Bacteriological investigation of the surface microlayer of the Gulf of Gdańsk*

OCEANOLOGIA, No. 30
pp. 93–103, 1991.
PL ISSN 0078–3234

Surface microlayer
Number of bacteria
Physiological activity
Southern Baltic

ZBIGNIEW MUDRYK
Pedagogical University,
Institute of Biology,
Słupsk

KRZYSZTOF KORZENIEWSKI,
LUCYNA FALKOWSKA
Gdańsk University,
Institute of Oceanography,
Gdynia

Manuscript received June 19, 1990, in final form April 16, 1991.

Abstract

The number of heterotrophic bacteria in the surface microlayer and the subsurface layer of the Southern Baltic was determined using the ZoBell medium. The ability of the bacteria isolated from the two water layers to perform certain physiological processes was also determined with the use of test substrates.

It has been established that the number of bacterioneouston was 1.5–2 times greater than the number of bacterioplankton. The ammonifying, lipolytic, proteolytic and amylolytic bacteria were the most numerous among the physiological groups, whereas the pectinolytic and cellulolytic bacteria were scarce.

1. Introduction

Organic compounds in the form of a soluble suspension in sea water constitute the main nutritional base and energy source for numerous heterotrophic microorganisms (Seki, 1982). The concentration and composition of these compounds determine, to a great extent, the number of microflora, while their uptake is the measure of metabolic activity of bacteria. The greatest concentration of the organics matter occurs in the surface layers of a water basin, mainly in the surface microlayer of 0.1 μm thickness

* The investigations were carried out under the research programme CPBP 03.03.C, co-ordinated by the Institute of Oceanology of the Polish Academy of Sciences.

(Sieburth, 1976). As a result, this layer is characterized by a great concentration of bacterioneuston (Norkrans, 1980; Sieburth, 1979). Apart from the bacteria, the nanoplankton of numerous, mainly photoautotrophic organisms also accumulate, to a large degree, in the surface microlayer. These organisms are: blue-green algae, green algae, diatoms, as well as fungi and protozoa (Dahlbäck, 1983; Liss, 1975; Whittle, 1977). Nanoplankton is the main factor of the primary production and constitutes *ca.* 50% of the total biomass of organisms inhabiting aqueous basins (Durbin *et al.*, 1975). Various simple and complex organic compounds, being mainly extracellular excretions, are abosrbed in the surface microlayer. From 2% to 21% of the primary production in the Baltic Sea is liberated to water in the form of excretions, 90% of which is taken up by the bacteria (Iturriaga and Hoppe, 1977). These substances stimulate the metabolic activity of the bacteria, thus influencing their respiration processes and cellular growth. At the same time the bacteria cause the biodegration and then mineralization of the organic compounds in water, due to their ability to perform numerous physiological processes (Saava, 1985). Therefore heterotrophic bacteria play an essential role in carbon and nitrogen circulation in the marine ecosystem. This paper aims to determine the number of bacteria and to recognize their physiological properties in order to gain information on the potential destruction of the organic matter caused by bacteria isolated from the surface microlayer of the Gdańsk Deep.

2. Materials and methods

Water samples were drawn on May 25 and 26, 1989, at two stations located at the Gdańsk Deep of the Baltic (Fig. 1). Water from the surface

