Trends in the development of ecological microbiology

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1 Introduction .......................................................... 1
2 Ecological niches .................................................... 5
3 The ecology of photosynthesis ...................................... 14
   3.1 Ecology of phytoplankton photosynthesis .................. 14
   3.2 Ecology of bacterial photosynthesis ....................... 19
4 Intensity of bacterial reproduction ................................ 22
5 Intensity of sulphate reduction and chemosynthesis in lakes 26
   5.1 Current interpretations of the concept of “chemosynthesis” 26
   5.2 Intensity of sulphate reduction in nature ............... 28
   5.3 Intensity of chemosynthetic processes in nature ....... 31
6 Intensity of molecular nitrogen fixation .......................... 34
7 Degradation of organic matter .................................... 38
8 Conclusions .................................................................. 43
   References ............................................................. 45

1 Introduction

Although the concept “ecology” may be variously formulated, the most general definition would apparently describe ecology as a science dealing with the interactions between the organism and its environment.

Some investigators, writes Brock (1966), emphasize the characteristics of the environment while others stress the relationships among the organisms. The former include limnologists, oceanographers and soil scientists, and the latter plant and animal ecologists who are chiefly concerned with relationships existing between the organisms themselves, and who take into account the environment in as much as it
enables them to account for the distribution of individual species of organisms in nature. As far as ecological microbiology is concerned, it covers both these areas of study, since microorganisms are so intimately linked up with the environment that the latter must be taken into serious consideration at all times. As regards microorganisms themselves, their interrelationships, both symbiotic and parasitic, have been repeatedly demonstrated.

As the development of given microorganisms in nature depends on the chemistry of the environment as well as on their relationships with other organisms, both these parameters may characterize the optimum ecological niche for microbial development. In the course of their development microorganisms in turn affect the environmental chemistry and provide conditions favourable for other species. The elucidation of these relationships constitutes a major interest of microbial ecology.

The origins of ecological microbiology are associated with the ideas of Louis Pasteur concerning the roles of the “infinitely small” in nature, while the foundations of that science were laid by Winogradsky and Beijerinck who considered microorganisms to be closely associated solely with the environment and believed that searches for living organisms should be started after a knowledge of phenomena occurring in nature had been gained.

After Pasteur, the work of Koch and his school greatly contributed to the development of research methods without, however, touching at all on problems concerning the dynamics of biological processes occurring in nature. Koch’s methods were employed to isolate new organisms and to study their morphology and physiology in pure culture, although those studies failed to give a clear idea as to what are microbial activities in nature. Such was the status of ecological microbiology at the beginning of this century.

Thereafter, microbiological studies of natural media developed at a rapid rate and went a long way to approach their goal. Those by Forel, Birge, Juday, Tinemann and others largely elucidated the physico-chemical properties of reservoirs.

Waksman (1927, 1941), ZoBell (1946), Senez (1951) and others have summarized the results of studies of the physiology and distribution of individual bacterial groups in the soil, silt deposits and lake and sea water and have arrived at a number of conclusions regarding the roles of microorganisms in biochemical processes occurring in those environments.
Microbiologists specializing in the field of microbial ecology of course sought to investigate the functions of true microflora in its natural state. In order to understand the responses of microorganisms to changes in physical and chemical properties of the environment, it was necessary to have a pure culture. The nature of pure cultures is, however, such that it precludes serious ecological generalizations.

Indeed, the absence of other organisms and, consequently, the lack of competition for energy and food sources create biologically unnatural conditions. The ecologist is interested in actual, real properties of the microorganism he studies rather than in those which the culture may acquire in an environment far from the natural one and in the absence of struggle for the substrate. Thus, while the study of pure cultures may elucidate its potentialities and point out the direction in which its morphology and physiology may alter, such investigations, which are confined to microbial physiology and biochemistry, cannot elucidate the activities of microflora in nature.

Winogradsky (1947) believed that such activities were made up not of individual processes but rather represented a single self-regulating process. Microbial functions are controlled by the competition for the energy-supplying substance and are limited by the physicochemical conditions of the environment. One cannot but agree with Winogradsky that the study of actual processes carried out by microbes must be based not only on the consideration of individual microbial species but, in the main, on the study of microbial communities as a whole acting directly in their natural environment.

Such an approach does not replace the method of pure cultures, which is still used whenever necessary. However, the study of microbial communities as a whole with due regard to physicochemical properties of the environment should contribute to a better understanding of ecological problems, which cannot be resolved by the classical methods involving microbial counts and physiological studies of pure cultures.

The foregoing also applies to morphological studies of microorganisms: on an artificial medium that is too rich for a given organism, one may observe what may be called hypertrophy of that organism. Morphological properties remain normal only if the organism is cultivated on a "poor" medium closely approaching the naturally occurring substrate.

Recent studies in microbial ecology have been reviewed in Brock's monograph (Brock, 1966) where it is pointed out that efforts in this
field have been concentrated on the following: (1) investigation and
determination of the limits of ecological conditions under which the
organism lives in nature; (2) studies, in pure culture, of the growth
and behaviour of the organism upon a change in individual ingredients
of the nutrient medium; (3) studies of responses of naturally occurring
microbial populations to environmental changes; and (4) morpho-
logical and behavioural studies of organisms placed in those biotopes
from which they had been previously absent. Brock (1966) adduces
several examples to show how a culture isolated from the natural
environment alters its morphology under laboratory conditions.

Experimental studies undertaken with the use of new techniques have
enabled Brock to conclude, after Winogradsky and Beijerink, that pure
culture studies are very important in ecological investigations, although
one must remember at all times that pure cultures may have great
ekological distinctions from natural ones. In other words, the inter-
pretation of laboratory investigations should always be adjusted to take
account of field observations. On the basis of Winogradsky's ideas
and their elaborations, the main principles of ecological microbiology
appear to be as follows:

1. Acquaintance with a given naturally occurring phenomenon, viz.
the determination of the physicochemical conditions in which it occurs
and identification of the microbial groups involved. To put it differ-
ently, it is necessary to characterize the ecological niche for particular
microbial groups.

2. Identification of members of the major microbial groups playing
principal roles in biological processes and the study of the more
important aspects of their physiology and relationships with other
organisms.

3. Determination of their activities in nature in communities with
other species, using a quantitative approach.

The present review is chiefly concerned with the characteristics of
ekological niches for individual microorganisms and with the deter-
mination of intensities of individual microbiological processes in the
course of turnover of various substances in reservoirs. Such a quantita-
tive evaluation of individual processes was largely impossible at the
time of Winogradsky and became a practical proposition only recently
with the advent of new methods of ecological microbiology. To attain
a complete understanding of microbial ecology one must possess a
totality of information on microbial physiology and morphology in conjunction with physical and chemical data on environmental properties.

2 Ecological niches

In nature, the chemical composition and physical properties of water varies over a wide range not only in different water bodies but also within the same lake. Since many microorganisms can grow within a very narrow range of fluctuations of water constituents, their natural development can proceed only in strictly limited biotopes. That is to say, certain "biological niches" are created in the reservoir where individual microbial species encounter minimal competition on the part of other members of the microflora as well as optimal physico-chemical conditions for their growth.

