*Polish Journal of Environmental Studies Vol. 6, No. 4 (1997), 23–28*

# Studies on Metabolic Activity of Planktonic Bacteria Isolated from Coastal Lake Łebsko

**Z. Mudryk**

Department of Experimental Biology, University of Education,
Arciszewskiego 22, 76-200 Słupsk, Poland

*Received 5 February, 1997*
*Accepted 4 April, 1997*

**Abstract**

Metabolic activity of heterotrophic bacteria isolated from coastal lake Łebsko was determined using the respirometric method. It has been shown that planktonic bacteria oxidized organic compounds with various intensity. Caseine hydrolyzate was the most intensively oxidized respiratory substrate, whereas cellobiose was oxidized the least intensively. In the studied lake, oxygen was taken up most actively by bacteria of the *Flavobacterium-Cytophaga* group and the genus *Pseudomonas* in their respiratory processes. The level of metabolic activity of bacteria from different parts of the lake was shown to differ. The oxygen uptake of bacterial mixtures was, as a rule, lower than the sum of oxygen uptake of single strains.

**Keywords:** lake Łebsko, planktonic bacteria, metabolic activity

## Introduction

In aquatic ecosystems, heterotrophic bacteria which utilize organic matter as the basic source of energy for their life processes constitute a dominant group of microorganisms [8, 10]. In water bodies this matter occurs as dissolved organic matter (DOM) and particulate organic matter (POM). Excretions and remains of dead fitoplankton and zooplankton constitute the main source of DOM and POM [2]. Because of a great variety of quantitative and qualitative composition of organic matter in aquatic ecosystems, many authors have drawn attention to significant differences in the trophic demands of bacteria, and in the level of their metabolitic activity [7, 29].

The level of metabolitic activity of bacteriocenoses is an indicator which well reflects intensity of the processes related to transformation of organic matter in water bodies [34]. In metabolic processes, heterotrophic bacteria can utilize as a source of energy a wide spectrum of various organic compounds, including monomers as well as polymers after their enzymatic hydrolysis [2, 3]. This is why the intensity of metabolic processes depends mainly on the variety and enzymatic activity of individual physiological groups of bacteria.

While determining the metabolic activity of bacteria is very important for understanding the ecological role of those organisms in aquatic ecosystems, it constitutes one of the major methodical problems. In spite of the fact that a number of various methods for determining metabolic activity of aquatic bacteria are currently used [13, 14, 31], none of them is free from methodical errors which could allow to determine precisely and correctly the level of that activity. In the current study, the respirometric method was applied to investigate the metabolic activity of bacteria. This method, being relatively simple, fast and sufficiently precise, is often used to measure metabolitic activity of bacterial microflora [23, 25, 29].

The aim of the study presented here was to gain information on the potential capability of the microflora of lake Łebsko to destruction organic compounds, which was achieved by determining the metabolitic activity of planktonic bacteria.

## Materials and Methods

### Study Area

The study was conducted at estuarine lake Łebsko, the largest Polish Baltic Sea coastal reservoir (Fig.1). This lake is situated in the Słowiński National Park, a World Biosphere Reserve. It is an extensive water body covering over 7,000 ha, characterized by a very small average depth (1.7 m). Conditions there are intermediate between marine and inland environment. The lake is fed by waters of the river Łeba while being connected with the Baltic Sea by a 2.75 km, narrow

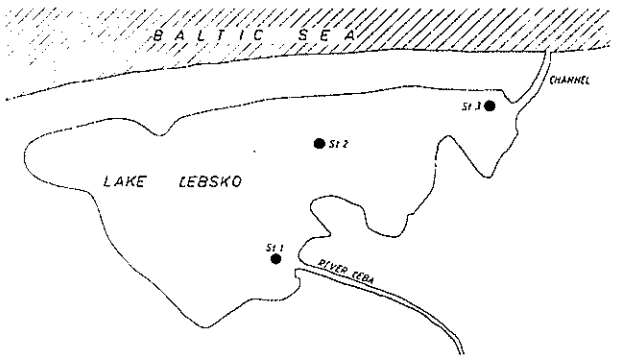



Fig. 1. Map of lake Łebsko with location of sampling sites.

channel. Through this channel water from the sea flows into the lake, causing the water of the lake or of its parts to acquire sea water characteristics, resulting in its 5‰ salinity [30]. According to the Venetian system, this level of water salinity classifies lake Łebsko as a mixo-oligohaline type, i.e. brackish water reservior (0.5-5‰) [6].