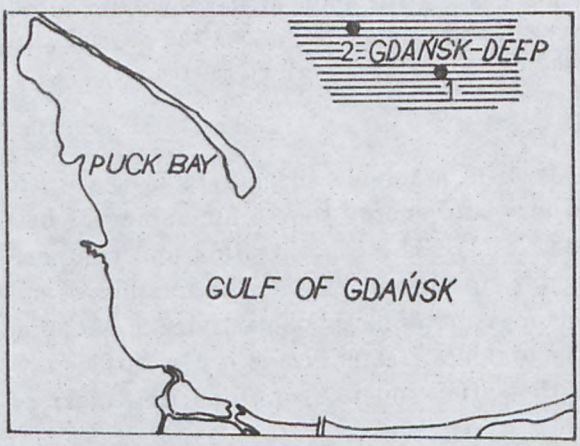

Fig. 1. Location of sampling stations

microlayer was sampled with a sterile Garret's net, while subsurface water was drawn directly into sterile glass bottles from a depth of 0.15 m. The samples were transported in ice-containing containers to the laboratory and subjected to bacteriological analysis. The number of heterotrophic bacteria in the two water layers was determined by the spread plate method in five parallel replicates, using the ZoBell 2216E medium (Rheinheimer, 1977). After ten-day incubation at 15°C the bacteria colonies were counted and recalculated per 1 cm^2 of water. Then, 35 colonies of each bacteria were split off the entire plate or a certain part of it, and transferred to a semisolid ZoBell 2216E medium. After examining their purity the strains were stored at 4°C and grafted to fresh media every 3 months in order to keep them for further investigations.

To determine the physiological activity of the isolated strains they were spread over a series of test media. The ability of the bacteria to perform the following biochemical processes was examined:

1. Ammonification investigated in a medium according to Katznelson (1946). The presence of ammonia was detected with Nessler reagent.
2. Formation of H_2S from organic compounds (Rodina, 1968). Hydrogen sulphide was detected by means of strips of filter paper impregnated with 10% Pb (II) acetate and placed over the medium in the test tubes.
3. Acidification of glucosis was investigated in Lochhead and Chase (1943) medium with and addition of 0.04% solution of bromocresol purple. A positive result was indicated by the change of the medium colour from violet to yellow.
4. The ability to hydrolyze fats, proteins and starch was examined in the Jones (1971) medium, containing 10 cm^3 tributyrin, 20.0 g gelatin and 5.0 g soluble starch, respectively. These organisms were classified as lipolytic, around whose colonies clear zones occurred. To visualize the decomposition of proteins the plates were treated with Frazier reagent, while starch hydrolysis was detected with Lugol's solution.
5. Pectin decomposition was determined according to Jayasankar and Graham (1970). After incubation the plate was treated with 1% Cetrimide solution. The appearance of clear zones was treated as a positive result of the test.
6. The ability of the bacteria to hydrolyze cellulose was determined using a medium prepared according to Donderski (1983). Colloidal cellulose was prepared from cellulose powder according to Halliwell (1962). The appearance of clear zones around the colonies, due to decomposition of cellulose, was treated as a positive result.

7. Deoxyribonucleic acid (DNA) decomposition was determined using the medium delivered by the Oxoid company. DNA hydrolysis was detected by treating the plates with 1 M HCl.
8. Ribonucleic acid (RNA) hydrolysis was determined according to the method of Jeffries *et al.* (1957). RNA decomposition was detected as in paragraph 7.

The reaction of all the media was adjusted to pH 7.0–7.6. They were then sterilized at 117°C for 20 min. The 48–72 hour bacteria cultures from the slants of the ZoBell 2216E medium were used as inocula. The results were read after 6-day incubation at 20°C. Hydrolysis of DNA and RNA was determined after 10 days, while pectin and cellulose decomposition was measured after 14 days.

3. Results and discussion

Data on the number of heterotrophic bacteria in the Gdańsk Deep are listed in Table 1. It follows from this data that the number of bacteria in the surface microlayer ranged from 6.5 to $11.8 \cdot 10^3$ per cm^3, and was 1.5–2 times higher than in the subsurface layer. Similar results have been obtained by Apine (1989) for the Gulf of Gdańsk and by Saava (1985) for the coastal waters of the Baltic, where the number of bacterioneouston was 1.5–4 times higher than in the subsurface layer. In addition the investigations carried out by Rheinheimer (1984) in the Kiel Bight prove that the number of saprophytic bacteria in the surface microlayer is twice as high as that determined for the depth of 1 m. A series of other investigations confirms that in general the number of bacteria in aqueous basins decreases with depth (Hardy, 1982; Kjelleberg *et al.*, 1979; Novitsky, 1983). Probably the high concentration of organic matter in the surface water layers creates optimal conditions for the bacteria growth. Hence, numerous bacterial cells inhabit the surface microlayer by adhesion. Stable adhesion depends on the amount of mucopeptides, glycoproteins and lecithin polymers occurring in the bacterial cell wall (Marshall, 1985; Zaidi *et al.*, 1984). These compounds