A biological niche cannot be regarded as a certain steady-state system. The organisms contained in it make use of definite substances for their metabolism, transforming them into other substances indifferent for them or inhibiting their development.

For that reason, the entire system of a biological niche must be open. There should be a dynamic equilibrium between the ingress and egress of individual substances required for a given organism. This may be associated with the fact that the microflora of a neighbouring biological niche provides conditions favourable for the development of the organism in question.

The formation of zones with the optimum development of particular species is affected most of all by the following factors: the degree of illumination and aeration; inflow of nutrients, of reduced compounds of sulphur, iron or manganese, of dissolved gases (methane or hydrogen); and the acidity and the oxidation potential of the medium.

Biological niches are observed most distinctly in meromictic lakes where deep-water layers are enriched with salts and do not take part in the spring or autumn water circulations. These layers usually do not contain dissolved oxygen and are enriched with hydrogen sulphide or ferrous salts. This creates a vertical gradation of oxidants and reductants causing the formation of distinct biological niches.

The characteristics of ecological niches with mass-scale development of particular phytoplankton species have been studied in detail by Findenegg (1971) in a series of 600 experiments staged in 30 lakes of
Austria. That author has shown that the mass development of species at a particular depth depends not only on light intensity and water temperature but also on the trophic status of the lake, while the rate of accumulation of carbon dioxide per unit biomass varies greatly from one species to another.

The ecological niche was found to be particularly distinct for *Oscillatoria rubescens* in the period of lake stratification. Mass development of that species was observed at depths of 10–12 m where the temperature was near 7 °C, light intensity did not exceed 10 per cent of that at the surface, and nutrient elements appear to have been flowing out from the hypolimnion.

The development of photosynthesizing sulphur bacteria in meromictic lakes at the depth of 10–12 m in the upper layers of the hydrogen sulphide zone was observed by Issatschenko (1914) in Lake Mogil'noje, Kuznetsov (1942), Jegorowa (1951), Dolgov (1955), Sorokin (1966a, 1970) and others in Lake Belovod, by Jimbo (1940), Takahashi and Ichimura (1968) in lakes of Japan, by Kuznetsov and Gorlenko (1973) in lakes of the Mari ASSR, and by many other authors. The distribution of these organisms was studied in greatest detail by Kuznetsov and Gorlenko (1973) and Gorlenko and Kuznetsov (1972) in Lake Kononier in the Mari ASSR and by Gorlenko et al. (1973) in Lake Gek-Gel in the Azerbaijan SSR.

Observations of the vertical distribution of photosynthesizing sulphur bacteria was observed in the chasm lake Kononier at the depth of 22 m and the results were considered in relation to the ecological conditions of the environment. During the observation period, in September 1970 (Fig. 1), water transparency by Secchi disc was 5.5 m, the thermocline was well marked, oxygen occurred down to the depth of 9 m, and hydrogen sulphide was detected in the hypolimnion below a depth of 10.5 m.

The ecological niche, where phytoplankton consisted of *Microcystis* and diatoms and production of organic matter in the process of photosynthesis reached its maximum of 50 μg C per litre, was found in the surface layer. At the depth of 10.5 m there occurred another niche with strong development of *Oscillatoria prolifica* and a water temperature of 7 °C. Dark oxidation of carbon dioxide attained its maximum, 17 μg C per litre, in the region of the junction of layers containing oxygen and hydrogen sulphide. As can be seen from Fig. 1, beginning with the depth of 10 m there occurred a sharp gradation of individual
elements of the environment: decreased illumination, increased hydrogen sulphide content, and appearance of dissolved manganese salts. All this created separate ecological niches with optimum conditions for development of particular microbial species.

Ten metres deep (Fig. 1, Table 1), under microaerophilic conditions, there was a zone favourable for mass development of Metallogenium personatum, which at a certain stage parasitized Osc. prolifica. Below 10-75 m the maximum development of Rhodothece conspicua was noted, while from 11-00–11-5 m green sulphur bacteria Pelodictyon luteolum predominated. At 11–12 m there also occurred very large numbers of aggregates of the brown symbionts Pelochromatium roseum and Pelochromatium roseo-viride. This was a consortium including a mobile heterotrophic bacterium covered with a layer of brown and a layer of green photosynthesizing bacteria. Chlorochromatium glebulum-consortium and Chlorochromatium aggregatum also developed there. In deep layers, filaments of a colourless Peloploca were found.

The distribution of individual species was dependent on the content of hydrogen sulphide, pH of the water, and the penetration of light of a definitive wavelength. Such a distribution of microorganisms well agrees with absorption of light rays by their respective pigments. Thus, the chlorophyll of Microcystis and diatoms absorbs light of wavelength
<table>
<thead>
<tr>
<th>Organism</th>
<th>Lake</th>
<th>Depth of location of biological niche</th>
<th>t °C</th>
<th>$O_2$ mg l$^{-1}$</th>
<th>$H_2S$ mg l$^{-1}$</th>
<th>pH</th>
<th>$rH_2$</th>
<th>Bacterial count 1000 ml$^{-1}$</th>
<th>Salinity g Cl$1$ l$^{-1}$</th>
</tr>
</thead>
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<td>Gek-Gel</td>
<td>27</td>
<td>5-0</td>
<td>0-0</td>
<td>0-15</td>
<td>7-5</td>
<td>17-7</td>
<td>900</td>
<td>Fresh</td>
</tr>
<tr>
<td><em>Chlorochromatium aggregatum</em></td>
<td>Okha-Lampi</td>
<td>5</td>
<td>10-0</td>
<td>weak</td>
<td>1-0</td>
<td>6-6</td>
<td>15-0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Pelodictyon luteolum</em></td>
<td>Kononier</td>
<td>10-7</td>
<td>5-0</td>
<td>0-0</td>
<td>0-5</td>
<td>7-4</td>
<td>13-0</td>
<td>180</td>
<td>—</td>
</tr>
<tr>
<td><em>Pelochromatium roseum</em></td>
<td>Kononier</td>
<td>11-25</td>
<td>4-0</td>
<td>0-0</td>
<td>0-6</td>
<td>7-15</td>
<td>12-1</td>
<td>70</td>
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</tr>
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<td>Berestianoje</td>
<td>11-00</td>
<td>6-4</td>
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</tr>
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<td>17-0</td>
<td>0-0</td>
<td>4-2</td>
<td>7-7</td>
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<td>9000</td>
<td>27-4</td>
</tr>
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<td><em>Pel. phaeovibrioides</em></td>
<td>Repnoje</td>
<td>5-75</td>
<td>4-0</td>
<td>0-0</td>
<td>50-0</td>
<td>7-2</td>
<td>14-3</td>
<td>33000</td>
<td>1-5</td>
</tr>
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<td>Thiocephsa roseopersicina</td>
<td>Repnoje</td>
<td>5-5</td>
<td>4-0</td>
<td>0-0</td>
<td>8-0</td>
<td>6-9</td>
<td>16-3</td>
<td>120</td>
<td>8-0</td>
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<tr>
<td><em>Chromatium vinonum</em></td>
<td>Repnoje</td>
<td>5-75</td>
<td>4-0</td>
<td>0-0</td>
<td>50-0</td>
<td>7-2</td>
<td>14-3</td>
<td>—</td>
<td>8-5</td>
</tr>
<tr>
<td>Thionic</td>
<td>Repnoje</td>
<td>1-25</td>
<td>—</td>
<td>0-0</td>
<td>4-2</td>
<td>7-4</td>
<td>16-4</td>
<td>10</td>
<td>26-6</td>
</tr>
<tr>
<td>Thiospira sp.</td>
<td>Veisovo</td>
<td>1-25</td>
<td>18-0</td>
<td>0-0</td>
<td>4-2</td>
<td>7-4</td>
<td>16-4</td>
<td>—</td>
<td>26-6</td>
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<tr>
<td>Thiovolum sp.</td>
<td>Veisovo</td>
<td>1-25</td>
<td>18-0</td>
<td>0-0</td>
<td>4-2</td>
<td>7-4</td>
<td>16-4</td>
<td>—</td>
<td>26-6</td>
</tr>
</tbody>
</table>
680 nm in the red region of the spectrum; purple bacteria absorb in the green and red regions at 550 and 800 nm respectively, while green sulphur bacteria absorb at 710–750 nm in the red region of the spectrum; brown bacteria (*Pelochromatium roseum*), which contain carotenoids, absorb from 460 to 580 nm, while bacteriochlorophyll-d absorbs light of wavelength 710–730 nm. Thus, each organism has its own biological niche, and at greater depth there occur organisms using light in the green-blue region of the spectrum, i.e. the light which can penetrate greater depths of water.