The studied estuary is a polymictic water basin of a considerable level of eutrophication. This high trophic level together with a high concentration of nutrients, and the penetration of light to the bottom of the lake create perfect conditions for the development of phytoplankton, whose bloom lasts practically from spring to autumn.

The shore of lake Łebsko is covered with numerous emergent macrophytes, among which *Phragmites australis.*, *Typha angustifolia*, *Scirpus lacustris*, *Schoenoplectus lacustris* predominate. This vegetation forms a belt, sometimes exceeding 100 m, parallel to the shore.

## Sampling

Water samples were collected from lake Łebsko at three sites (Fig. 1). One of the sites (St. 2) was located mid-lake, the other two were set in the area of a tributary of the river Łeba (St. 1), and in the zone of the channel connecting the lake with the sea (St. 3). Water samples were taken directly into sterile glass bottles at depths of 15 - 20 cm, and were transported to the laboratory in ice containers not exceeding $+7^0$C. Time interval between the taking of a sample and its analysis did not exceed 6 hours.

## Experiments

Strains of planktonic bacteria used in the presented study were isolated from water on agar medium prepared according to Jones [17]. Bacteria were indentified on the basis of data suggested by Buchanan and Gibbons [4] and Oliver [22]. The following characteristics are basic for the identification of bacteria: motility, pigment production, morphology, Gram staining, Hugh-Leifson's test, arginine hydrolyzing ability, cytochrome oxidase formation, test API 20.

Metabolic activity of 43 strains of planktonic bacteria was determined by the respirometric method in a Warburg apparatus. Bacteria were multiplied on agar slants (Jones 1971) for 72 h at $26^0$C. Subsequently, they were washed off from the slants with 0.01 M of the phosphate buffer (pH 7.0), centrifuged at 15,000 rpm for 15 minutes and washed twice with the buffer. The washed bacteria were resuspended in the same buffer to 200 Klett units in a Klett-Summerson cololimeter with blue filter (No. 42.), corresponding to $10^9$ bacterial cells per $cm^3$. Casein hydrolyzate (Casamino acids vitamin-free, Difco), glucose, fructose and cellobiose were used as substrates in the study on metabolic activity of bacteria.

Metabolic activity was measured as oxygen uptake in a Warburg apparatus. To each reaction vessel two or one $cm^3$ (depending on experiments with single or mixed cultures) of individual cell suspensions were added; 0.3 $cm^3$ of 20% KOH was placed in the center well together with a fluted filter paper; the side-arm contained 1 $cm^3$ of substrate (10$\mu$M) dissolved in 0.01 M of phosphate buffer of pH 7.0. In the case of casein hydrolyzate 5.0 mg of these substrates was used for each vessel. The gas phase was air, the temperature was maintained at $26^0$C. The reaction vessels were allowed to reach equilibrium (15 minutes), and substrate was tipped into the main chamber. The oxygen uptake was measured for 2 houres while the vessel was shaken at 120 oscillations per minute. The rate of endogenous respiration was substracted, and the uptake converted to $\mu l\ O_2 \cdot h^{-1}$ per $10^9$ cells.

## Results

Results of the study on the metabolic activity of planktonic bacteria isolated from lake Łebsko are presented in Fig. 2. On the basis of those data it has been determined that casein hydrolyzate was the most actively utilized respiratory substrate. The amino acid mixture constituting casein hydrolysate was utilized in oxidation processes by over 80% of the studied strains. Considerably lower was the use of oxygen in the bacterial respiratory processes in the presence of glucose and fructose. The planktonic bacteria of lake Łebsko utilized cellobiose as exogenic respiratory substrate with the lowest intensity.

From the data presented in Fig. 3 it can be concluded that the intensity of assimilation of respiratory substrates by planktonic bacteria inhabiting various parts of the lake was different. Although in all study sites caseine hydrolyzate was an actively utilized respiratory substrate, it was utilized with the greatest intensity by the bacteria inhabiting the mid-lake and seawa-

ter zone (St. 2 and St. 3). Apart from caseine hydrolyzate, bacteria relatively actively in their metabolic processes utilized fructose (St. 1 and St. 2) and glucose (St. 3). At all three study sites, cellobiose as a respiratory substrate was utilized with a low activity.