Table 1. Number of heterotrophic bacteria isolated on ZoBell 2216E medium (bacteria $10^3 \cdot cm^{-3}$)

Date of sampling	Sampling station	Surface microlayer	Chromogenic bacteria in %	Subsurface water	Chromogenic bacteria in %
1989.05.25	1	8.25	52.1	4.70	38.3
	2	6.5	70.8	3.04	44.4
1989.05.26	1	11.8	56.8	8.95	27.9
	2	7.25	64.8	5.04	53.5

are hydrophobic and reveal a great affinity to the surface water microlayer through their polar groups. At the same time numerous bacteria, particularly trichous, can move to the surface microlayer from deeper water layers by chemotaxis and form colonies there (Dählback, 1983).

The occurrence of a large number of bacteria draws attention in sea water. According to ZoBell (1946) over 50% of marine bacteria are chromogens. Also the observations by Rheinheimer (1987) revealed that chromogens constitute a large percentage of the microflora of the North and the Baltic Seas. In our investigations chromogens in the surface microlayer consituted 50%–70% of all the examined bacteria and their amount was higher by 20% than at the depth of 0.5 m.

Seas are inhabited by microorganisms capable of performing numerous biochemical-physiological processes with various activity. The level of this activity is a function of physico-chemical and biological gradients characterizing the given basin.

It follows from our investigations that organisms performing deamination of amino acids were the most numerous physiological group of bacteria both among the bacterioneouston and the bacterioplankton. Also according to ZoBell (1946) the ammonifying bacteria consitute one of the most numerous groups of microorganisms. They play an essential role in the processes of organic nitrogen mineralization in water. Saava (1985) established that the number of ammonifying bacteria in the Baltic is very high and can exceed 1 million in 1 cm^3. Investigations of Bőlter and Rheinheimer (1987) carried out at the western and the central Baltic revealed that the ammonifying bacteria constituted *ca.* 90% of the total examined microflora, which is consistent with the results obtained in this work. Such a large number of bacteria from this physiological group is undoubtedly related to the high concentration of free and combined amino acids, sometimes exceeding 800 $nM \cdot dm^{-3}$ (Dawson and Pritchard, 1978). Amino acids are taken up very vigorously by the heterotrophic bacteria, which is indicated by the short time of turnover of these compounds (Billen *et al.*, 1980).

Lipolytic organisms constituted *ca.* 70% of the bacteria isolated from the surface microlayer. Investigations carried out in other regions of the Baltic also confirm the presence of a large number of lipolytic bacteria. In the eastern part of the Baltic the number of lipolytic bacteria was *ca.* 100.000 cm^3, while in the western and central part these organisms constituted 94% of the microflora (Bőlter and Rheinheimer, 1987; Saava, 1985). Lipolytic bacteria are also very numerous in other sea basins. In the North Sea 41% of the strains hydrolyzed fats, in the Beaufort Sea 80%–85%, while in the Atlantic Ocean as much as 95% of the microflora actively synthesized lipases (Boeye *et al.*, 1975; Kaneko *et al.*, 1978; Sieburth, 1971).