Similar patterns in the distribution of phytoplankton and photosynthesizing bacteria have been reported from observations in Lake Repnoje (Fig. 2).

![Fig. 2. Water chemistry and vertical distribution of photosynthesizing bacteria in Lake Repnoje. Thiocapsa roseopersicina (1), Chlorobium phaeovibrioides (2), Chromatium sp. (3).](image)

The degree to which light is absorbed by *Pelodictyon luteolum* during its mass development in Lake Chernoje-Kicheer, can be seen from Fig. 3 (Gorlenko, 1969).

Lake Kicheer is meromictic. It is 10 m deep and to some extent atrophic. The upper limit of the hydrogen sulphide layer is situated at a depth of 3.5 m. At a depth of 4 m *Pelodictyon luteolum* numbers attained 7 million cells per ml. Photometric measurement showed that at the upper limit for sulphur bacteria illumination was 30 per cent of that in the surface layer and that it fell to 3 per cent at the lower limit for *Pelodictyon*.

Of particular interest from the ecological viewpoint is the con-
sortium *Pelochromatium roseum* (Fig. 4). Studies by Gorlenko have shown that the mobile bacterium located inside this consortium appears to belong to the sulphate-reducers. Residing in the light zone of the lake under anaerobic conditions, the green sulphur bacteria supply organic matter in the light to the central sulphate-reducing bacterium, which then releases hydrogen sulphide to the microzone of the consortium, thereby providing the photosynthesizing bacteria with a hydrogen donor. This creates favourable conditions for *Pelochromatium roseum* in such lakes as Kuznechikha in the Mari ASSR where only traces of hydrogen sulphide are detectable with analytical methods.

Well-defined biological niches with mass-scale development of *Chlorobium phaeobacteroides, Metallogenium personatum* and *Sidercapsa*, were reported by Gorlenko et al. (1973) in the meromictic lake Gek-Gel (Fig. 5). This lake is located in Transcaucasia 1650 m above sea level; its maximum depth is 70 m, and the chemocline is situated 30 to 40 m deep. Results of chemical and microbiological analysis are presented in Fig. 5 and Table 2.

At the end of September 1971, oxygen had completely disappeared from a depth of 28 m. The concentration of hydrogen sulphide in the zone below this depth did not exceed 2.5 mg per litre. Fe$^{2+}$ in the epilimnion
Fig. 4. Schematic structure of the consortium *Pelochromatium roseo-viride* (I) and *P. roseum* (II). Sulphate-reducing bacteria (1), *Chlorobium phaeobacteroides* (2), *Pelodictyon luteolum* (3).

Fig. 5. Water chemistry and distribution of *Metallogenium* (1), *Siderocapsa* (2), and *Chlorobium phaeobacteroides* (3) in Lake Gek-Gel in September 1970. (Gorlenko et al., 1973.)
TABLE 2
Characteristics of biological niches of mass development of iron- and manganese-oxidizing bacteria

<table>
<thead>
<tr>
<th>Organism</th>
<th>Lake</th>
<th>Depth of location of biological niche</th>
<th>$t^\circ{C}$</th>
<th>$O_2$ mg l$^{-1}$</th>
<th>Mn$^{2+}$ mg l$^{-1}$</th>
<th>Fe$^{2+}$ mg l$^{-1}$</th>
<th>pH</th>
<th>Number of iron bacteria 1000 ml$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metallogenium</td>
<td>Gek-Gel, 1971</td>
<td>25</td>
<td>4·9</td>
<td>0·32</td>
<td>0·29</td>
<td>—</td>
<td>7·5</td>
<td>55·6</td>
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<tr>
<td>Siderocapsa sp.</td>
<td>Gek-Gel, 1971</td>
<td>30</td>
<td>5·0</td>
<td>0</td>
<td>0·70</td>
<td>—</td>
<td>—</td>
<td>18·7</td>
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<tr>
<td>Metallogenium</td>
<td>Gek-Gel, 1970</td>
<td>23</td>
<td>5·4</td>
<td>0·27</td>
<td>1·08</td>
<td>0·23</td>
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<td>—</td>
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<td></td>
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</tbody>
</table>
did not exceed 0.27 mg per litre, and manganese 0.004 mg per litre, but the concentration of these elements increased sharply downwards from the limit of the hydrogen sulphide zone to reach 2.45 ml per litre for Fe$^{2+}$ and 4.25 per litre for Mn$^{2+}$ at the bottom.

Water from various horizons and particular that from the oxygen-hydrogen sulphide junction was membrane filtered in 10-ml portions for microscopic examination after appropriate treatment. It was found that in 1970 *Metallogenium personatum* developed in a very narrow water layer at the depth of 26 m, at the boundary of oxygen disappearance and appearance of bivalent manganese. Here *Metallogenium* numbers reached 60000 coenobia per ml and fell sharply in the underlying layers. Inoculation from the same layer into moist agar revealed large numbers of mould fungi on which parasitized *Metallogenium personatum*.

A biological niche was found at depths between 27 and 29.5 m with mass development of a *Siderocapsa* sp. morphologically close to that of *Siderocapsa anulata*. The dissolved oxygen content was lower and that of ferrous and manganous salt was higher. Finally, at the depth of 30 m there occurred a distinct narrow ecological niche occupied by *Chlorobium phaeobacteroides*. Growth of this organism at such a depth was due to the fact that the light absorption maximum by its carotenoids occurs at 450–470 nm, light of which wavelengths penetrates to the greatest depths of water.

