barren formations is indeterminate.

MICROBIAL MIGRATION IN MARINE SEDIMENTS

Propelled by flagella or perhaps more important by protoplasmic extrusion during growth, bacteria tend to move through water-wet sediments. The rate of movement is rather slow ranging from a few microns to as much as a few centimetres per day. The rate is influenced by the nutrient content of interstitial fluids, the size of the pore spaces, the kind of bacteria, temperature, and many other factors. In certain formations through which water percolates, bacteria are effectively filtered out by various mechanisms.

As might be expected, bacteria do not migrate into an environment which is unfavourable for their growth. Extremes of temperature, pressure, salinity, pH, redox potential, toxic constituents, and adsorptive capacity of solids may constitute barriers to the migration of bacteria. There is no evidence that bacteria either move into or survive long in marine sediments lacking in nutrients essential for growth.

ACTION OF BACTERIA IN SEDIMENTS

There are many ways in which bacterial activities in recently deposited marine sediments affect geochemical properties and diagenetic processes (Beerstecher, 1954). Besides creating reducing conditions, affecting the redox potential (ZoBell, 1946b), reducing sulphates to sulphides (ZoBell and Rittenberg, 1948), and oxidising molecular hydrogen (ZoBell, 1947), the principal effect of bacteria is on the organic content of sediments. Virtually all kinds of organic compounds are susceptible to microbial modification in sediments. The general tendency is for proteins, lipids, and cellulosic components to be attacked to give simpler compounds such as carbon dioxide, methane, hydrogen sulphide, hydrogen, and ammonia.

Heterotrophic bacteria grow or reproduce at the expense of the organic compounds which they assimilate. Large quantities are consumed as a source of energy and an average of roughly one-third of the carbon content may be converted into bacterial cell substance. The latter consists largely of complex proteins and lipids. Following their death the less refractory bacterial protoplasm undergoes autolysis or degradation. The more refractory remainder, together with the modified remains of the originally deposited marine organic matter, constitutes humus. With increasing age and depth of burial, marine humus generally contains proportionately progressively decreasing percentages of oxygen, nitrogen, and sulphur. The direction and magnitude of these changes in the chemical composition of the carbonaceous constituents in marine materials as compared with crude oil are illustrated by data in table 1.

TABLE 1. Proximate Analysis of Carbonaceous Constituents in Some Marine Materials

	Marine Sapropel	Marine Humus	Ancient Sediments	Crude Oils
	%	%	%	%
Carbon	45-52	52-58	60-75	82-87
Hydrogen	5-9	6-10	9-12	11-15
Oxygen	25-30	12-20	0.7-10	0.1-4.9
Nitrogen	9-15	2-5	0.5-2	0.05-1.2

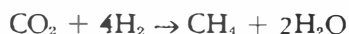
Sapropel consists largely of the remains of marine plants and animals recently deposited at the mud-water interface. Marine humus is the organic matter found in cores of recent marine sediments ranging from 10 to 10⁶ years old. Ancient sediments were represented by rocks exposed by man or by weathering and estimated to be from 10⁶ to 10⁸ years old. Exclusive

of oil pools the total carbon content of sedimentary materials decreases with increasing geological age, but in the residual carbonaceous matter the carbon and hydrogen content increases as a result of decreasing content of oxygen, nitrogen, and sulphur.

The general tendency is for marine humus to approach the elementary composition of crude oil. Similar modifications of lesser magnitude result from the microbial degradation of marine organic matter maintained in the laboratory under anaerobic conditions. A small fraction of the residual carbonaceous material proves to be hydrocarbons similar to those characteristic of crude oil. According to Smith (1954) and Stevens *et al.* (1956), complex hydrocarbons are common constituents of recent marine sediments, the concentration increasing with age or depth of burial. Bacterial activity could account for these changes, but no one has shown that the changes depend upon bacteria.

PETROLEUM CONSTITUENTS PRODUCED BY BACTERIA

Methane in large quantities is produced by bacteria. Methane production occurs in many anaerobic environments where bacteria are present and active. From 5 to 25 per cent or more of the carbon content of certain organic compounds undergoing anaerobic fermentation may be converted into methane. This common constituent of natural gas and petroleum is also produced by the bacterial reduction of carbon dioxide in the presence of hydrogen (Barker, 1936; ZoBell, 1947):



If methane can help to explain the origin of oil, the microbiologist can account for its formation in sufficient quantity in widespread locations.

The bacterial production of ethane in small quantities has been reported by Neave and Busswell (1928), Rawn *et al.* (1939), and others. According to Davis and Squires (1954), gases produced by the microbial fermentation of cellulose contained, besides large quantities of methane, from 3.2 to 7 p.p.m. ethane, 3.9 to 4.7 p.p.m. ethylene, 0.06 to 0.14 p.p.m. propane, and 0.13 to 0.21 p.p.m. propylene.

A good many bacterial species produce as part of their cell substance carotene, flavorodin, and other complex pigmented hydrocarbons having the empirical formula $\text{C}_{40}\text{H}_{54-58}$ (Porter, 1946). Although these unsaturated hydrocarbons are not necessarily associated with petroleum, they are susceptible to hydrogenation and some of these compounds do have a benzene nucleus. Phenol, cresol, and other aromatic compounds commonly occurring in crude oil are produced by marine bacteria from the degradation of certain amino acids and proteins (Stone *et al.*, 1949; Updegraff, 1948).

Incorporated in the cells of most bacteria are small amounts of liquid and solid hydrocarbons. From sulphate-reducing bacteria cultivated on fatty-acid medium we harvested ether-soluble unsaponifiable extracts amounting to 0.08 per cent by weight of the dried cells. Carbon and hydrogen analyses showed that the extracts corresponded closely to the chemical composition of hydrocarbons ranging from C_{10} to C_{25} . In several subsequent experiments cells of *Desulfovibrio* species cultivated on various organic substrates under difficult conditions were found to have converted from 0.002 to 0.15 per cent of the organic carbon consumed into liquid and solid hydrocarbons.

Autotrophic sulphate reducers, utilising molecular hydrogen as a sole source of energy and carbon dioxide or carbonates as the sole source of carbon, also produced appreciable quantities of hydrocarbons (ZoBell, 1945). Approximately 0.42 per cent of the carbon content of the cells was extracted as an oily substance, 26 per cent of which consisted of predominantly paraffinic hydrocarbons. From another batch of autotrophic sulphate reducers which had consumed 33.92 litres of H_2 was extracted 1,640 mg of oily material. Microchromatographic separations, spectral analyses, and elemental analyses indicated that 458 mg of the oily material consisted largely of paraffinic hydrocarbons, and there was presumptive evidence of the presence of 367 mg of aromatic compounds.

Evidence that from 0.03 to 0.95 per cent of the carbon content of certain other bacterial cells from marine materials consists of liquid and solid hydrocarbons has been cited by Stone and ZoBell (1952). The amount is adequate to account for the hydrocarbon content of recent marine sediments (Smith, 1954). Hydrocarbons from such sources could help to account for the origin of oil, but the amount would have to be supplemented substantially by hydrocarbons from other sources unless as yet undiscovered bacteria or cultural conditions result in far greater yields.

BACTERIAL LIBERATION OF OIL

An increase in the amount of liquid and solid hydrocarbons recoverable from diatoms undergoing degradation by anaerobic bacteria has been recorded by Clarke and Mazur (1941). While part of this hydrocarbon may be produced by the bacteria, most of it was probably merely released from the diatom matrix by the bacterial decomposition of the protein, lipid, and carbohydrate structures. Similarly, bacteria are believed to be instrumental in the release of bound hydrocarbons from the decomposing remains of plants and animals, many of which are known to contain hydrocarbons (Chibnall *et al.*, 1934; Sanders, 1937). Hydrocarbons thus released would be free to migrate and to accumulate in oil pools.

ZoBell (1952) has described a second mechanism by which bacteria may liberate oil by dissolving carbonate or sulphate rocks on which oil is absorbed. Besides releasing adherent oil the bacterial dissolution of such sedimentary materials may favour the flow and accumulation of oil by the formation or enlargement of void spaces and channels.

The formation of carbon dioxide, methane, or hydrogen in microtraps or elsewhere in oil-bearing sediments is another mechanism by which bacteria may promote the liberation, migration, and accumulation of oil.

MICROBIAL MODIFICATION OF OIL

Under favourable conditions, virtually all kinds of hydrocarbons are susceptible to attack by bacteria, yeasts, or fungi imperfecti (ZoBell, 1946c; 1950). Nearly 200 microbial species representing 40 genera have been shown to oxidise hydrocarbons. Among the substances shown to be oxidised by microbes are several pure hydrocarbons ranging in complexity from methane to anthracene, as well as a great variety of waxes, tars, asphalts, crude oils, petrols, kerosenes, lubricating oils, and other petroleum products. Besides microbial cell substances synthesised at the expense of the hydrocarbons oxidised, the principal oxidation products appear to be carbon dioxide and lesser amounts of organic acids, alcohols, and other intermediate compounds.