Kjelleberg and Hakansson (1977) explain this phenomenon by the fact that numerous fatty compounds, like triglycerides, phospholipids, lipoproteins, free fatty acids, waxy esters and glycerol, accumulate at the water surface in an emulsified form and stimulate the optimal conditions of lipolytic bacteria growth. Decaying remnants of phyto- and zooplankton are the main source of these compounds. Particularly large amounts of fat are accumulated in the cells of blue-green algae, brown algae and green algae, as well as Copepoda in the case of zooplankton (Jacobsen and Azam, 1984; Whittle, 1977). The number of lipolytic bacteria in the subsurface water of the Gdańsk Deep was 3.5 times smaller than in the surface microlayer. Kim (after Rheinheimer, 1987) established that the number of lipolytic bacteria in the bacterioneouston of the Kiel Fiord was as much as 6 times higher than at 1 m. Also, in the investigations by Kjelleberg *et al.* (1979), carried out on the Swedish coast, the number of lipolytic bacteria in the surface microlayer was 2–45 times higher than in the subsurface layer.

Proteolytic organisms also occurred in great number in the water of the Gdańsk Deep. More than half of the bacteria isolated from the two water layers was capable of hydrolytic protein decomposition. It was established in the investigations carried out at the western and central Baltic that 75% of the bacteria hydrolyzed gelatin; up to 17,000 bacteria from this physiological group per 1 cm^3 was recorded in the Gulf of Finland (Bőlter and Rheinheimer, 1987; Saava, 1985). According to Little *et al.* (1979), the occurrence of a large number of proteolytic bacteria is related to the fact that apart from carbohydrates, the main components of the organic matter in aqueous basins are proteins, polypeptides and amino acids. They can constitute over 50% of dry organic matter.

Amylolytic bacteria were quite numerous, particularly among the surface microlayer organisms. In his investigations in the Kiel Bight, Mayer-Reil (1983) observed active decomposition of carbohydrates by the bacteria, mediated mainly by α-amylases. Bacteria from this physiological group occur in, high concentration, in other marine basins. In the Irish Sea and in the Indian Ocean they constitute 30%, while in the Aral Sea even 58% of the isolated strains (Johnson *et al.*, 1968; Litchfield and Floodgate, 1975; Novozhilova, 1973). It follows from the data presented in Figure 2 that starch-hydrolyzing bacteria were twice as numerous in the surface microlayer as at a depth of 0.5 m. Similar results have been obtained by Kim (after Rheinheimer, 1987) in the Kiel Bight.

The number of glucose acidifying bacteria among the bacterioneouston and bacterioplantkon was identical. They constituted 37% of the total examined microflora. Bőlter and Rheinheimer (1987) found the number of these bacteria twice as high in the investigations carried out in the western