Biological niches with mass development of individual species of iron bacteria have been observed by Salimovskaja-Rodina (1936) in Lake Chainoje, by Sokolova (1961) in Lake Glubokoje, by Drabkova and Stravinskaja (1969) in Lake Punnus-Yarvi and by others.

Lake Glubokoye is of a fairly rich mesotrophic type; by the end of stratification periods oxygen deficiency occurs and soluble forms of iron increase in the lower hypolimnion layers. The physicochemical features of this lake have been described in detail by Shcherbakov (1967), and the microbiological data on the distribution of iron bacteria have been reviewed by Sokolova (1961). Thus, *Metallogenium* predominantly developed in deep layers with temperature 3–5 °C, oxygen levels from 0 to 1 mg per litre and Fe$^{2+}$ 0.05–0.1 mg per litre. At higher oxygen levels, there were successively observed biological niches of *Ochrobium tectum*, *Gallionella* sp. and *Siderocapsa* sp.

The formation of a biological niche with mass development of sulphate-reducing bacteria is associated with the establishment of
anaerobic conditions, the presence of sulphates and the inflow of organic matter or hydrogen.

The diffusion of hydrogen sulphide from bottom deposits into water converts iron and manganese into their reduced forms, thereby creating an ecological niche favourable for bacteria that oxidize ferrous and manganous compounds. Further increases in hydrogen sulphide concentration result in the disappearance of oxygen and, provided illumination is adequate, conditions are created favourable for the growth of photosynthesizing sulphur bacteria, while the iron and manganese-oxidizing bacteria move upward to the zone with a minimum content of dissolved oxygen, as has been observed in Lake Kononier and Lake Gek-Gel (cf. Tables 1 and 2).

3 The ecology of photosynthesis

The primary factor necessary for photosynthesis is light. The different regions of the solar spectrum are absorbed by water to varying degrees depending on the presence of coloured humic substances, mechanical suspensions, etc. As an example one may take Lake Black Oak with its water of low chromaticity, 9° according to the platinum–cobalt scale, and with great transparency. As shown by Birge and Juday (1932), the deepest penetration is by green light of wavelength of 500–550 nm, while greatest absorption is in the ultraviolet and infrared regions.

Apart from light, factors responsible for the formation of biological niches favouring individual particular species of photosynthesizing microorganisms include, among others, biogenic elements and water temperature. To elucidate the influence of individual factors, the latter should be delimited in some way.

3.1 Ecology of phytoplankton photosynthesis

The effects of light on Asterionella formosa was studied by Talling (1966) during a period of temperature stratification in Lake Windermere when the penetration of nutrient elements from the bottom was hindered by a temperature discontinuity. The experiments used samples of lake water in which A. formosa was practically in a monoculture. It was found that at a given temperature photosynthesis increased with increasing illumination up to 10000 lux where light saturation occurred as can be seen from Fig. 6.
Photosynthesis also increases with increasing temperature, but to a certain limit.

Thus, the optimum light intensity for the development and photosynthesis of phytoplankton occurs in the range of 3000–10000 lux, while photosynthesizing bacteria can grow in another ecological niche with lower temperatures and illumination.

The conditions most favourable for the growth of phytoplankton are found in surface layers, predominantly at depths of 20–50 cm, where sunlight intensity approaches the optimum for algae (Lund, 1967). This has been shown particularly clearly by Talling (1965) for African lakes, by Romanenko et al. (1971) for different lakes of Latvia, and by Findenegg (1971) for lakes of Austria.

Another important factor in phytoplankton development is the amount of nutrient elements supplied to the lake. Lakes are often classified into eutrophic and oligotrophic, although such a classification can provide only a fairly rough idea of the biological processes occurring in them. Eutrophic lakes are those with a constant inflow of nutrient elements and thus a high potential productivity. The reverse is true of oligotrophic lakes. The more eutrophic a lake is the richer one would expect it to be in phytoplankton, but the magnitude of production of organic matter is limited by the quantity of light that can be used in photosynthesis. For that reason, in eutrophic lakes rich in phytoplankton all primary production is concentrated in the surface
where so much light is absorbed because of the large quantity of algae that little penetrates into underlying layers. In oligotrophic lakes, where the inflow of nutrient salts is small, algal development per unit volume is much slower so that light can penetrate much further into the water mass. As a result algae can grow in deeper layers. Thus, when recalculated in terms of unit surface area of the lake, the production of organic substances in oligotrophic lakes approaches that of eutrophic ones. A good example of this has been reported by Talling (1965) from African lakes. As can be seen from Fig. 7, the areas delineated by the depth of photosynthesis and by the curve describing the quantity of oxygen released per hour per m³ of water are identical for Lake Victoria and Lake George. Comparison of the amount of synthesized organic matter in Lake Victoria with that in the Kasinga Canal or Lake George shows identical values of primary production under 1 m² of water despite the fact that in Lake Victoria the 1 per cent level of light penetration is 13 m while in Lake George it is only 70-80 cm.

Fig. 7. Extent of photosynthesis in African lakes (oligotrophic Lake Victoria and eutrophic Lake George) as related to depth of light penetration (in per cent of the surface illumination) according to Talling (1965).

Studies into the effect of light inhibition on photosynthesis in a number of oligotrophic lakes of Karelia with highly transparent water have shown that in Lake Urozero and Lake Pertozero where water transparency by Secchi disc was 8 and 4 m respectively, the maximum of photosynthesis occurred at the depth of 2 m, the intensity of photosynthesis in Urozero exceeding 7.5-fold that in the surface
In all mesotrophic lakes, maximum photosynthesis was observed at the depth of 0.5 m and did not exceed 1.5 to 2.5 times that in the surface layer.

Light inhibition and decreased photosynthesis are particularly marked in eutrophic lakes with mass development of phytoplankton. One example is Lake Dotkas in the Latvian SSR (Fig. 8) as compared with the oligotrophic lakes Dolgoje and Inesis. Light is thus an important factor determining the location of the photosynthesizing layer. Photosynthetic intensity in different lakes depends on the depth of the photic zone and the amount of phytoplankton. Table 3 presents relevant data for several Latvian lakes.