Most active in oxidising hydrocarbons are species of *Pseudomonas*, *Mycobacterium*, *Proactinomyces*, *Micrococcus*, *Bacterium*, *Bacillus*, *Sarcina*, *Serratia*, *Penicillium*, and *Aspergillus*. They have been shown to be active only in the presence of free oxygen or nitrate, which restricts the environments where they could be expected to modify oil. There is little or no free oxygen or nitrate below the topmost few millimetres of organic-rich sediments or in reservoir fluids. Whether there are other oxygen donors to provide for the oxidation of hydrocarbons in the absence of free oxygen or nitrate has not been demonstrated except for certain sulphate-reducing bacteria which slowly oxidise some of the higher (C_{12} to C_{31}) paraffinic hydrocarbons and a few aromatic compounds.

The widespread occurrence of hydrocarbon-oxidising bacteria in soil, sediments, and water suggests that they probably destroy or modify large quantities of oil or its components in aerobic environments. Of practical importance is the disappearance of oil from surface sediments, soil, and natural waters. Also petroleum or products refined therefrom may be susceptible to microbial modification when in contact with water such as, for example, machine coolants consisting of oil mixed with water or kerosene stored in tanks over water bottoms. To what extent bacteria may modify oil after its burial or formation in anaerobic environments is a subject for speculation.

According to Tausson and Aleshina (1932), certain sulphate-reducing bacteria tend to convert paraffinic hydrocarbons into naphthenic compounds. Tausz (1919) noted an increase in the asphaltic residue of Mendoza crude oil from an initial content of 0.3 per cent to 5.2 per cent as a result of prolonged bacterial activity under controlled laboratory conditions of experimentation. Maliyantz (1935) attributed observed decreases in the sulphur content of Russian crude oils to the selective action of sulphate-reducing bacteria.

The work of Tausson and Shapiro (1934) is representative of the observations of several workers who have demonstrated that bacterial activity affected the physical appearance, saponification number, iodine number, density, index of refraction, and other properties of petroleum held in the presence of free oxygen and water with dissolved minerals essential for bacterial growth. In the absence of water, bacteria have no effect on oil. From this it follows that after individual droplets of oil coalesce and collect in sufficient quantity to present a limited surface, the danger of destruction or microbial modification decreases.

In our laboratories at La Jolla experiments of 12 years' duration are in progress during which time strictly anaerobic sulphate-reducing bacteria have altered the properties of oil barely beyond the limits of recognized experimental errors. Definitely the bacteria have grown, reduced sulphate, and produced hydrogen sulphide, thus showing that oil components have been oxidised. Observed changes in the appearance, amount, and density of the residual oil, however, are so small that these changes might be attributed to the presence of bacterial protoplasm, emulsification of oil in water or water in oil, adsorption, loss of volatile constituents, or to experimental errors or artefacts.

PRESERVATIVE ACTION

Although bacterial activities might result in the destruction or modification of oil in certain sediments, elsewhere they may help to protect it from oxidation. The most obvious protective action is the consumption of free oxygen and the creation of reducing conditions. Likewise early in the history of oil microbes may produce antibiotics or other bacteriostatic substances which inhibit the growth of oil-oxidising organisms. Such bacteriostatic substances have been detected in source sediments of petroleum, but whether the presence of such substances is purely coincidental or prerequisite to the accumulation of oil is highly problematical.

Toxic metabolic products of bacteria, notably hydrogen sulphide, or the depletion of essential nutrients undoubtedly tend to limit bacterial activity. In a closed system such as a petroleum deposit bacterial activity would be self limiting by these factors. A state of dynamic equilibrium would soon be established in which the rate of reproduction or metabolism would be limited by the rate at which essential nutrients reach the bacteria and toxic products are carried away. All rates might be affected by the movement of reservoir fluids, but at best the rates are believed to be extremely slow.

A good many samples of reservoir fluids, including both crude oils and oil-well brines, have been examined for their growth-inhibiting or growth-promoting properties. Generalisations are difficult because there are so many kinds of microbes, fluids, and experimental conditions. Most reservoir fluids contain substances or possess properties which render them inhibitory or even lethal to many microbial cultures owing to the presence of phenols, benzene, hydrogen sulphide, high salinity and heavy metals. We have yet to find, however, a reservoir fluid which was either bactericidal or bacteriostatic for all bacteria. From this it might be inferred that reservoir fluids have a selective action on the growth of bacteria.

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GEOLOGY AND MICROBIOLOGY

By L. G. M. BAAS BECKING, Bureau of Mineral Resources, Canberra, Australia

INTRODUCTION

1. Biology and Geology

At the interphases of lithosphere, hydrosphere, and atmosphere, life has developed as a thin film, levying toll on the inflowing solar energy – a specific self-perpetuation still far beyond our understanding. This living state of matter is a veritable countermould of its environment, of the "milieu ambiant". The influence of the environmental factor upon the living systems is described in physiology. The inverse, a description of the influence of the living system upon its environment has, thus far, not been considered as a separate discipline. We are increasingly aware, however, of the power of living things to mould their environment. Biology, in its widest sense, merges here with geology, in its widest sense. An attempt will be made here to give a brief outline of this boundary between geology and biology.

2. The Actuality Principle

Geology has been able to develop to its present state by the realisation, a century ago, of certain fundamental principles. One of these concepts, often ascribed to Lyell, is the "actuality" of geological processes. This was already demonstrated by Goethe (in the second part of Faust) in his description of the fight between Plutonists and Neptunists. We accept now as self-evident the fact that there is not much difference between the nature of geological processes in the present and in the past, with one restriction, however. We should not extrapolate too far into the past, before our planet had grown beyond its early, juvenile, stages. However, a coral reef in the Devonian shows great similarity to its recent counterpart; the component organisms may have been specifically, or even generically, different, but the biocoenosis, the "facies" of the reef, has remained the same. Especially the microbiological processes taking place in this environment almost certainly have remained identical. This application of the actuality principle holds for most environments; whether geothermal, evaporate, marine, estuarine, high-moor or limestone. This means that oil and coal are now formed under our feet, that the accumulation of phosphate, of iodide, of limestone and of flint proceed at a rate comparable to the intensity of these processes in the past. Without the concept of actuality there would be no possible microbiological approach to geological processes.

3. Beijerinck's Laws

M. W. Beijerinck, the founder of the Delft school of microbiology, derived much of his inspiration from the belief that most microbes are omnipresent. This belief was foreshadowed by Pasteur and by Darwin, and it may be traced even to Leeuwenhoek. As far as microbes are concerned we might state this as "everything is everywhere". We do not need to go on long expeditions to look for purple bacteria or for iron bacteria. They are always there. If, however, the microbe is bound, by any form of symbiosis (be it parasitic, helotic or mutualistic) to other creatures, its distribution depends on that of its partner, host, or victim, and the "first rule of Beijerinck" does not apply.

How can we coax the dormant organism from its sleep? Beijerinck did this by creating the environmental conditions necessary for its development. *Sarcina ventriculi*, observed by Goodsir, in 1842, in the contents of the human stomach was isolated by Beijerinck from sand. The conditions under which the organisms appeared were: blood temperature, dearth of oxygen, high acidity, copious organic material. We might extend the thesis, "everything is everywhere" by "the milieu selects" (Beijerinck, 1921).

4. Metabiosis

In a natural sequence of microbiological processes most of the actors are off-stage. There is a sequence of monologues or dialogues. There is often also something like the old Greek "chorus" in the background.

If filterpaper be put in a stoppered bottle filled with seawater and some marine mud, the cellulose will start to decompose. On the decomposition products the sulphate reducers thrive. (Baas Becking *et al.*, 1955). If now the bottle is placed in the light, purple bacteria will develop, and if the cork is removed *Beggiatoaceae* will develop at the surface, followed by iron bacteria. When most of the reduced sulphur is gone, green algae follow. This process Derx (1947) has called "metabiosis".

By our trust in the actuality principle and in Beijerinck's laws we may now proceed to approach certain geological processes as forms of metabiosis.

5. The Reduced Inorganic Milieu

Igneous rocks and volcanic exhalations contain reduced substances such as ferrous iron, hydrogen, and sulphides. They are formed, for the largest part, by abiological processes. On the contrary, the reduced substances in sedimentary rock, in soil and in the hydrosphere are nearly all of biological origin, as the igneous source material has been oxidised by weathering. The reduced state of both organic and inorganic substances in the biosphere is caused by one process only; the photosynthesis of the green cells. The author cannot conceive of other causes. Ultraviolet radiation however, may be operative above the troposphere in the decomposition of water.

Hydrogen and all terrestrial organic substances, coal, oil, the ferrous and manganous state, sulphide, sulphur, thiosulphate and sulphite, ammonia, hydroxylamine, nitrous oxide, nitrate, methane and carbon monoxide are all, ultimately, of photosynthetic origin. In the following it is assumed that the reduced substances present in volcanic exhalations are of secondary importance. In elaborating this thesis we have to lean heavily on the classical work of the late A. J. Kluyver (Kluyver and Donker, 1926) at Delft, who was Beijerinck's successor, and of Kluyver's pupil, C. B. van Niel (1949).