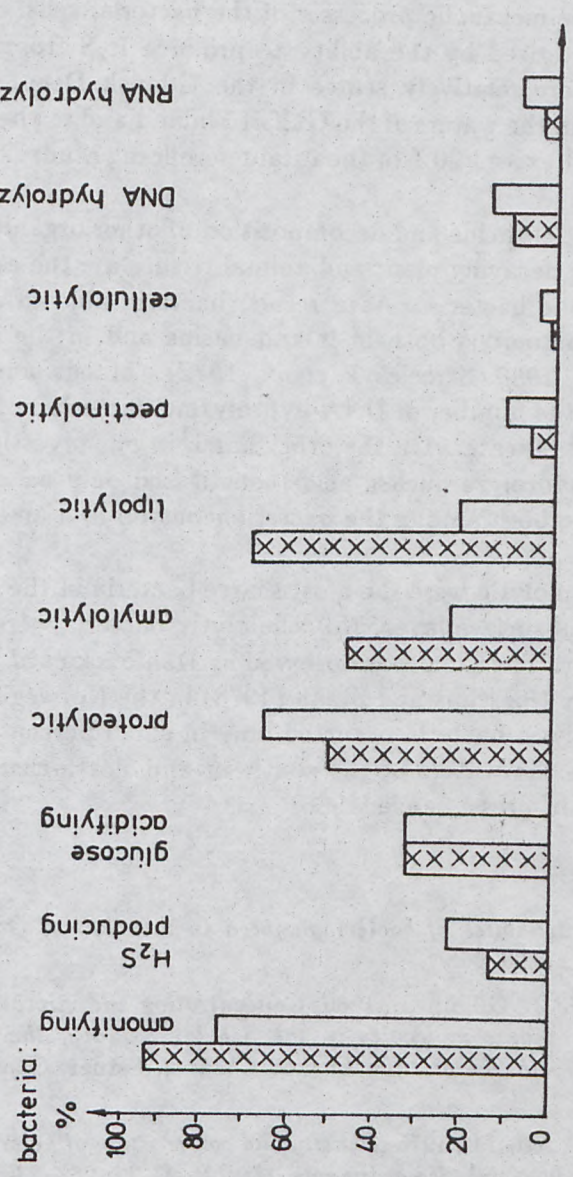

Fig. 2. Physiological groups among bacteria isolated from surface microlayer and subsurface seawater of the Gdańsk Deep (average of two stations)
⊠ surface microlayer, □ subsurface water

and central Baltic. Gocke (1975) reports that 75% – 90% of glucose dissolved in water of Kiel Bight was actively assimilated by the bacteria. 30% of the glucose assimilated was consumed in respiratory processes, the remaining 70% being utilized in the metabolic processes of the bacterial cells.

The bacteria characterized by the ability to produce H_2S from partly decomposed proteins were relatively scarce in the Gdańsk Deep. Their number were small also in the waters of the Gulf of Finland and in the Baltic coastal zone, and did not exceed 20% of the total microflora (Mudryk, 1989; Väätänen, 1980).

Mineralization of nucleic acids and decomposition of other organic phosphorus compounds from decaying plant and animal tissues are the essential functions of heterotrophic bacteria. As a result, bacteria able to decompose nucleic acids are numerous both in inland basins and in the marine environment (Niewolak, 1980; Strzelczyk *et al.*, 1972). Maeda and Taga (1974) established that the number of DNA-hydrolyzing bacteria in the Pacific reached 100.00 cm^3 of water. On the other hand, in our investigations the organisms able to hydrolyze nucleic acids constituted only *ca.* 10% of all the examined strains, both among the bacterioneouston and among the bacterioplankton.

Pectinolytic and cellulolytic were the most scarce bacteria in the surface microlayer and in the subsurface layer. No cellulolytic bacteria were found in the surface microlayer. The same was observed by Dahlbäck *et al.* (1982) in the Arctic Sea and by Norkrans and Stehn (1978) in the Norwegian Sea. Pectinolytic and cellulolytic bacteria occurred only in small percentages in the Bight Fiord and in the waters of the southern and north-east Baltic (Bölter, 1977; Mudryk, in press; Saava, 1985).

References

Apine S., 1989, *Diurnal dynamics of bacterioplankton in the Gulf of Gdańsk in spring*, Oceanologia, 27, 93–96.

Billen G., Joiris G., Wijant J., Gillain G., 1980, *Concentration and microbiological utilization of small organic molecules in the Scheldt Estuary, the Belgian coastal zone of the North Sea and the English Channel*, Estuar. Coast. Mar. Sci., 11, 279–294.

Boeye A., Wayenbergh M., Arts M., 1975, *Density and composition of heterotrophic bacterial populations in North Sea sediments*, Mar. Biol., 32, 263–270.

Bőlter N., 1977, *Numerical taxonomy and character analysis of saprophytic bacteria isolated from the Kiel Fjord and Kiel Bight*, Ecol. Stud., 25, 148–176.

Bőlter N., Rheinheimer G., 1987, *Numerical analysis of microbial and chemical characters and of saprophytic bacteria from the Baltic Sea*, Bot. Mar., 30, 535–544.

Dahlbäck B., 1983, *Microbial accumulation at interfaces. Marine microorganisms and interfaces*, Univ. of Göteborg, 12–50.

Dahlbäck B., Gunnarsson L., Hermansson M., Kjelleberg S., 1982, *Microbial investigations of surface microlayers, water column, ice and sediment in the Arctic Ocean*, Mar. Biol., 9, 101–109.