The poorest phytoplankton in the latter half of June 1967 was found in the oligotrophic lakes, the diurnal photosynthesis for the average sample from the photic zone was 0.01 mg C per litre. Phytoplankton was richest in eutrophic lakes and synthesized 7.5 and 11.7 mg C per litre per day, that is 750 and 1170 times as much as in the oligotrophic Lake Dridzas. However, in the oligotrophic lakes the photic zone was more than 15 m thick while in the eutrophic lakes, considering the reverse course of the light beam when determining Secchi disc transparency, it hardly attained 1.2 m. In view of this the total quantity of organic matter formed through photosynthesis in the eutrophic lakes under 1 m² was only 75–90-fold that produced in the oligotrophic lakes. It should be noted that characterization of lakes according to their trophic status is tentative. Most likely to be correct is
### TABLE 3

Magnitude of photosynthesis in different lakes of Latvia (from Romanenko et al., 1971)

<table>
<thead>
<tr>
<th>Type of lake</th>
<th>Name</th>
<th>Depth m</th>
<th>Average for photic zone</th>
<th>Under 1 m$^3$ of water column under 1 m$^2$</th>
<th>Ratio of photosynthesis in the lake under study to that in Lake Dridzas Average ratio for photic zone</th>
<th>Under 1 m$^2$</th>
</tr>
</thead>
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<tr>
<td>Oligotrophic</td>
<td>Dridzas</td>
<td>65</td>
<td>0.01</td>
<td>130</td>
<td>0.01</td>
<td>1</td>
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<tr>
<td></td>
<td>Dolgoje</td>
<td>44</td>
<td>0.012</td>
<td>120</td>
<td>0.012</td>
<td>1.2</td>
</tr>
<tr>
<td>Mesotrophic</td>
<td>Alaukstas</td>
<td>7</td>
<td>0.12</td>
<td>360</td>
<td>0.12</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Inesis</td>
<td>7</td>
<td>0.20</td>
<td>600</td>
<td>0.20</td>
<td>2</td>
</tr>
<tr>
<td>Eutrophic</td>
<td>Shengeidas</td>
<td>5</td>
<td>7.5</td>
<td>9800</td>
<td>7.5</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Dotkas</td>
<td>5</td>
<td>11.7</td>
<td>11700</td>
<td>11.7</td>
<td>90</td>
</tr>
<tr>
<td>Dystrophic</td>
<td>Melnezers</td>
<td>4</td>
<td>0.05</td>
<td>100</td>
<td>0.05</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Slokas</td>
<td>1.5</td>
<td>0.12</td>
<td>100</td>
<td>0.12</td>
<td>0.8</td>
</tr>
</tbody>
</table>
TRENDS IN ECOLOGICAL MICROBIOLOGY

the proposal by Elster (1958) to judge the trophic status of a reservoir by the extent of primary production in 1 litre of the surface layer. It can be seen from Table 3 that phytoplankton production in eutrophic lakes when recalculated per litre of the photic zone considerably exceeds that in oligotrophic lakes. But these figures are levelled out to a great extent when one calculates production under 1 m². In effect the trophic level of lakes depends on both photosynthetic processes and on the degradation of organic matter (Ohle, 1958). The latter occurs not only in the photic zone but throughout the water column. For that reason, it is better to assess phytoplankton production from the average value of photosynthesis recalculated per m³ of the water column under 1 m² of the lake. In this case the difference between the different types of lake becomes more distinct.

3.2 ECOLOGY OF BACTERIAL PHOTOSYNTHESIS

Winogradsky (1949) devoted much attention to coloured sulphur bacteria. He described a large number of purple sulphur bacteria and showed that the oxidation of hydrogen sulphide is vital for them.

Later Van Niel (1931) studied their physiology and demonstrated their capacity for photosynthesis. Light is therefore an important ecological factor for photosynthesizing sulphur bacteria. All photosynthesizing sulphur bacteria are anaerobes with hydrogen sulphide serving as their hydrogen donor.

Different authors have measured light saturation at different light intensities for photosynthesizing bacteria. Takahashi and Ichimura (1970) studied the photosynthesis of pure cultures of Chromatium D and Chlorobium sp. in relation to light intensity and expressed this relationship in relative units, having taken the value of light saturation as 100 per cent. As can be seen from Fig. 9, the photosynthetic curve at 25 °C in Chromatium attains saturation at 2000 lux after which there is light inhibition; in Chlorobium sp. light saturation occurs at 5000 lux, although they are capable of photosynthesis at illumination levels below 500 lux. Larsen (1953) determined this minimum value for Chlorobium thiosulfatophilum as 200–260 lux, Lippert and Pfennig (1969) as 700–1000 lux; for Chromatium sp. this value was estimated to range from 210 to 790 lux by Wassink et al. (1942) and 1000 to 2000 lux by Lippert and Pfennig. Obviously, such differences in values of light saturation could be due to the use of different light sources and to
Fig. 9. Effect of light intensity on photosynthesis in *Chlorobium* (1) and *Chromatium* (2) according to Takahashi and Ishimura, 1970.

Fig. 10. Photosynthetic intensity for phytoplankton (1), photosynthezing sulphur bacteria (2), dark fixation of carbon dioxide (3) in Lake Weisovoje in July 1971. (Gorlenko et al., in press.)
different states of the cultures employed. At any rate, purple and green photosynthesizing sulphur bacteria are capable of developing at much lower light intensities than are required for phytoplankton.

If sunlight penetrates the upper limit of the hydrogen sulphide zone in summer, there occurs a mass development of photosynthesizing bacteria, as observed by many investigators.

The production of organic matter by these bacteria has been studied relatively little. Mention may be made of studies by Lyalikova (1957), Sorokin (1966a, b), Ivanov (1957), Gorlenko (1969), Gorlenko et al. (1973), Kuznetsov (1970), and Takahashi and Ichimura (1968).

As an example, we may cite data on photosynthesis in Lake Belovod in July 1958, in the salt lakes Weisovoje and Repnoje in June 1970 (Fig. 10), and in Lake Chernoje-Kicheer in the summer of 1968.

Production of organic matter by photosynthesizing bacteria attained 700 μg C per litre per day in Belovod, 190 in Weisovo and 160 in Repnoje, the maximum values of photosynthesis by phytoplankton being 400 μg C per litre per day at the depth of 1 m. Still higher values were recorded in Chernoje-Kicheer (Fig. 11). It is of interest that the zone of maximum photosynthesis coincides with the zone of maximum numbers of Pelodictyon luteolum. Above this layer, no green sulphur bacteria developed because of the presence of dissolved oxygen, while below that layer they

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![Graph](image-url)  

**Fig. 11.** Photosynthetic intensity in Lake Chernoje-Kicheer in July 1969. Algal photosynthesis (1), bacterial photosynthesis (2). (Gorlenko, 1969.)
failed to grow because of the lack of light due to complete absorption by the bacterial layer.

A similar picture was reported by Takahashi and Ichimura (1968) in nine Japanese lakes. In Lake Kiseratsu in August 1965, the maximum phytoplankton photosynthesis occurred at the depth of 3 m and corresponded to 9·2 \( \mu \text{g C} \) per litre per hour, while bacterial photosynthesis attained 154 \( \mu \text{g C} \) per litre per hour at the depth of 6 m. Dark fixation was also observed in that layer and corresponded to 3·31 \( \mu \text{g C} \) per litre per hour. Hydrogen sulphide was found in that lake already at the depth of 5 m.

Thus, the maximum production of organic matter by photosynthesizing sulphur bacteria may in certain cases exceed the photosynthetic production of organic matter by phytoplankton. However, since photosynthesizing bacteria occur in a very thin stratum limited by the presence of oxygen and by lack of light below, phytoplankton photosynthesis at all times exceeds bacterial photosynthesis when recalculated per unit area of the lake.