6. The Reduction of Carbon Dioxide

It was realised by van Niel (1949, and later papers) that the essence of the reduction of carbon dioxide is the transfer of hydrogen to the carbon dioxide molecule;

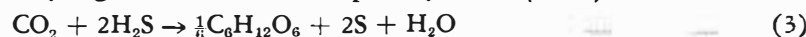


Green cells and purple bacteria may persist on hydrogen alone. At 25° C at equilibrium, this reaction yields 0.55 kcal of free energy (values from Latimer (1952)). In principle, if hydrogen is available, carbon dioxide may be reduced by the cell, if the proper enzyme systems are available.

In photosynthesis of the green cell, containing α chlorophyll, water is the source of hydrogen. The chlorophyll catalyses the photolysis of water into oxygen and hydrogen. This reaction may proceed without such a catalyst in the ultra-violet. Oxygen is actually formed from water in



This reaction is, of course, endergonic. It requires, at 25° C at equilibrium, 112.83 kcal of free energy. Van Niel found that this equation would also apply to another group of photosynthents, the purple bacteria, which contain bacteriochlorophyll instead of α chlorophyll. Instead of photolysing water, these bacteria photolyse H_2S (SH^-).



This reaction would require, at standard state, only 11.53 kcal of free energy.

Finally, van Niel and his pupils were able to show that certain purple bacteria may obtain their hydrogen from organic substances.



By substituting in (4) sulphur for A we obtain equation (3); oxygen for A, equation (2), and for equation (1) $\text{A} = \text{O}$. From the above it follows that purple bacteria requiring H_2S make use of a reduced sulphur compound, which, in itself, is usually of biological origin. The green cells do not require a reduced substance so with them the story starts.

7. Heterotrophs

Photosynthesis reduces the CO_2 to various reduction levels and originates a very large number of carbon compounds, which, by incorporating sulphur, nitrogen, phosphate, and other compounds into their molecules, may create the structure of living matter. Still, the hydrogen in these molecules remains, according to Kluver, as the primary fuel. Organisms which require organic compounds for their development we call heterotrophs. In the laboratory these creatures are usually overfed. In nature they have to persist on "a lenten fare", a fact which is sometimes not realised by bacteriologists.

8. Organic Matter as Hydrogen Donor and as Hydrogen Acceptor

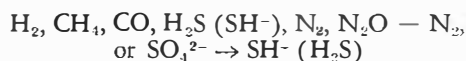
The organic matter, created by the photosynthetic reduction of carbon dioxide might oxidise as well as reduce the inorganic environment. Clarke (1916, pp. 647-707) mentions many examples of such processes. Gold salts are reduced to gold by "organic matter".* The same is true of copper and lead salts (CuSO_4 , PbCO_3), which are reduced to the native elements by carbon compounds. HgS is reduced to native mercury on a bituminous substrate in the presence of methane. Between CO_2 and CH_4 there is an increase of 8 electrons (Baas Becking, 1947). Taking the oxidation state of CO_2 as 0, we can calculate the oxidation state of any C-H-O compound by putting C = 4, H = 1, and O = - 2; e.g. for "one-C compounds",

$$\begin{array}{rcl} \text{CO}_2 & = 4 - 4 & = 0 \\ \text{HCOOH} & = 4 + 2 - 4 & = 2 \\ \frac{1}{6} \text{C}_6\text{H}_{12}\text{O}_6 & = 4 + 2 - 2 & = 4 \\ \text{CH}_3\text{OH} & = 4 + 4 - 2 & = 6 \\ \text{CH}_4 & = 4 + 4 & = 8 \end{array}$$

As most naturally occurring C compounds show a reduction value near 4 it is obvious that they may serve both as H donors and as H acceptors.

9. The Reduction of the Inorganic Environment

The most common reactions, however, are the biological reductions. Ferric to ferrous, and manganic to manganous, may be partly abiological and analogous to the reactions mentioned by Clarke (1916). In most cases, however, the living cell with its enzyme systems has to be present to achieve these reductions. There are many such reactions described in the literature giving rise to



The causative organisms are autotrophs in the sense that Winogradsky (1949) used the term; they are independent of the organic environment, with the restriction that the hydrogen they use is (outside volcanic regions) of biological origin.

10. Oxidations Yield Hydrogen

It would be tempting to consider the other autotrophs, those that live on the oxidation of the inorganic reduced matter, as primarily utilising hydrogen as well, the substrate, whether NH_4^+ , NO_2^- , H_2S , S, or ferrous iron, being of secondary importance. There are several facts that support this view. In the first place from the behaviour of iron bacteria and thiobacteria, the substrates (reduced iron salts, sulphide, thiosulphate or sulphur) seem, in certain cases, to be interchangeable. Moreover, a survey of the literature shows that inorganic oxidations yield hydrogen, sometimes at room temperature. (A list of these reactions may be found in a paper by the author (1957)).

The oxidation of inorganic substances supports a large number of autotrophs, called chemosynthonts or "anorgoxydants" (Winogradsky, 1949).

*Read "microbiological processes".

11. Reductions Yield Oxygen

While inorganic oxidations yield hydrogen, inorganic reductions, by the same token, yield oxygen. It is therefore questionable whether true anaerobiosis occurs in a preponderantly inorganic environment. Preliminary experiments carried out in collaboration with Mr E. J. Ferguson Wood indicate that sulphate reducers might require (or at least find their optimum in) low, but demonstrable oxygen tensions.

Still we will call the hydrogenase-containing sulphate reducer an "anaerobic chemo-autotroph", as it lives at very low oxygen tensions.

12. The Central Position of Hydrogen

The facts mentioned above have been gathered in a diagram (fig. 1), showing that the primary photo-autotroph (I) containing α chlorophyll photolyses water by the acceptance of electrons. The hydrogen produced reduces the carbon dioxide to the reduction level, let us say, of a carbohydrate. Actually a great number of compounds are generated by this process almost immediately. The organic carbon compounds, produced in this way, may serve either as hydrogen donor or as hydrogen acceptor, as described in paragraph 8. Both reactions function in metabolism (Kluyver and Donker, 1926), and both reactions have their abiological counterpart. The organic matter *per se* functions as a hydrogen source for the heterotrophs

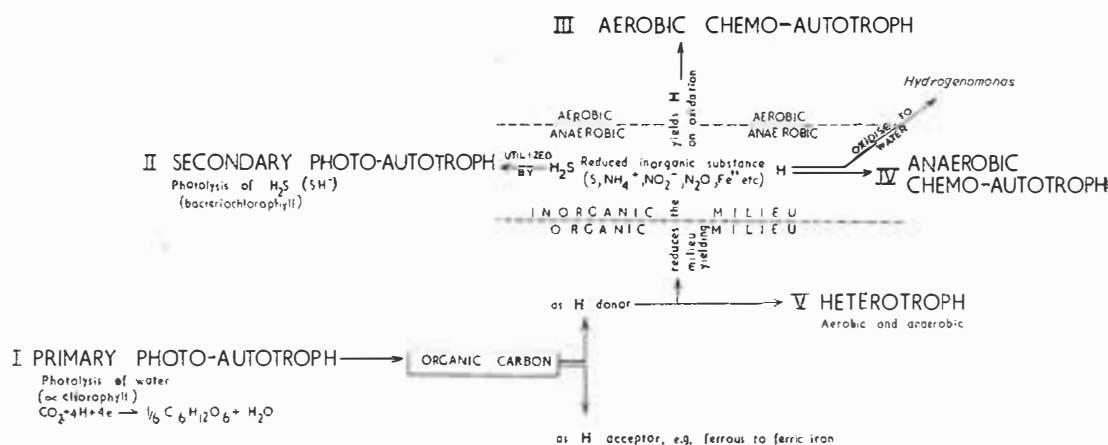


Fig. 1. Hydrogen as the central substance in microbial metabolism

(marked V in fig. 1), while the hydrogen may also be accepted by oxidised, inorganic substances and reduce them. At this reduced level, these inorganic substances become, in their turn, sources of energy (= sources of hydrogen). The organic matter as an energy source becomes oxidised and so creates a reduced inorganic energy source. One of these reduction products is the hydrogen itself. It is the fuel for (IV) the anaerobic chemo-autotrophs (see section 9) as well as for the aerobic hydrogen bacteria. One other, inorganic, reduction product deserves special mention. H₂S (SH⁻) may be photolysed to hydrogen and sulphur (just as water was photolysed into hydrogen and oxygen by the green cells) by purple and green bacteria. The purple bacteria contain bacteriochlorophyll; they are called in fig. 1 "secondary photo-autotrophs" (II) because the H₂S itself is, in almost all cases, a product of biological sulphate reduction (section 6).

The reduced inorganic environment yields, on oxidation, hydrogen which may serve as a fuel for (III), the aerobic chemo-autotrophs (section 10). For the sake of unification, the diagram is over-simplified. Although hydrogen, undoubtedly, will play a universal role, there may be cases where it does not appear "in person" while it still makes its influence felt. Figure 1 will serve as a basis of discussion in the following paragraphs.