Dawson R., Pritchard R., 1978, *The determination of amino acids in seawater using a fluorimetric analyser*, Mar. Chem., 6, 27–40.

Donderski W., 1983, *Tlenowe bakterie heterotroficzne jezior o różnej trofii (Heterotrophic aerobic bacteria of lakes of various trophicity)*, UMK Toruń, 105 pp., (in Polish).

Durbin E., Krawiec R., Samayda T., 1975, *Seasonal studies on the relative importance of different size fractions of phytoplankton in Narragansett Bay (U.S.A.)*, Mar. Biol., 32, 271–287.

Gocke K., 1975, *Untersuchungen über die Aufnahme von gelöster Glukose unter natürlichen Verhältnissen durch grössenfraktionierts Nano und Ultrananoplankton*, Kieler Meeresforsch., 31, 87–94.

Hardy J., 1982, *The sea surface microlayer: biology, chemistry and anthropogenic enrichment*, Prog. Oceanogr., 11, 307–328.

Halliwell C., 1962, *Methods of enzymatic analysis.* [In:] *Cellulox*, Bergmeyer H. V. (Ed.), Academic Press, New York – London, 64–71.

Iturriaga R., Hoppe H. G., 1977, *Observations of heterotrophic activity on photoassimilated organic matter*, Mar. Biol., 40, 101–108.

Jacobsen T., Azam F., 1984, *Role of bacteria in copepod faecal pellet decomposition. Colonization, growth rates and mineralization*, Bull. Mar. Sci., 35, 495–502.

Jayasankar N., Graham P., 1970, *An agar plate method for screening and enumerating pectinolytic microorganisms*, Can. J. Microbiol., 16, 1023 p.

Jeffries Ch., Holtman D., Gure D., 1957, *Rapid method for determining the activity of microorganisms an nucleic acids*, J. Bacteriol., 73, 590–596.

Johnson R., Schwent R., Press W., 1968, *The characteristic and distribution of marine bacteria isolated from the Indian Ocean*, Limnol. Oceanogr., 84, 656–664.

Jones J., 1971, *Studies on freshwater bacteria: factors which influence the population and its activity*, J. Ecol., 59, 593–613.

Kaneko T., Hauxhurst J., Krichowsky N., Atlas R., 1978, *Numerical taxonomic studies of bacteria isolated from arctic and subarctic marine environments.* [In:] Lautit M.W., Miles J.A.R. (Eds.), *Microbial Ecology*, Springer Verlag, Berlin – Heidelberg – New York, 26–30.

Katznelson H., 1946, *The rhizosphere effect of mangles on certain of soil microorganisms*, Soil Sci., 5, 343 p.

Kjelleberg S., Hakansson N., 1977, *Distribution of lipolytic, proteolytic and amylolytic marine bacteria between the film and subsurface water*, Mar. Biol., 39, 103–109.

Kjelleberg S., Stenström T., Odham G., 1979, *Comparative study of different hydrophobic devices for sampling lipid surface films and adherent microorganisms*, Mar. Biol., 53, 21–25.

Liss P., 1975, *Chemistry of the sea surface microlayer*, Chem. Oceanogr., 2, 193–243.

Litchfield C., Floodgate G., 1975, *Biochemistry and microbiology of some Irish Sea sediments. II. Bacteriological analysis*, Mar. Biol., 30, 97–103.

Little J., Sjogren R., Carson G., 1979, *Measurement of proteolysis in natural waters*, Appl. Microbiol., 37, 900–908.

Lochhead A., Chase F., 1943, *Qualitative studies of soil microorganisms. V. Nutritional requirements of the predominant bacterial flora*, Soil Sci., 55, 185.

Maeda M., Taga N., 1974, *Occurrence and distribution of deoxyribonucleic acid hydrolyzing bacteria in sea water*, J. Biol. Ecol., 14, 157–169.

Marshall K., 1985, *Bacterial adhesion in oligotrophic habitas*, Microbiol. Sci., 2, 321–326.