4 Intensity of bacterial reproduction

Evidently, the most important ecological factors responsible for the development of bacteria in reservoirs are temperature and energy sources: assimilable organic matter for heterotrophic bacteria and oxidizable mineral compounds as a source of free energy for autotrophs.

Winogradsky considered the study of activities of microbial communities in the natural environment as a major task of microbiology. Such studies have become to a great extent possible following the development and practical application of methods based on the use of labelled atoms of carbon, sulphur, nitrogen and other elements.

Observations by a number of authors have shown that *Bacillus subtilis*, *Staphylococcus*, *Clostridium welchii* and some other organisms fail to grow in the absence of carbon dioxide. Wood and Werkman (1936) noticed that when glycerol is fermented by *Propionibacterium pentosaccum* culture, the content of bicarbonate is reduced in the culture medium with a concurrent increase of succinic acid (recalculated in terms of carbon).

\[
\text{CH}_2\text{OH}-\text{CHOH}-\text{CH}_2\text{OH} + \text{CO}_2 \rightarrow \text{COOH}-\text{CH}_2-\text{CH}_2-\text{COOH}
\]

Because pyruvic acid could be isolated from the fermenting liquid, Wood concluded that this acid whose carboxylation results in the
formation of oxalacetic acid, is an intermediate product of fermentation. This so-called Wood–Werkman reaction is catalysed by the enzyme phosphoenolpyruvate carboxylase.

\[
\text{CH}_2 = \text{C} - \text{COOH} + \text{CO}_2 \rightarrow \text{COOH} - \text{CH}_2 - \text{C} - \text{COOH} + \text{P}_{\text{inorg}}
\]

\[
\underline{\text{O}} - \text{PO}_3 \text{H}_2 \quad \underline{\text{O}}
\]

(Phosphoenolpyruvic acid) (Oxalacetic acid)

 Practically all organic substances when assimilated by microorganisms are degraded to pyruvic acid as a result of oxidative and reductive processes. Pyruvic acid, by combining with carbon dioxide in the Wood–Werkman reaction, forms oxalacetic acid, which is a key link in the Krebs cycle of tricarboxylic acids.

As is known, the entire synthesis of amino acids necessary for cell anabolism passes indirectly through the Krebs cycle.

Thus, the above reactions of heterotrophic fixation of carbon dioxide involved the direct uptake of carbon from pyruvic acid by incorporating it into the structure of organic acids or of amino acids, i.e. when the carbon of fixed free carbon dioxide is largely used to build up cellular material.

Experiments of Romanenko (1964a, b, 1971) and Sorokin (1964) with both pure cultures and natural bacterial populations of the Rybinsk artificial lake have shown that heterotrophic assimilation of carbon dioxide accounts on average for 6–7 per cent of the carbon incorporated into bacterial biomass from the uptake of preformed organic compounds.

The constancy of this value makes it possible to determine, by means of isotopes, the heterotrophic assimilation of CO\(_2\) per unit time and thus to evaluate the quantity of bacterial biomass formed and the bacterial generation time (Romanenko, 1969).

The rate of reproduction of individual bacterial species varies and depends on the amount of assimilated organic matter, on the competition for its utilization by individual species, on water temperature and, probably, also on a number of other factors stimulating or inhibiting bacterial development. For that reason, one can speak with more justification, with reference to water reservoirs, of the time necessary for the number of bacteria to double rather than of the bacterial generation time. Calculations have shown that the reproduction rate
<table>
<thead>
<tr>
<th>Year</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>Average from May to October</th>
</tr>
</thead>
<tbody>
<tr>
<td>1964</td>
<td>82.0</td>
<td>62.1</td>
<td>23.7</td>
<td>20.6</td>
<td>54.8</td>
<td>39</td>
<td>47.2</td>
</tr>
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<td>43.0</td>
<td>23.8</td>
<td>17.6</td>
<td>19.2</td>
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<td>25.9</td>
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</tr>
<tr>
<td>1966</td>
<td>41.2</td>
<td>26.4</td>
<td>22.3</td>
<td>28.4</td>
<td>37.0</td>
<td>61.0</td>
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<td>86.0</td>
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<td>41.4</td>
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<td>56.8</td>
</tr>
<tr>
<td>Average</td>
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<td>21.5</td>
<td>27.4</td>
<td>45.8</td>
<td>35.4</td>
<td>37.3</td>
</tr>
</tbody>
</table>
of bacteria in Lake Rybinsk depends to a large extent on water temperature. Average data for 5 years are summarized in Table 4.

Each entry is the average of 12 assays of water samples taken at different points of the lake. The mean bacterial doubling time between May and October is 41 hours, i.e. the bacterial number should double every two days if no bacteria were consumed by zooplankton or died naturally. In midsummer at temperatures of 22–24 °C, the bacterial doubling time lies between 15 and 27 hours. In December, bacteria practically cease to multiply, and the generation time has been calculated as 600 hours. Analyses by Romanenko have shown that from December to April, when the temperature of the uppermost water layer remained almost constant, close to 0 °C, the reproduction rate of bacteria progressively increased, apparently owing to the appearance of psychrophilic forms.

It was possible on the basis of the magnitude of heterotrophic assimilation of carbon dioxide also to calculate the amount of bacterial biomass produced in the lake at the expense of dissolved organic matter. Such calculations have been undertaken by Kuznetsov et al. (1966) in 1964 and are given in Fig. 12.

Concurrently with the determination of the biomass of heterotrophic bacteria developing due to autochthonous and allochthonous organic

![Graph](image.png)

Fig. 12. Rybinsk artificial lake in 1964. Diurnal production of bacterial biomass (1), diurnal phytoplankton photosynthesis (2). (Kuznetsov et al., 1966.)
substances, determinations were also carried out on the production of organic matter as a result of phytoplankton photosynthesis. Over the growth period of 1964, the production of bacterial biomass in Lake Rybinsk was 117,000 tons of carbon throughout the lake, or 33 g of carbon per m², while photosynthesis accounted for 102,000 tons of carbon throughout the lake, or 29 g of carbon per m².

5 Intensity of sulphate reduction and chemosynthesis in lakes

5.1 Current interpretations of the concept of “chemosynthesis”

One of Winogradsky’s major discoveries was the finding that nitrifying and some other types of bacteria used the energy released upon the oxidation of inorganic compounds to assimilate free carbon dioxide as their source of cell carbon. This process, known as chemosynthesis, is widely distributed, and the organisms capable of chemosynthesis are termed chemolithotrophs.

In characterizing the fundamentals of ecological microbiology, Winogradsky (1947) wrote that since the time of his discovery of the capacity of microorganisms for chemosynthesis, that problem had received wide attention and his own conclusions were no longer categorical.

Rittenberg (1972), in his review devoted to the physiology of autotrophic bacteria, has attempted to demonstrate that there are no bacteria that would correspond to Winogradsky’s category “anorgoxidants”, or “strict autotrophs”. He thinks it unlikely that such bacteria exist or are detectable, although their existence cannot be denied a priori. He proposes to modify Winogradsky’s concept of “obligate autotrophs” and to refer to them as to organisms with a special type of physiology. Nevertheless, while refusing to accept the concept of “obligate autotrophs”, Rittenberg in no way belittles the historic significance of the brilliant studies carried out by Winogradsky.