PHOTOSYNTHESIS

13. Oxygen

On the overall reaction $6\text{CO}_2 + 6\text{H}_2\text{O} + n h\nu \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$ there is, per square centimetre of terrestrial surface, a gain of ca. 30 mg organic carbon per annum corresponding to 106 mg $\text{CO}_2 + 43 \text{ mg H}_2\text{O} = 72 \text{ mg carbohydrate} + 77 \text{ mg oxygen}$. As there is 230 g atmospheric oxygen per cm^2 there would be a cyclic renewal of the atmospheric oxygen in ca. 3,000 years, if we assume that the atmospheric oxygen originated entirely by photosynthesis (as was already assumed by Koene (in Kuiper, 1951), from Brussels, in 1856). The abiological photolysis of water vapour, taking place at a region below the cold tropopause (210°K , 12 to 20 km) is probably also of great importance, although we cannot, at present, estimate its quantitative importance (Poole (1950) 10 per cent of the total, Kuiper (1951) 25 per cent of the total). A thermal dissociation of the water molecules, giving rise to oxygen, as proposed by Tammann in 1924 (Baas Becking *et al.*, 1957), seems on many grounds to be most unlikely.

The amount of oxygen, chemically bound by weathered rock, has been estimated by Goldschmidt (1954) as several times that present in the atmosphere. Goldschmidt (1954) has gathered data on the amounts of ferrous and ferric iron (expressed as FeO and Fe_2O_3) present in magmatic and sedimentary rocks, as an index of oxidation.

Table 1 shows figures for Fe_2O_3 and FeO (Clarke, 1916) re-expressed as ferric and ferrous iron.

TABLE 1. Ferric and Ferrous Iron in Magmatic and Sedimentary Rocks
(After Clarke, 1916)

	Magmatic	Shales	Ferruginous Marine Mud	Sandstone	Red Marine Clay
	%	%	%	%	%
Fe_2O_3	3.08	4.03	5.07	1.08	8.66
FeO	3.80	2.46	2.30	0.30	0.84
Fe, ferric	2.14	2.49	3.54	0.75	6.05
Fe, ferrous	2.96	1.92	1.79	0.24	0.66
ferric/ferrous	0.72	1.30	2.97	3.13	9.20

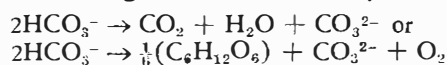
Although these figures are suggestive, it should be remembered that the relations ferric/ferrous iron, apart from the magmatic rock, is certainly not fixed and may change over-night when conditions are favourable to sulphate reduction. The shales are, in part, fossil estuarine black muds often subjected to intensive sulphate reduction. Sandstones and ferruginous marine muds may be periodically subjected to sulphate reduction (e.g., *Zostera*, eelgrass, on the sand flats), while, probably due to the paucity of organic matter and a continuous oxygenation, sulphate reduction is virtually absent from the red clay. Oxygen, of course, is consumed in biological, catabolic processes as well as in industrial processes. Samples of black mud kept for even a short time before analysis have become oxidised.

This is not the place to speculate upon the state of the oxygen in the pre-biological period of the earth. According to Goldschmidt the lithosphere is a veritable "oxygen lattice" and a large part of the earth's surface was in an oxidised state before life began.

14. Carbon Dioxide

Considering the figures given in the table in section 13, the carbon dioxide cycle should be much more rapid than the oxygen cycle. However, the number of abiological factors entering into this cycle is so great and so variable that we will confine ourselves to the direct influence of photosynthesis on the CO_2 equilibrium in natural waters.

In sea water the carbon dioxide is preponderantly in the bicarbonate form and in photosynthesis this bicarbonate will be changed into carbonate by removal of its CO_2 .



There is a concomitant increase in alkalinity. That this increase is due to the removal of the CO_2 may be shown by bubbling a neutral gas, like nitrogen, through sea water. The pH increases, in a short time, from 8.1 to 8.2 to 9.4 to 9.5. The pK_2 of carbonic acid would be (at 25°C) at a pH of 10.3. Near pH 9.4 there is still a preponderance of bicarbonate but pH 9.4 seems to be the endpoint of photosynthesis in sea water. (The experimentally determined value for the pH of a suspension of solid calcium carbonate in N/1 sodium chloride in equilibrium with CO_2 -free air is 9.5. When brought to equilibrium with normal air the pH is lowered to 8.4. In pure water in the absence of CO_2 the equilibrium pH of a CaCO_3 suspension is 9.6.*) At this pH, CaCO_3 starts to precipitate. This precipitation is common in nature and of the utmost importance in the formation of coral and coralline reefs and in the genesis of sediments consisting of shell fragments or of other lime-depositing organisms. That the increase in pH is an important factor in this precipitation may be inferred from the fact that lime-depositing plants often excrete $\text{Ca}(\text{OH})_2$ and that the secretion of ammonia and amines has been reported in lime-depositing animals.

Coralline algae deposit calcium carbonate much faster in the light than in the dark. The whole subject, geochemically of prime importance, is still awaiting further research (Baas Becking, 1956b).

If calcium and magnesium are absent from a water, photosynthesis may cause the pH to rise to 10.55. A saturated solution of Na_2CO_3 yields this pH. As the pH is quite near to the pK_2 of carbonic acid (pH 10.33) there should be still bicarbonate present. Thio-iron bacteria, present in acid mine waters at a pH of < 2.00 are apparently still capable of carbon-dioxide reduction, using either or both CO_2 and H_2CO_3 .

15. Organic Carbon

Carbon accumulates by photosynthesis chiefly in or near reduction state 4 (reduction state $\text{CO}_2 = 0$, $\text{CH}_4 = 8$). This means that the photosynthate may be either further reduced or further oxidised. Reductions may yield fatty acids and even hydrocarbons and are, on the

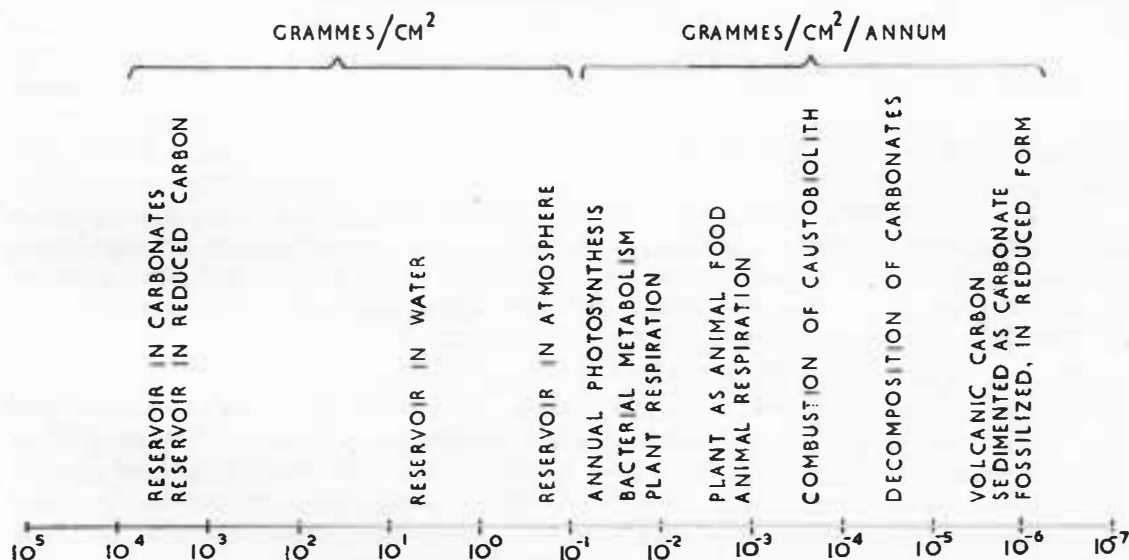


Fig. 2. Reservoirs and utilisation of carbon

whole, endergonic processes. Oxidations will yield a great number of organic acids and, finally, carbon dioxide. Figures 2 and 3 show the amounts of organic carbon entering into various microbiological and abiological processes.

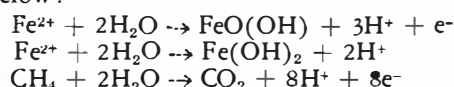
*Mr A. D. Haldane, Bureau of Mineral Resources, kindly supplied this information from unpublished experiments.

These processes are, for a large part, cyclic. Temporarily (on a geological time scale) removed from the cycle are photosynthetic accumulations, such as coal and oil.

The utilisation of organic carbon is illustrated in fig. 2. The processes of breakdown (katabolic processes) with a concomitant mineralisation of the environment may end with the carbon either completely reduced to CH_4 , or completely oxidised to CO_2 . CH_4 is, however, fuel and may yield, on oxidation (or dehydrogenation), hydrogen.