Mayer-Reil L., 1983, *Benthic response to sedimentation events during autumn to spring in a shallow water station in the western Kiel Bight. II. Analysis of benthic bacteria populations*, Mar. Biol., 77, 247–256.

Mudryk Z., 1989, *Badania bakteriologiczne w strefie kontaktowej estuariowego jeziora Gardno z Morzem Bałtyckim (Bacteriological investigations in the contact zone between the estuarine Gardno Lake and the Baltic Sea)*, Kom. Mikrobiol. PAN, Gdańsk, 67–83, (in Polish).

Mudryk Z., (in press), *Badania heterotroficznych bakterii planktonowych wyizolowanych z przybrzeżnej strefy Morza Bałtyckiego (Investigations on heterotrophic plankton bacteria isolated from the coastal zone of the Baltic)*, Słupskie Prace Mat.-Przyr., (in Polish).

Niewolak S., 1980, *Occurrence of microorganisms in fertilized lakes. I. Ribonucleic acid-hydrolyzing microorganisms*, Pol. Arch. Hydrobiol., 27, 37–52.

Norkrans B., 1980, *Surface microlayers in aquatic environments*, Adv. Microbiol. Ecol., 4, 51–85.

Norkrans B., Stehn B., 1978, *Sediment bacteria in the deep Norwegian Sea*, Mar. Biol., 47, 201–209.

Novitsky J., 1983, *Heterotrophic activity throughout a vertical profile of seawater and sediment in Halifax Harbour*, Appl. Environ. Microbiol., 45, 1753–1760.

Novozhilova M., 1973, *Mikrobiologiya Aralskovo morya*, Nauka, Ałma-Ata, 160 pp., (in Russien).

Rheinheimer G., 1977, *Microbial ecology of a brackish water environment*, Ecological Studies, 25, Heidelberg – Berlin – New York – Springer, 291 pp.

Rheinheimer G., 1984, *Bacterial ecology of the North and Baltic Seas*, Bot. Mar., 27, 277–299.

Rheinheimer G., 1987, *Mikrobiologia wód (Microbiology of water)*, PWRiL, Warszawa, 327 pp. (in Polish).

Rodina A., 1968, *Mikrobiologiczne metody badania wód (Methods of microbiological water examination)*, PWRiL, Warszawa, 443 pp.

Saava A., 1985, *First periodic assessment of the state of the marine environment of the Baltic Sea area. The background document for the first periodic assessment.* [In:] *Baltic Marine Environment Protection Comission*, Helsinki, Rostock – Warneműnde, 76–83.

Seki H., 1982, *Monitoring of eutrophication by microbial uptake kinetics of dissolved organic matter in water*, Environ. Monit. Assessment, 2, 387–391.

Sieburth J., 1971, *Distribution and activity of oceanic bacteria*, Deep Sea Res., 18, 1111–1121.

Sieburth J., 1976, *Bacterial substrates and productivity in marine ecosystems*, Ann. Rev. Ecol. Syst., 7, 259–285.

Sieburth J., 1979, *Sea microbes*, Oxford Univ. Press, New York, 491 pp.

Strzelczyk E., Donderski W., Lewosz W., 1972, *Occurrence of microorganisms capable of decomposing organisms phosphorus compounds in two types of bottom sediments of the eutrophic lake Jeziorak*, Acta Microbiol. Pol., 4, 101–110.

Väätänen P., 1980, *Effects of environmental factors on microbial populations in brackish waters of the southern coast of Finland*, Appl. Environ. Microbiol., 40, 48–54.

Whittle K., 1977, *Marine organisms and their contribution to organic matter in the ocean*, Mar. Chem., 5, 381–411.

Zaidi B., Bard R., Tosteson T., 1984, *Microbial specificity of metallic surfaces exposed to ambient seawater*, Appl. Environ. Microbiol., 48, 519–524.

ZoBell C., 1946, *Marine microbiology. A monograph on hydrobacteriology*, Chronica Botanica Co., Waltham, Mass. U.S.A., 240 pp.