It seems more accurate to speak, as Zavarsin (1972) does, of “an autotrophic way of life” than of “anorgoxidants” or “obligate autotrophs”.

Peck (1968) believes that three groups of organisms are capable of the autotrophic mode of life:
1. Obligate autotrophs, organisms having an active carboxydismutase and capable of growing on strictly mineral media in the presence of
reduced inorganic compounds. The latter provides them with energy for growth and assimilation of carbon dioxide, which serves as the only source of carbon for biosynthesis. These organisms are unable to assimilate simple organic carbon compounds such as acetate.

2. Facultative autotrophs, organisms capable of using both inorganic and organic substances as sources of energy and growth.

3. Organisms that may be referred to as "assimilating autotrophs". These can be supplied with energy for growth solely from the oxidation of inorganic compounds but are also capable of anabolizing simple organic substances of the same type as the products of CO₂ fixation. An example of such an organism is *Methylococcus methanooxidans*.

The question as to whether sulphate-reducing bacteria should be referred to as facultative autotrophs has been a subject of intensive investigation. On the one hand, sulphate reduction in multiplying cultures proceeds actively on mineral media with the energy source in the form of molecular hydrogen, which makes it possible to consider those organisms as autotrophs. On the other hand, as shown by Postgate (1960), Senez (1962) and others, sulphate-reducing bacteria fail to grow in pure culture under the same conditions, and Rittenberg (1972) was unable to detect ribulose-diphosphate carboxylase in them.

Mechalas and Rittenberg (1960) cultivated *Desulfovibrio desulfuricans* on a mineral medium with different additions of labelled carbon dioxide and yeast extract. Their results have clearly indicated that good growth occurred only due to the organic matter of the yeast extract in the presence of hydrogen. Growth was minimal without hydrogen. Hence they came to the conclusion that the energy required for growth is produced chemolithotrophically, as a result of oxidation of hydrogen, and it is utilized to assimilate organic compounds of the yeast extract.

Sorokin (1966b) has observed the reduction of sulphates by a pure culture of *Desulfovibrio desulfuricans* in a hydrogen atmosphere, adding to the medium minimal quantities of sodium acetate, which is believed by that author to be essential for the formation of acetyl coenzyme A through the Krebs cycle. The energy for anabolism was produced from the oxidation of hydrogen by the oxygen of sulphates, and up to 30 per cent of bacterial biomass was formed from the fixation of free CO₂.
5.2 INTENSITY OF SULPHATE REDUCTION IN NATURE

Experiments with pure cultures of sulphate-reducing bacteria (Postgate, 1960) have shown these organisms capable of selective uptake of only certain hydroxy acids, alcohols, etc. This process, on the other hand, is widely distributed in nature and occurs anaerobically in the presence of sulphate and most diverse organic substances with the pH of the environment close to neutral. Evidently, sulphate reduction can result from the joint activities of a number of microorganisms.

There arose the question of determining the intensity of sulphate reduction processes in natural environments. Appropriate observations were conducted after the introduction of minimal amounts of sulphur-labelled sodium sulphate, \( \text{Na}_2^{35}\text{SO}_4 \), the natural substrate (Ivanov, 1957, 1959; Sorokin, 1966b, 1970).

Ivanov and Terebkova (1959) showed that the quantity of hydrogen sulphide produced due to sulphate reduction in Lake Solenoje near the town of Solvychegodsk in 1957 and 1958 did not exceed 0.01–0.05 mg \( \text{H}_2\text{S} \) per litre per day in the water mass compared with 1 to 19 mg \( \text{H}_2\text{S} \) per litre per day in the surface mud layer (Fig. 13), being at its maxi-

![Diagram](image)

Fig. 13. Intensity of sulphate reduction in silt deposits of Lake Solenoje, in mg of \( \text{H}_2\text{S} \) \( \text{l}^{-1} \text{day}^{-1} \) (after Ivanov and Terebkova, 1959).
mum in that part of the lake where the inflow of organic substances from the coastal zone was at its peak.

Sulphate reduction is particularly intensive in bottom deposits of the salt lake Sivash where enormous quantities of dead algae are carried over by wind to the littoral zone. As can be seen from Table 5, the

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Cl− g l−1 of water</th>
<th>S as SO4²− mg l−1 of water</th>
<th>Intensity of sulphate reduction in mg of H2S l−1 of silt for 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Sivash:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>near Lake Genicheskoje</td>
<td>11.6</td>
<td>57</td>
<td>26</td>
</tr>
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<td>near Papanin Is</td>
<td>24.9</td>
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<td>near Balki village</td>
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<td>southern extremity</td>
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<td>near Sivash village</td>
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<td>2630</td>
<td>74</td>
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</tbody>
</table>

concentration of salts in the southern part of Sivash, particularly in the evaporation reservoirs of the salt mines of Lake Genicheskoje, is in excess of 310 g per litre when calculated in terms of NaCl. It is interesting to note that even at such high concentrations of NaCl, when salt begins to precipitate from the solution, the amount of hydrogen sulphide formed in silt deposits as a result of sulphate reduction ranges from 33 to 75 mg per litre per day (Kuznetsov and Romanenko, 1968). Evidently, this process involves a complex of halophilic microorganisms, the principal role being played by Desulfo-vibrio salexigenes or other halophilic species of sulphate-reducing bacteria.

More or less significant concentrations of hydrogen sulphide are found in the water mass of meromictic lakes. Apparently, sulphate reduction may occur in such lakes also in the water mass. Thus Sorokin (1964) has reported sulphate reduction in Lake Belovod, in the upper part of the hydrogen sulphate zone of Lake Belovod and also in Lake Gek-Gel (Sorokin, 1966a, 1970). In Lake Belovod, sulphate reduction occurred in two horizons. One of these was located directly over the
zone of intensive production of organic matter by photosynthesizing bacteria at depths of 13.5 to 16 m. Sorokin explains the existence of that zone by the neoformation of readily assimilable organic matter as a result of bacterial biosynthesis occurring in the overlying layer, where the intensity of sulphate reduction attained nearly 30 μg H₂S per litre per day. The other zone of sulphate reduction lay in the hypolimnion where 160 μg of hydrogen sulphide formed per litre per day. Organic matter appears to have accumulated in that zone by sedimenting from the water mass or diffusing from silt.

A similar pattern of sulphate reduction was reported by Sorokin (1970) for Lake Gek-Gel. As in Lake Belovod, sulphate reduction had two maxima: one at the depth of 35 m, just above the layer of chemoo- and photosynthesis where 6 μg of H₂S formed per litre per day, and the other, more intensive, in the bottom layer 68 m deep, where water

Fig. 14. Intensity of sulphate reduction in Lake Gek-Gel. Zone of bacterial photosynthesis (2), intensity of sulphate reduction (1). (Sorokin, 1970.)
was enriched with organic substances as a result of anaerobic breakdown of silt deposits. The distribution of sulphate-reducing bacteria followed the same pattern (Fig. 14).