From the data presented in Table 1 it can be concluded that in the presence of the substrates used in this study, bacteria of the genus *Pseudomonas* and of the *Flavobacterium -Cytophaga* group were metabolicaly the most active. Those bacteria utilized as a source of energy all the respiratory substrates used in this study, whereas bacteria of the family *Enterobacteriaceae* were able to utilize only caseine hydrolyzate. In the presence of amino acid mixture (caseine hydrolysate) bacteria of the *Arthrobacter-Corynebacterium* group and of the genus *Acinetobacter* were the most active, whereas in the presence of monosaccharides the most active were bacteria of the *Areomonas-Vibrio* group. Apart from bacteria of the genus *Pseudomonas* and of the *Flavobacterium- Cytophaga* group, only bacteria of the genus *Acinetobacter* utilized cellobiose as an exogenous respiratory substrate.

Fig. 4 presents the results of the study concerning metabolic activity of planktonic bacteria and their one- and two-genera mixtures determined in the presence of caseine hydrolyzate, the substrate most actively utilized by bacteria in their respiratory processes. Those data show that respiratory activity of bacterial mixtures was as a rule considerably lower than the sum of activities of the strains making up those mixtures. It has also been determined that the intensity of oxygen uptake by bacterial mixtures comprising strains of various genera was higher than by one-genera mixtures. Among heterogenous mixtures, cases of complete lack of respiratory activity were noted.

## Discussion

In water bodies, development of bacteria is limited mainly by the accessibility of nutritional substrate [28]. According to Niewolak [21] and Gossens et al. [12], heterotrophic bacteria respirate 40-80% of the substrate taken up, the remaining part transforming into their own biomass. Zmysłowska [34] draws attention to the fact that in studies concerning determination of metabolic activity of bacteria it is important to use properly selected respiratory substrates. In the study presented here in order to determine metabolic activity of bacteria, caseine hydrolysate and carbohydrates in the form of monosaccharides (glucose, fructose) and polysaccharides (cellobiose) were used as respiratory substrates. Caseine hydrolyzate, a mixture of various amino acids, was chosen because in many water bodies amino acids occur in often high concentrations, and are actively taken up by bacteria [20, 24]. Glucose and fructose were used because of the fact that of all soluble monosaccharides occurring in aquatic ecosystems they reach the highest concentrations and are actively assimilated by bacterial microflora [3]. On the other hand, cellobiose, which is more difficult to assimilate by bacteria, was used in this study for comparative reasons, as it is utilized only by specific physiological groups of bacteria [10].

The results of this study indicate that populations of coastal lake Łebsko assimilated organic compounds with various intensity. Of all respiratory substrates used in this study, caseine hydrolyzate was metabolized most actively. Active utilization of this amino acid mixture by bacteria was also determined in coastal sea waters [33], estuaries [19], rivers [23], and lakes [8, 29]. The ability to actively assimilate amino acids is a common characteristic in bacterial populations. Those low molecular weight organic compounds are a universal substrate preferred by heterotrophic bacteria as a source of carbon, nitrogen, and energy [27].

In spite of the fact that the results of many studies [8, 11, 26, 28] indicate that glucose and fructose are actively assimilated by bacteria as respiratory substrates, those sugars were utilized considerably less intensively than an amino acid mixture in respiratory processes by the microflora of lake Łebsko. Those data

Table1. Metabolic activity of different genera bacteria isolated from lake Łebsko

| Bacteria | Number of strains studied | Oxygen uptake in $\mu l\ O_2.h^{-1}$ (average) | | | |
|---|---|---|---|---|---|
| | | substrates | | | |
| | | caseine | glucose | fructose | cellobiose |
| *Aeromonas - Vibrio* | 10 | 66.72 | 15.49 | 25.12 | 0.00 |
| *Arthrobacter - Corynebacterium* | 5 | 225.51 | 13.41 | 4.59 | 0.00 |
| *Acinetobacter* | 5 | 143.05 | 0.00 | 0.00 | 15.21 |
| *Enterobacteriaceae* | 2 | 28.62 | 0.00 | 0.00 | 0.00 |
| *Flavobacterium - Cytophaga* | 11 | 68.79 | 6.45 | 23.10 | 8.89 |
| *Pseudomonas* | 7 | 89.62 | 1.31 | 0.58 | 15.01 |
| *Bacillus* | 1 | 3.34 | 0.00 | 0.00 | 0.00 |
| *Alcaligenes* | 1 | 86.30 | 0.00 | 16.53 | 0.00 |