16. Water

As mentioned in section 13, photosynthetic water amounts to $43 \text{ mg/cm}^2/\text{annum}$. For land plants we know that 99 per cent of the absorbed radiation is used in evaporation, and ca. 1 per cent in photosynthesis. As the synthesis of 72 mg carbohydrate would require 0.0456 kcal free energy, a land plant in daytime will use $4.56 \text{ kcal/cm}^2/\text{annum}$ in the evaporation of water. As it takes ca. 0.580 kcal to evaporate 1 ml of water this corresponds to roughly 8 cm precipitation. We see, therefore that an appreciable percentage of the terrestrial water passes through organisms, and that, in its primary aspect, the water cycle has important biological components. Moreover, as in this paper most oxido-reductions are considered as a lysis of the water molecule (for which theory there is considerable evidence) the importance of water still is enhanced. Examples of this are given below:



The above reactions are thermodynamically possible in the natural aqueous environment. From the above it appears that water may be a product of reductions. It is equally a product of oxidation, e.g. if we invert the equation for photosynthesis (katabolism). A curious bacteriological production of water is caused by *Hydrogenomonas*, which oxidises hydrogen directly.

THE ACCUMULATION OF MATTER

17. Introduction

A gold nugget represents a high concentration of atoms of Au. This nugget dissolved in aqua regia still represents a fair concentration. But when this solution of AuCl_3 is thrown into the sea, the gold is dissipated. Harvesting and mining are examples of processes by which the dissipation of matter (in a way, a material analogue of entropy) is reduced. Living matter possesses such "antidissipatory" properties. In the land plant it takes the carbon present in more than four hundred litres of air to synthesise one gramme of carbohydrate. In the plant the organic carbon may be more than 10 per cent of wet weight. This presents a concentration factor of more than 300 as compared to the 0.03 per cent CO_2 present in the atmosphere. In fig. 3 the range of concentration factors are shown when the composition of organisms is compared to that of a common environment—sea water. On the diagonal 10^0 there is neither concentration nor dissipation; the parallel lines show concentrations up to 10^{+6} and dissipation to 10^{-2} . In certain organisms the concentrations may actually be a million-fold. The internal milieu may be, therefore, of entirely different composition from the outward environment.

We have mentioned already one accumulation, that of the carbonates of calcium and magnesium, and the possible role played by photosynthesis in this process. There are a whole series of such accumulations, however, which involve internal metabolism. Alkali and alkaline-earth metals seem to penetrate freely into the living cell. Heavy metal ions are usually soluble only in fairly acid solutions. In an acid environment they may penetrate directly, in a more alkaline environment they may be dissolved by acids secreted by the cell, but it seems more likely that they may be chelated by oxyacids present in the outer pellicle of living cells (Clarke, 1916). Penetration of anions requires respiratory energy and is, therefore, more closely connected with cell metabolism than the penetration of cations. The energy required presupposes a reservoir of organic carbon. Accumulation of anions, such as iodide, bromide, phosphate, sulphate and borate have to be considered under this heading. They are secondary to the accumulation of organic carbon compounds by photosynthesis.

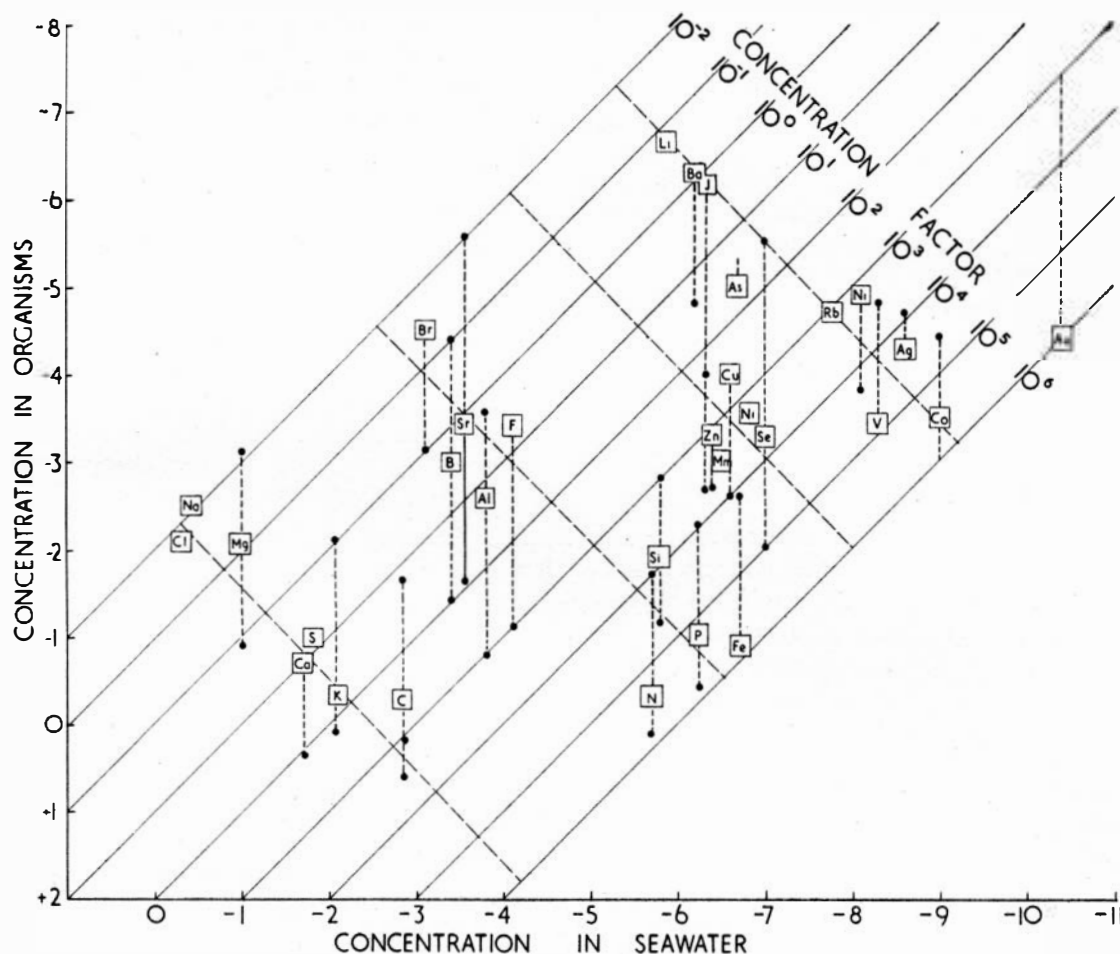


Fig. 3. Comparison of the concentration factors of elements in organisms and in sea water

Apart from the enrichments caused by biological processes, some seem to be of inorganic nature (formation of phosphate, calcite, aragonite). The author, however, is inclined to adhere to Linnaeus' "Omne calx ex vivo".

The organic accumulations will be dealt with later as they have undergone oxido-reductive changes.

18. Accumulation of Anions

(1) Silicate: Radiolaria and diatoms accumulate silica as opal. Grasses and Equisetae among the higher plants show this in a lesser degree. Sponges and silicoflagellates are well known as Si accumulators. Silica has been found, in organic combination, in egg yolk. There is very little evidence as to the processes involved. The influence of the acidity on the solubility of silicates, often invoked in older literature, seems to be little over a large pH range. The concentration of silicate from sea water into living cells may be 1,000 to 50,000 fold.

(2) Phosphate is originally present in magmatic rock, especially in basaltic intrusions and chiefly in the form of apatite. While it is highly concentrated in the skeletal material of vertebrates also as apatite (concentration factor from sea water 10^4 to 10^6) there may be, however, inorganic marine processes forming phosphorites not unlike those which give rise to lime deposition. The phosphates, already accumulated in bone, are further concentrated by bird or bat colonies (guano, Hutchinson, 1950) and a large number of phosphate minerals are associated with this guano.

(3) Marine organisms concentrate iodine and bromine, but not only as organic compounds. Iodine is accumulated chiefly as iodide (by brown seaweeds). The concentration from surrounding sea water may be more than 5,000 fold. The "fossil" water in certain oil regions (central Java) contains enough iodine for commercial exploitation, an additional argument for the marine origin of petroleum.

(4) Boron is concentrated from sea water (which is the main source) by a variety of organisms. The sulphate (see below) is found in organisms in up to 10 times the concentration in sea water. Nitrogen is concentrated in large amounts by marine and terrestrial organisms to a degree only equalled by phosphates. Of the anions, only the chloride ion seems to be dissipated rather than concentrated by living matter in relation to the ocean. This is due to the fact that whereas the blood of invertebrates contains an amount of Cl^- comparable to that of sea water, the blood and tissues of vertebrates contain much less.

(5) Fluorine is concentrated in dentine and in bone, and the fluorisation of fossil bone increases with age. Certain poisonous plants contain organic fluorine.