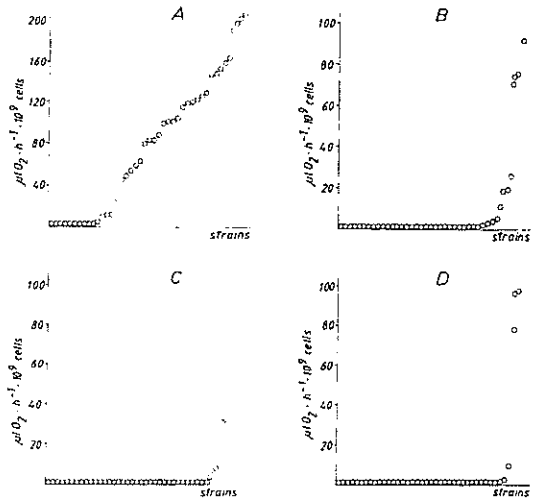



Fig.2. Oxygen uptake by bacteria in the presence of casamino acids (A), fructose (B), glucose (C) and cellobiose (D).

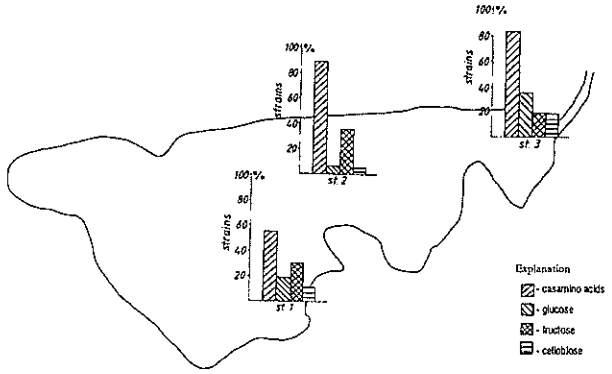



Fig. 3. Percent respiring strains of bacteria in various parts of lake Łebsko

are consistent with the results of Hoppe [15] and Mudryk [19], which determined that bacteria of brackish waters were characterized by considerably higher metabolic activity in relation to amino acids than to monosaccharides.

Cellobiose as a product of the hydrolysis of cellulose was the least suitable respiratory substrate for the bacteria inhabiting lake Łebsko. These data fully correspond with the results obtained by Godlewska-Lipowa [10] for Mazurian lakes, and by Strzelczyk et al. [29] for lakes Tynwał, Jasne, and Jeziorak.

The data presented in this paper show that the intensity of assimilating respiratory substrates by bacteria inhabiting different parts of lake Łebsko was different. Salinity changes in this water ecosystem might have been a significant factor affecting those differences. According to Palumbo et al. [24] and Griffiths et al. [13], salinity changes can influence directly the utilization of amino acids and monosaccharides by bacteria. The activity of accumulating carbohydrates by heterotrophic bacteria in the Fraser River estuary [1] and amino acids in estuarine lake Jamno [20] was increasing with the increasing salinity of water. The

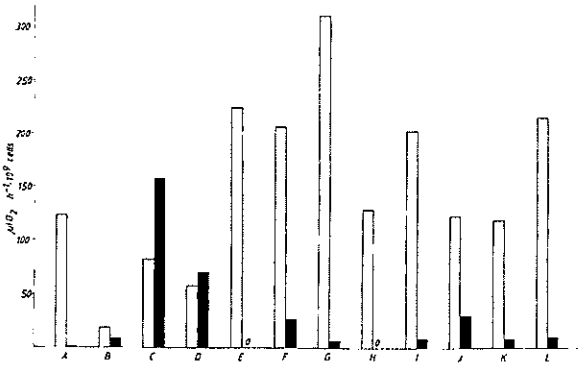



Fig.4. Respiratory activity of single strains bacteria and their one - and two-genera mixtures in the presence of casamino acids

Explanations:
□ – sum of oxygen uptake by single strains
■ – oxygen uptake by bacterial mixtures

A - *Flavobacterium* + *Flavobacterium*
B - *Aeromonas* + *Aeromonas*
C - *Pseudomonas* + *Pseudomonas*
D - *Enterobacteriaceae* + *Enterobacteriaceae*
E - *Acinetobacter* + *Pseudomonas*
F - *Arthrobacter* + *Areomonas*
G - *Arthrobacter* + *Acinetobacter*
H - *Flavobacterium* + *Areomonas*
I - *Flavobacterium* + *Arthrobacter*
J - *Pseudomonas* + *Aeromonas*
K - *Pseudomonas* + *Enterobacteriaceae*
L - *Aeromonas* + *Acinetobacter*

results of the current study also confirm this tendency in relation to amino acids, as caseine hydrolyzate was more actively utilized by bacteria inhabiting the brackish (St. 2 and St. 3) than the freshwater (St. 1) part of the estuarine lake Łebsko.