19. Accumulation of Cations

In some cases concentrations may be a millionfold (Au and Fe), but in other cases (Na) the cation is dissipated rather than concentrated. Iron is an element of most respiratory pigments, copper forms part of the respiratory pigment of molluscs and of many crustaceans. Zinc and manganese are present in important enzymes. Also in the case of cobalt (cobalamin) we can find a reason for its accumulations. In many cases, (as in the anions) the reason escapes us. Why gold or silver are concentrated is an open question, and nickel is accumulated for no apparent reason. The strangest is the case of vanadium, present in sometimes very large amounts in bitumen, asphalt, and oil. It is known to accumulate in ascidians, although this may be due to concentration of this metal by some unknown (perhaps microbial) food factor. Vanadium seems to enter the porphyrin ring and may perhaps function as a respiratory enzyme.

20. The Role of Microbes in Accumulation

Most of the analyses mentioned in this section have been carried out with algae, fishes, sponges, tunicates, and the like, and this for the obvious reason that it is easier to obtain large quantities of such organisms than microbes. Bacteria may concentrate phosphate from surrounding sea water two hundred fold. We know that most of the active plankton is truly microbial.

THE INFLUENCE OF ORGANIC MATTER, REDUCTIONS

21. Introduction

While it is conceivable that organic matter will exert an oxidative influence on the inorganic environment, e.g.



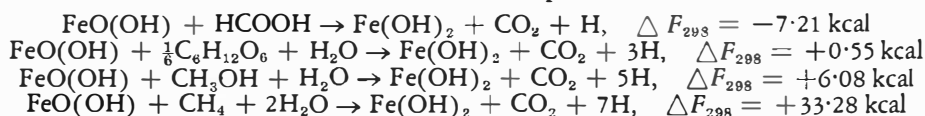
and that the search for such processes may be profitable, in most cases the carbon compounds formed directly and indirectly by photosynthesis, cause, by their oxidation, a reduction of the inorganic environment. This reduction may be only in the cation, only in the anion, or in both component parts of the molecule. Often the reduced state of one ionic component is incompatible with the oxidised state of the other as in Fe_2S_3 (Baas Becking, 1956a), while the opposite, FeSO_4 , is stable in an anaerobic environment. Borates, phosphates, and silicates exist, in nature, apparently at one oxidation level, the highest. The chief metals of variable valence in the natural environment are iron and manganese, while of the non-metals we find a great number of reduction levels in compounds of carbon, sulphur, and nitrogen. The halogens occur almost exclusively as simple halides but may be found both in the reduced and in the (complex) oxidised state. The highest oxidation states in which iodates, periodates, perchlorates, chromates and manganates occur (Chilean "caliche"), will not be treated in this paper.

While a great many of the reactions described are also plutonic and, as such, abiogenic, the limit between juvenile and vadose plutonic phenomena is not sharp, as volcanic material may be obtained from sediments. In most cases, however, we are concerned with sedimentary processes, at biological temperatures.

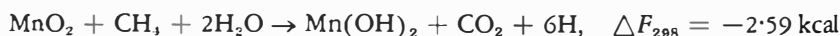
The reactions to be described in this section are conceived to be due to the acceptance of hydrogen (generated by the oxidation of organic material) by inorganic compounds.

22. The Reduction of Ferric and Manganic Salts

At the root level (the rhizosphere) in otherwise aerated soil, and in water at a certain depth (still outside the region of sulphate reduction), ferric iron is reduced to ferrous. We can write the reactions for the fictitious case of one-carbon compounds, as follows:



Faintly endergonic, these reactions are possible electro-chemically in the natural environment. The reduction of pyrolusite is always exergonic, even with methane as a hydrogen donor ($\text{Mn}^{4+} \rightarrow \text{Mn}^{2+}$).



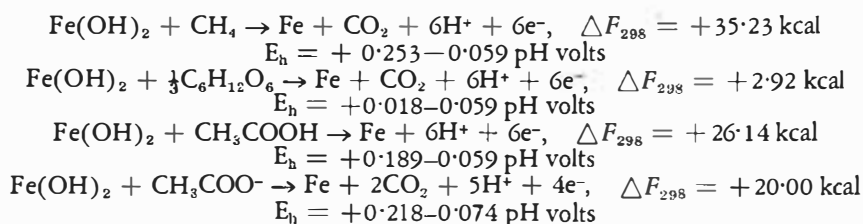
These reactions, however, do not require, or liberate, much energy.

23. Native Elements

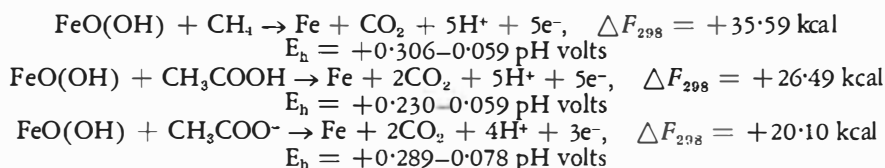
In section 8 it was stated that "organic matter" (read microbiological reactions of organic matter) may cause salts of copper and mercury to be reduced to the native metals. The same holds for lead.

Reliable observers have reported on the presence of non-meteoritic, non-metallurgic, native iron. Now the reaction $\text{Fe}^{2+} = \text{Fe} + 2\text{e}^-$ shows an equilibrium potential of -0.434 volts. This is much lower than any potential observed by either Mr Wood, Mr Kaplan or by myself in the natural environment (Baas Becking *et al.*, 1957). Here we have to invoke organic carbon, which, as in a metallurgical process, will reduce the iron compound to iron. At the low temperature, however, enzyme systems of living matter have to take the place of the high temperatures in the furnaces. Such reduction may take place well within the natural environment:

(1) Ferrous iron to native iron



(2) Ferric iron to native iron



From the above it appears that the native iron may be formed by a process analogous to one of those given above within the framework of the natural environment and that the reduction of both the ferric lepidocrocite and of the ferrous hydro-oxide yield similar potentials. Reduction of both nickel and cobalt salts may be conceived to occur in a similar fashion. The electrode potentials of both zinc and manganese are too low to be sufficiently elevated by an organic hydrogen donor.

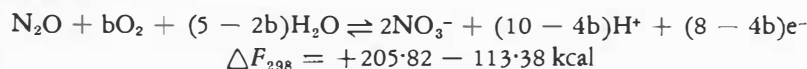
Sulphur, selenium and tellurium all may occur native in the natural environment. The occurrence of native iodine has been reported. The formation of the last mentioned elements, however, require oxidation. Formation of free carbon is a problem in itself and will be dealt with later in this section.

24. The Reduction of Nitrate and of Nitrogen

The importance of the chief reactions in the nitrogen cycle are shown in fig. 5. The following reactions will be considered:

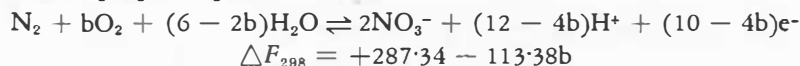
(1) *Denitrification*. Adel (1951) discovered, by means of infra-red spectroscopy, the presence of appreciable quantities of nitrous oxide (about 5 mm n.t.p.) in the terrestrial atmosphere. Later, Taylor and collaborators (Kuiper, 1951) showed the presence of this compound in soil air. Since 1910, when Beijerinck and Minkman discovered the bacterial formation of N_2O , we have known that this compound is an important link in the nitrogen cycle, but that it would be of cosmic significance nobody had even guessed! The amount in the atmosphere would correspond to ca. $8\gamma/cm^3$. Any consideration of the denitrifications has to include nitrous oxide. About the quantity of nitrogen liberated by denitrification we know nothing. It would, at most, amount to a very small fraction of the vast nitrogen reservoir of the atmosphere.

The reactions concerned are:



The reduction will proceed with oxygen values lower than $b = 1.81$

The denitrification proper implies:

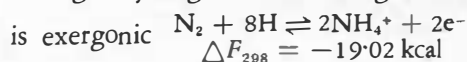


The reduction will proceed with oxygen values lower than $b = 2.53$.

This reaction is highly exergonic with organic matter, even with methane:



(2) *Nitrogen Fixation*. The fixation of atmospheric or hydrospheric nitrogen occurs only amongst hydrogenase-containing microbes, both aerobic and anaerobic. The overall reaction



25. Reduction of the Sulphate

Sir John Murray in 1891 (Clarke, 1916) ascribed the origin of the sulphide in marine muds to the action of "organic matter" on sulphates. In view of what has been said above, this does not seem unreasonable, were it not that Beijerinck, in 1895, showed the reaction to be due to bacteria. If a reaction is thermodynamically possible either in the organic or in the inorganic milieu, there will be microbes that make use of this reaction (perhaps with the exception of carbon, which seems singularly inert).

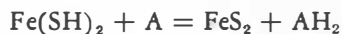
Like nitrogen, sulphur occurs in nature in a great many reduction stages, as shown in table 2. While both thiosulphate and sulphate are present in the natural aqueous environment, geochemically the reduction levels 8, 6, and 0, sulphide, sulphur, and sulphate are the most important.

TABLE 2. Reduction Stages of Sulphur

Reduction Stage	Compound	Oxidised to	Free Energy of Reaction	Maximal H Produced	Maximal b for Oxidation	Minimal b for Oxidation
8-6	H ₂ S	S	+6.54	2	0.50	0.05
8-6	SH ⁻	S	-3.01	1	0.25	0
6-4	2S	S ₂ O ₃ ²⁻	+23.05	6	1.50	0.40
4-2	S ₂ O ₃ ²⁻	2S O ₄ ²⁻	+30.95	6	1.50	0.55
4-2	S ₂ O ₃ ²⁻	2HSO ₃ ⁻	+21.05	4	1.00	0.37
2-0	SO ₃ ²⁻	SO ₄ ²⁻	-4.55	2	0.50	0
2-0	HSO ₃ ⁻	HSO ₄ ⁻	+2.75	1	0.50	0.03
8-0	H ₂ S	SO ₄ ²⁻	+55.96	10	2.50	0.49
8-0	SH ⁻	SO ₄ ²⁻	+46.41	9	2.25	0.41

At the sulphide level sulphur occurs as H₂S in acid solutions, and as SH⁻ in alkaline solutions (equilibrium at pH *ca.* 7.0). Sulphide ion S²⁻, in spite of many statements in the literature, does not occur in the natural environment. It is formed only in highly alkaline solutions. The natural sulphides will react with metal compounds and several hundred sulphide minerals are known, all owing their existence to sulphate reduction.

In the sequence of the electrochemical series, native metals, like mercury, silver, copper, and lead may be found by the exchange of sulphides (Clarke, 1916). Some of the iron sulphides were discussed in a previous paper (Baas Becking, 1956a). There is good evidence to assume that there are four important iron sulphides. Two of them only persist in aqueous suspension; the hydrotroilite Fe(OH)SH black, but non-smelling, and the disulphydryl iron Fe(SH)₂, a component of the "fetid" black mud. From the latter compound pyrite or marcasite may be formed by dehydrogenation, the hydrogen acceptor (A) being either sulphur, oxygen, or organic matter:



It was found (Baas Becking and Mackay, 1956) that the formation of iron sulphide will solubilise phosphate, e.g.



Enteromorpha, the pellicle of which contains much phosphate, may yield much soluble phosphate when treated with H₂S. If the H₂S tension is high enough, Fe(SH)₂ will persist even at the low pH generated (pH < 5.0). There is good evidence to assume that a similar liberation of phosphate will occur in natural sulphate reduction. Ore concentration may be brought about by sulphate reduction if the solubility product of the sulphide of a certain metal is lower than that of the compound in which the metal occurs. The importance of such concentrations was recognised at least a century ago. The sulphate reduction does not give rise, in nature, to important accumulations of intermediates. Native sulphur is a product of sulphide oxidation and will be treated later.

26. Reduction of C Compounds

Organic matter may act as a hydrogen donor to carbonic acid. The formation of methane from higher oxidation-stages of the carbon is a case in point. Well known is the Lieske process, by which the noxious carbon monoxide in illuminating gas is changed into methane by bacterial action, at the same time increasing the calorific value of the gas.

In certain cases hydrogen itself may be formed from organic matter.

27. The Formation of Coal

(1) *Graphical Representation* (fig. 4). In order to represent changes in composition of organic matter (simplifying the processes to changes in C, H, and O) we may represent the relations in a triangular diagram (Baas Becking, 1947) if the corners represent C, 4H, and 2O respectively. The point representing CO₂ will be halfway on the C – 2O line, while CH₄ and H₂O occupy analogous positions on the other sides. It is easy to show that all substances with a reduction level 0 will be situated on the line H₂O – CO₂, all substances with a reduction level 8 on the line H₂O – CH₄ while the line C – H₂O ("carbo – hydrate") shows the reduction level 4.

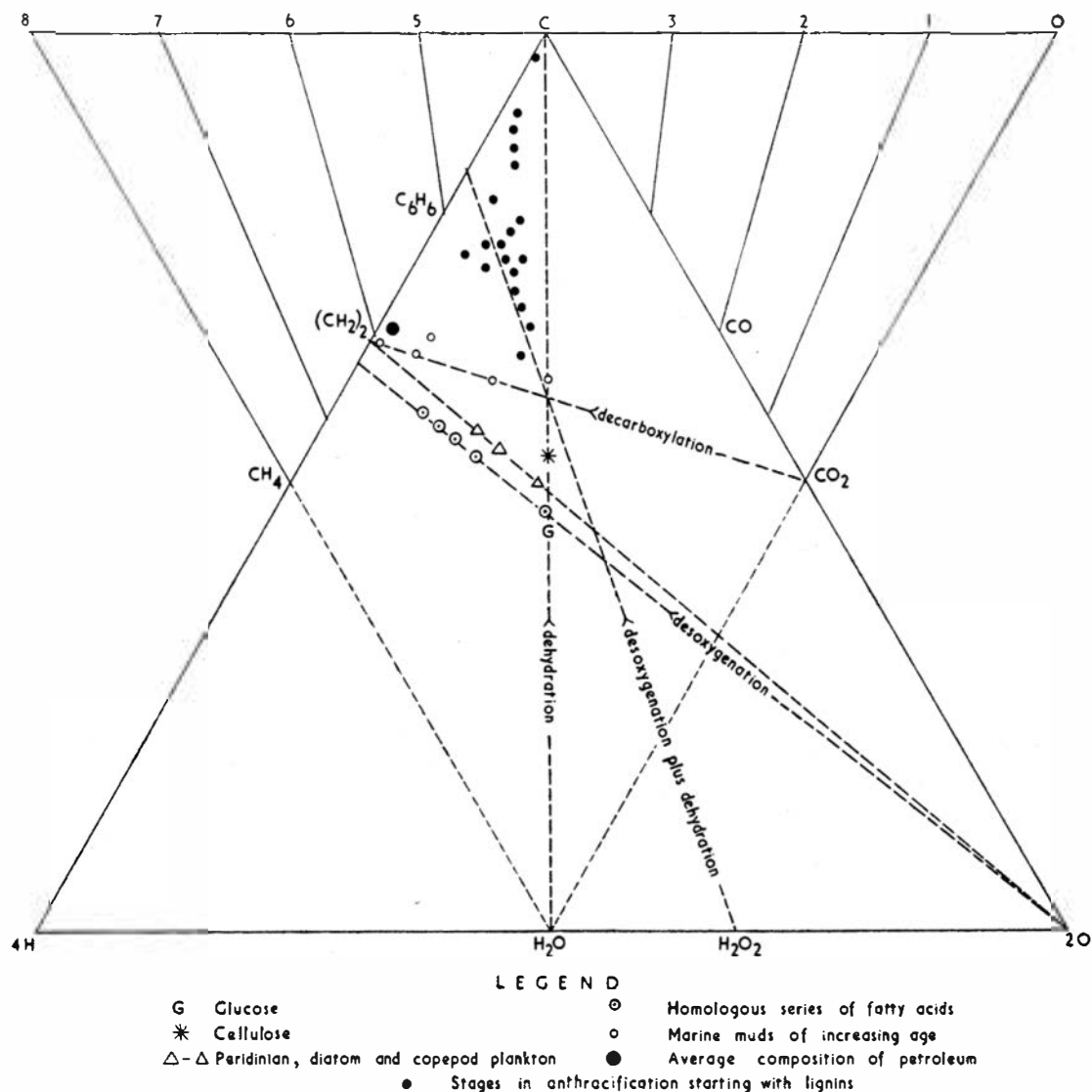


Fig. 4. Reduction stages of carbon

A bundle of lines passing through H_2O represents (de)hydrations, a bundle through $4H$ represents (de)hydrogenations, while the bundle through CO_2 represents (de)carboxylations and that through $2O$ (des)oxygenations.

(2) *Anthracification*. The formation of coal, anthracification, probably takes place in a fresh-water environment. The waters appear to have been mainly soft, often atmospheric (ombrogenic). While certain microbiological processes in the wet fresh humus (or in the *Sphagnum* bog) continue, others seem suppressed (Baas Becking and Nicolai, 1934). It is probable that under the acid anaerobic conditions fungi will become increasingly important. Peat, pollen grains, and wood may be considered as the chief starting points of the anthracification. If this anthracification were a simple dehydration, glucose (G in fig. 4) would simply form caramel, and with further dehydration finally amorphous carbon would appear. In the seed coat of the sunflower and of the African daisies such a caramelisation may take place in a few days, with the formation of black phytomelanins. However, if the composition of cellulose and of lignin, sphagnum moss, lignite, brown coal, bituminous coal and anthracite are plotted on the triangular diagram, fig. 4, a series of points is obtained expressing, apart from dehydration, desoxy-

genation (line through H_2O_2). This shows that the process is of a complex nature. It may be that other overall reactions play a role, but then it is clear that the process consists, apart from the preponderating dehydration, of a progressive desoxygenation.

28. Petrolification

Like anthracification petrolification is chiefly anaerobic but the environment must have been nearly always marine, and, at least at the start, alkaline. Without indulging in any speculation as to the role of microbes in this process it may be said that certain microbiological processes, as well as the planktological food chain show sequences analogous to the C-H-O composition of marine sediments of increasing age. In Fig. 4, points on the line G – $(\text{CH}_2)_2$ represent the composition of various organic acids with increasing CH_2 content, while the three triangles immediately above represent the average composition (from right to left) of peridinian, diatom, and copepod plankton. In both cases these series represent desoxygenations. The open circles on the line through CO_2 represent the composition of marine sediments of increasing age. The black, filled circle represents the average compositions of petroleum. This series represents a decarboxylation. Both desoxygenation and decarboxylation may play the major roles in petrolification.