Studies on metabolic activity of one- and two-genera mixtures of planktonic bacteria show that, as a rule, the uptake of oxygen by combined strains was lower than the sum of oxygen taken up by individual strains. According to Strzelczyk et al. [29], a decrease in the respiratory activity of bacterial mixtures could result from the fact that in a mixture a double number of bacteria were competing for the same amount of substrate as in the case of a single bacterial culture. Additionally, respiratory substrates as organic compounds could have been incorporated by bacteria into their cell structures instead of being oxidized. Also, metabolites of the bacterial cells themselves can block the uptake of respiratory substrates. Lemos et al. [18] draw attention to the possibility of inhibition of the respiratory activity of bacteria by substances of bacteriocine character, which are secreted by certain groups of microorganisms. Bacteriocines have a narrow spectrum of activity, and are active only against organisms competing for nutrients with bacteria synthesizing bacteriocines [16].

Wiliams and Vickers [32] suggest that in the case of homogenous as well as heterogenous bacterial mixtures, antagonistic relationships connected with the production of compounds of antibiotic character can occur. They are usually polypeptides linked to organic or fatty acids [9]. As has been shown by Lemos et al. [18], the antagonistic effect of antibiotics between individual groups of bacteria is a phenomenon often occurring among bacteria inhabiting salty and brackish water bodies. That is why the possibility of this phenomenon taking place in the case of the brackish lake Łebsko cannot be excluded.

Chodyniecki [5], while studying various combinations of bacteria of the genus *Pseudomonas*, *Aeromonas* , *Vibrio,* and *Flavobacterium,* determined that 12% of interactions between strains of those bacteria were antagonistic in character, whereas 8% were stimulating. In mixtures of bacteria from the coastal lake Łebsko antagonistic interactions were definitely more pronounced than stimulating ones.

The author of the presented study is fully aware of the fact that the respirometric method applied here allowed to measure only the potential metabolic activity of bacteria, which never is identical with that in the natural environment. In the laboratory where the study of the metabolic activity was conducted, it was not possible to include nor to monitor changes in short time intervals and in a wide range environmental factors that affect the functioning of bacteria in *in situ* conditions. The obtained data and their ecological interpretation can, however, be a significant source of information on the role of bacterial microflora in the processes of degradation of organic matter, and in maintaining the homeostasis of the costal lake Łebsko.