From the above it appears that the two chief processes by which fossil organic matter is stored show definite trends. We are still very far from a microbiological explanation. It may well be that, at least for petrolification, advance will be made by the study of organic S compounds, accompanying sulphate reduction.

THE OXIDATION OF THE REDUCED INORGANIC ENVIRONMENT

29. Introduction

The inorganic environment, originally (in the extra-plutonic environment) in the oxidised state, becomes reduced by the products of photosynthesis. This reduced inorganic environment will, in its turn, be capable of liberating hydrogen on oxidation. I know that the textbooks speak of "the liberation of energy" and all of us use this term almost indiscriminately. Here we will substitute "hydrogen" for "energy" as I must confess that I never actually understood what was meant by this energy apart from the conclusion that a certain reaction might be thermodynamically possible. Apart from this there are good reasons to prefer a material to an energetical consideration.

A great number of bacteria, as said before, will thrive on hydrogen as an exclusive fuel. Their activity presupposes the presence of hydrogen, an oxidation product of an environment reduced by organisms requiring photosynthate. They are aerobic autotrophic chemosynthonts, but their fuel is ultimately derived from organic sources.

30. The Oxidation of H_2S (SH^-)

The reaction $\text{H}_2\text{S} \rightarrow \text{S}$ is endergonic in the absence of oxygen below pH 7 (see table, paragraph 25). However, it requires but little oxygen to make it possible (one molecule of oxygen per 20 molecules of H_2S). Although the oxidation of SH^- is exergonic, also in the absence of oxygen, it has been, and still is of great geochemical importance (Baas Becking and Kaplan, 1956). Accumulations of sulphur are widespread. As shown by Verhoop (1940), these accumulations are, at least in part, abiological. A great many organisms, however, amongst those the purple and green bacteria, are able to perform the oxidation of the sulphide. Sulphur is not always a halfway house in this oxidation. While the black mud oxidises abiologically to sulphur and lepidocrocite;



the pyrite (probably a dehydrogenation product of the black mud) oxidises, biologically, to the sulphate, according to

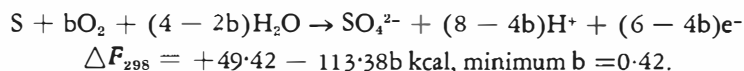


creating a highly acid environment (acid mine waters). Even negative pH's have been recorded in these waters; some free H_2SO_4 is apparently formed.

*In this and the following equation the oxygen has been left out for simplicity's sake.

31. The Oxidation of the Sulphur

As in the oxidation of pyrite, sulphate (with traces of intermediates such as thiosulphate and sulphite) is formed. The microbes concerned are again thiobacteria, in many cases related to iron bacteria. The thiobacteria also may make some HSO_4^- , as in *Thiobacillus concretivorus* Parker. The decrease of the pH in these oxidations, however, never goes further than pH 1.2. It is apparently the extra sulphur in the pyrite that causes the high acidity in its oxidation. Apart from the thiobacteria, purple bacteria as well as the *Beggiatoaceae* (about which group we know very little) oxidise S to SO_4^{2-} .

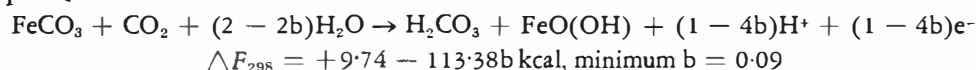


There are intermediate reactions. It seems that sometimes the sulphur is reduced before it is oxidised to the sulphate stage. We have known since the beginning of this century that most cells are capable of reducing sulphur to H_2S (SH^-).

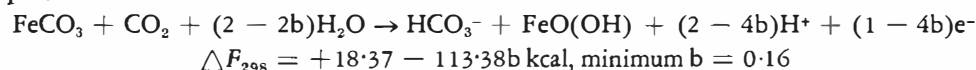
32. The Oxidation of Ferrous Iron

The sulphur and the iron cycle in nature intersect at several places. Moreover, the true iron bacteria, such as *Sphaerotilus* and *Gallionella* (Baas Becking *et al.*, 1956), like the *Beggiatoaceae* develop in a redox gradient, be it at different potential levels. It seems most probable that the iron compound oxidised serves simultaneously as a carbon dioxide and as a hydrogen donor.

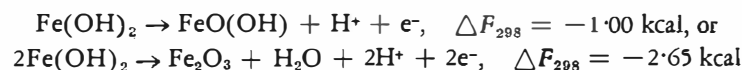
For pH < 6.4:



For pH > 6.4:



The oxygen tension required is low. Ferrous hydroxide is another possible source of hydrogen, but this substance may oxidise already in the absence of air and is stable only at potentials below those observed in nature

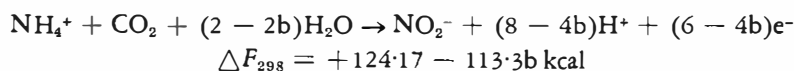


and may, therefore, be of secondary importance.

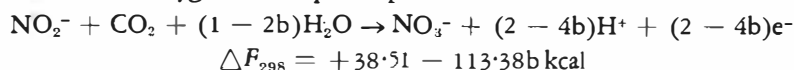
33. Oxidations in the Nitrogen Cycle

Aerobic chemo-autotrophs in this cycle are the nitroso- and the nitrobacteria.

In the former,



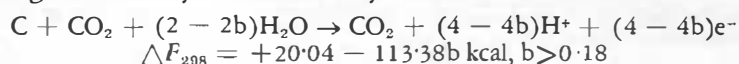
at least 1.08 molecules of oxygen are required per ammonia ion oxidised, while the nitrobacteria



require only one molecule of oxygen for three nitrate ions in order to make the reaction thermodynamically possible. As said before hydrogen generated by these, and similar, oxidations may be directly used by other secondary autotrophs, such as denitrifiers and the curious nitrous-oxide organisms (Beijerinck, 1921).

34. Other Oxidations

A great many other inorganic oxidations could be mentioned. They do not add anything novel to our general consideration, however. Thirty years ago, the author expressed his belief that all reactions in the environment that were thermodynamically possible had at least one organism dependent on such a reaction for its "energy" (Baas Becking, 1934). On the whole, this thesis has been confirmed, with at least one exception. Carbon, ideally suited (with its reduction state 4) to act both as hydrogen acceptor and as hydrogen donor, has remained singularly inactive. The great difficulty met in the study of the reaction



is the fact that I was unable to obtain pure carbon. There was always (bacteriologically active) carbon monoxide adsorbed on it. It may be that there is an intrinsic difficulty for organisms to tackle native carbon; and it may be that accumulations of carbon occur because of this fact. Here is another question that could be profitably approached by modern techniques.

CONCLUSIONS

From this outline of some microbiological aspects of geology it appears that the microbes have entered geology to stay. In any work involving sediments, recent or ancient, in any study of aqueous or atmospheric environments, we now realise fully that we have to take vital phenomena into consideration. Figure 5 shows the geological importance of certain biological processes.

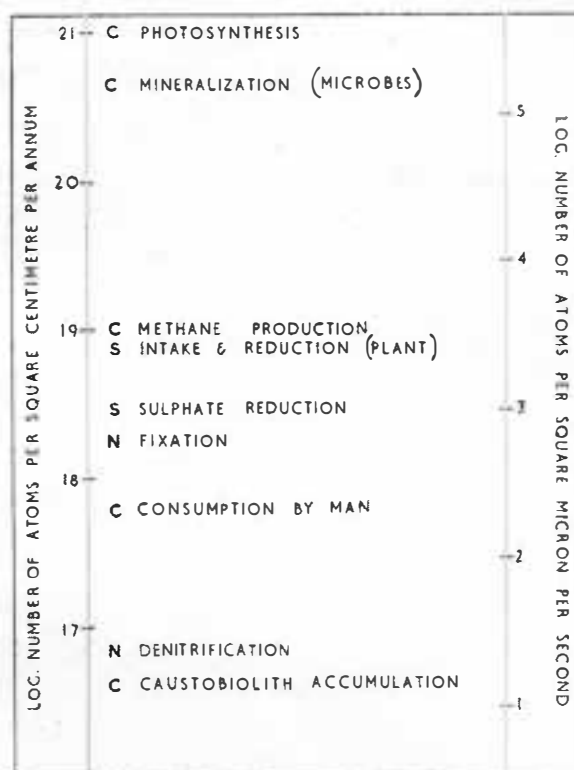


Fig. 5. The geological importance of certain biological processes

Just as organic biochemistry has given great impetus to bacteriology in the study of the internal milieu of the microbes, there is an inorganic biochemistry which has to describe the geochemical reactions taking place on this earth in relation to the activity of its microbes. I am convinced that such a biochemical approach will be profitable.

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