## References


1. BELL C. R., ALBRIGHT L. J. Attached and free-floating bacteria in the Fraser River estuary, British Columbia, Canada. Mar. Ecol. Prog. Ser. **6**, 317, **1981.**
2. BILLEN G., FONTIGNY A. Dynamics of phaeocystis-dominant spring bloom in Belgian coastal waters. II. Bacterioplankton dynamics. Mar. Ecol. Prog. Ser. **37**, 249, **1987.**
3. BÖLTER M. Submodels of a brackish water environment II. Remineralization rates of carbohydrates and oxygen consumption by pelagic microheterotrophs. Mar. Ecol.Prog. Ser. **3**, 233, **1982.**
4. BUCHANAN R. E., GIBBONS N. E. *Bergey's manual of determinative bacteriology.* Baltimore, The Williams and Wilkins Comp. pp 1268, **1974.**
5. CHODYNIECKI A. Antybioza i symbioza wśród bakterii wodnych. Ed. WSR Szczecin. **1968.**
6. DETHIER M. N. Classifying marine and estuarine natural communities: An alternative to the coword in system. J. Nat. Areas. 12, 90-100, **1992.**
7. DONDERSKI W. Tlenowe bakterie hetrotroficzne jezior o róznej trofii. Ed. UMK Toruń pp 147, **1983.**
8. DONDERSKI W., STRZELCZYK E. Metabolic activity of planktonic bacteria in three lakes of different trophy. Bull. Acad. Polon. Sci. 28, 39, **1980.**
9. GAUTHIER M. J. FLATAU G. N. Antibacterial activity of marine violet-pigment *Altromonas* with special reference to the production of brominated compounds. Can. J. Microbiol. 22, 1612, **1976.**
10. GODLEWSKA-LIPOWA W. Heterotrophic activity of bacterial microflora in Mazurian lakes of various trophy. Pol. Arch. Hydrobiol. 21, 51, **1974.**
11. GODLEWSKA-LIPOWA W. $O_2$-consumption as an indicator of heterotrophic activity of bacteria in lakes of different trophic conditions. Arch. Hydrobiol. Beih. 12, **11**, **1979.**
12. GOSSENS H., MINNAAR R. S., VERPLANKE H. Carbon mineralization in the water of lake Grevelingen as measured with the oxygen consumption method. Neth. J. Res. 18, 480, **1984.**
13. GRIFFITHS R. P., CADWELL B. A., MORITA R. Y. Observations on microbial percent respiration values in Arctic and Subarctic waters and sediments. Microb. Ecol 10, 151, **1984.**
14. GUNDERSEN J. K., JORGENSEN B. B. Fine scale in situ measurements of oxygen distribution in marine sediments. Kiel Meer. Sond. 8, 376, **1991.**
15. HOPPE H. G. Analysis of actively metabolizing bacterial populations with the autoradiographic method. Ecol. Stud. **25**, 179-196, **1977.**
16. HOYT P. R., SIZEMORE R. L. Competitive dominance by bacteriocidin-producing *Vibrio harveyi* strain. Appl. Environm. Microbiol. **44**, 653, **1982.**
17. JONES J. G. Studies on freshwater bacteria factors which influence the population and its activity. J. Ecol. **59**, 593, **1971.**
18. LEMOS M. L., DOPAZO C. P., TORANZO A. E., BARJA J. L. Competitive dominance of antibiotic-producing marine bacteria in mixed cultures. J. Appl. Bacteriol. **71**, 228, **1991.**
19. MUDRYK Z. Determination of the metabolic activity of heterotrophic bacteria isolated from estuarine Gardno lake. Pol. Arch. Hydrobiol. **36**, 97, **1989.**
20. MUDRYK Z. Bakterie heterotroficzne w procesach transformacji materii organicznej w jeziorach estuariowych . Ed. WSP Słupsk, pp. 175, **1994.**
21. NIEWOLAK S. Production of bacterial biomass in the water of the Iława Lakes. Acta Hydrobiol. **16**, 101, **1974.**
22. OLIVER J. D. Taxonomic scheme for the identification of marine bacteria. Deep-Sea. Res. **29**, 795. **1982.**
23. OSSOWSKA-CYPRYK K., Microbiological studies of waters and bottom sediments of Utrata river. Pol. Arch. Hydrobiol. **28**, 357, **1981.**
24. PALUMBO A. V., FERGUSON R. L., RUBLEE P. A. Efficient utilization of dissolved free amino acids by suspended marine bacteria. J. Exp. Mar. Biol. Ecol. **69**, 257, **1983.**
25. PARKINSON D., COLEMAN D. C. Microbial communities, activity and biomass. Agro. Ecos. Environm. **34**, 3, **1991.**
26. RHEINHEIMER G. Bacterial ecology of the North and Baltic Seas. Bot. Mar. **27**, 277, **1984.**
27. SIMON M. Isotope dilution of intracellular amino acids as a tracer of carbon and nitrogen sources of marine planktonic bacteria. Mar. Ecol. Prog. Ser. **14**, 295, **1991.**
28. STRZELCZYK E., LENIARSKA U., DONDERSKI W. Studies on metabolic activity of benthic bacteria isolated from three lakes. Acta Microbiol. Polon. **6**, 125, **1974.**
29. STRZELCZYK E., STOPIŃSKI M., MYZYK G. Sudies on metabolic activity of single and mixed cultures of planktonic and benthic bacteria of two lakes of different trophy. AUNC, Toruń, Limnol. Papers. **16**, 3, **1988.**

30. SZMIDT K. Hydrologia jezior przybałtyckich ze szczególnym uwzględnieniem jeziora Jamno. Stud. Mat. Oceanol. 3, 21-34, **1972.**
31. VOSJAN J. H., NIEUWLAND G., RUYITNO E. Monsoonal differences in microbial biomass, respiratory activity and bacterial numbers in the Banda Sea. Neth. J. Sea Res. **25**, 501, **1990.**
32. WILLIAMS S. T., VICKERS J. C. The ecology of antibiotic production. Microbiol. Ecol. **12**, 43, **1986.**
33. WIRSEN C. O., JANNASCH H. W. Microbial transformations of some $^{14}C$ labeled substrates in coastal water and sediment. Microbiol. Ecol. **1**, 25, **1974.**
34. ZMYSŁOWSKA I. Wpływ usuwania wód hypolimnionu na mikroflorę bakteryjną Jeziora Kortowskiego. Ed. ART Olsztyn pp 63, **1987.**