5.3 INTENSITY OF CHEMOSYNTHETIC PROCESSES IN NATURE

There exist a number of reactions whereby inorganic substances are oxidized with the release of free energy, which may be utilized by organisms in the process of chemosynthesis.

One of the more likely processes occurring in a lake is the oxidation of methane and hydrogen together with the oxidation of reduced mineral sulphur compounds.

The method of radioactive isotopes has been employed to evaluate these processes, which is to proceed from the principles of ecological microbiology laid down by Winogradsky, which state that such evaluations should be carried out in the natural environment, i.e. in the lake itself, or under conditions which approach the natural ones as closely as possible.

In determining the magnitude of chemosynthesis (Kuznetsov, 1958) in order to exclude photosynthetic processes, a certain volume of water is isolated in a dark bottle to which a minimal quantity of radioactive isotope of carbon in the form of NaH\textsuperscript{14}CO\textsubscript{3} is placed. The bottle with the test water is then placed into the lake and is incubated at the same depth from which the water had been taken for analysis. In other words, such ecological factors as water temperature and chemical composition remain unchanged. Since the experiment lasts 24 hours or even less, it is assumed and has also been confirmed experimentally (Topolov, 1970) that the remaining environmental factors also correspond to those of the lake, while the possible alterations are within limits of the experimental errors of the order of 10 per cent.

Chemosynthesis in water reservoirs is associated with the oxidation of such gases as methane, hydrogen and hydrogen sulphide, while processes of nitrification do not have any great importance as far as the enrichment of natural lakes with organic matter is concerned. This has been confirmed in experiments that have shown no burst of chemosynthesis following incorporation of ammonium salts into natural water (Sorokin, 1961). From an ecological point of view a very important process occurring in lakes is the oxidation of methane released from bottom deposits to the water mass. This is a process that leads to
complete disappearance of dissolved oxygen from deep layers of eutrophic lakes involving the release of enormous quantities of free energy.

The magnitude of carbon dioxide fixation is the course of methane oxidation has been estimated by Sorokin (1964) in the Rybinsk artificial lake above the former bed of the Mologa River in the region of Breitov during winter months when oxygen is found down to 11 m and is absent from the lower layers. The uptake of carbon dioxide was maximal at 9.5 m, at the lower border of oxygen distribution, and rose to 13.6 μg C per litre per day at a water temperature of around 2 °C. Taking into account the heterotrophic assimilation of carbon dioxide which was 2.8 μg C per litre per day in the surface water layer under ice, chemosynthesis due to methane oxidation amounted to 10.8 μg C per litre per day.

Introduction of a bubble of methane or hydrogen into test bottles containing natural water from the lake greatly accelerated the rate of production of organic matter in the process of chemosynthesis, particularly in summer months.

Total chemosynthesis due to methane and hydrogen sulphide oxidation has been assessed by Sorokin (1964) in Suskansk Bay on
<table>
<thead>
<tr>
<th>Depth m</th>
<th>Natural Content of H₂S in water (mg l⁻¹)</th>
<th>After addition of H₂S</th>
<th>Ratio of sulphur forms at the end of experiment (%) in dark bottle</th>
<th>Oxidation of S²⁻ to S μg l⁻¹ Biological per 28 h</th>
<th>Chemosynthesis μg C1⁻¹ per 28 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0</td>
<td>0.5</td>
<td>26</td>
<td>300</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>0.05</td>
<td>0.55</td>
<td>28</td>
<td>303</td>
<td>117</td>
</tr>
<tr>
<td>11</td>
<td>0.15</td>
<td>0.65</td>
<td>52</td>
<td>260</td>
<td>104</td>
</tr>
<tr>
<td>12</td>
<td>0.20</td>
<td>0.70</td>
<td>68</td>
<td>147</td>
<td>56</td>
</tr>
<tr>
<td>13</td>
<td>0.80</td>
<td>1.30</td>
<td>83</td>
<td>130</td>
<td>39</td>
</tr>
<tr>
<td>14</td>
<td>2.2</td>
<td>2.7</td>
<td>93</td>
<td>45</td>
<td>18</td>
</tr>
</tbody>
</table>
the Kuibyshev artificial lake. As will be seen from Fig. 15, the most intensive chemosynthesis, up to $30 \mu g$ C per litre per day, was observed at 10 m at the place where dissolved oxygen, hydrogen sulphide and methane were present simultaneously.

The intensity of chemosynthesis has been studied in greatest detail for thiobacteria, owing to the availability of adequate methods of analysing the intensity of chemosynthetic processes using compounds containing labelled sulphur $^{35}$S and Na$_2^{14}$CO$_3$ (Ivanov, 1959).

These studies were carried out by Sorokin (1966a, 1970) in the meromictic Lake Belovod in the Vladimir region.

The results of the analyses are presented in Table 6. The Table shows that the incorporation of sodium sulphide into the lower layer of the oxygen zone results in very intensive oxidation of hydrogen sulphide by a purely chemical agency, particularly at the depth of 9 m where in the lake itself hydrogen sulphide is absent and the population of thiobacteria is less active. The same occurs at the depth of 13 m and below, where the population of sulphur bacteria is weakened by oxygen deficiency. In the layer where sulphur bacteria are active, between depths of 10 and 13 m where oxidants enter from above due to turbulent mixing of water, while hydrogen sulphide is supplied from below, thiobacteria oxidize c. $100 \mu g$ H$_2$S per litre in 30 hours, which constitutes some 40 per cent of the total sulphides present in the water.

6 Intensity of molecular nitrogen fixation

The intensity of molecular nitrogen fixation by blue-green algae has been studied using both conventional methods whereby the increment of total nitrogen is estimated in algal culture and by means of the method of stable isotopes. It has been found that many of the blue-green planktonic algae from the families Anabaenaceae and Nostocaceae as well as from certain other families are capable of assimilating molecular nitrogen.

The $^{15}$N stable isotope method was first used by Dugdale and co-workers (Dugdale et al., 1959; Neess et al., 1962) for determining the extent of free nitrogen fixation by naturally occurring phytoplankton in Lake Sanctuary in Pennsylvania.

These authors have discovered a direct correlation between the quantity of fixed nitrogen and the overall growth in the plankton of *Anabaena flos-aquae*, *A. circinalis* and *A. spiroides*. A close relationship has
been found between photosynthesis and nitrogen uptake. In darkness, nitrogen fixation was practically nonexistent; it was maximal in the surface water layer. For 24 hours, the amount of fixed nitrogen in the 0–1 m layer attained 3 per cent of the total nitrogen of the water. Since the publication of this work determinations of free nitrogen fixation have been carried out in many lakes. A summary of the results obtained has been prepared by Fogg (1971) and is reproduced in Table 7.

The possibility of nitrogen fixation in the dark zone has been usually neglected or considered insignificant. Thus, Stewart et al. (1967) point out, for example, that nitrogen fixation diminishes abruptly with depth in Lake Mendota. However, indications that there are present in the water body organisms potentially capable of nitrogen fixation led Brezonik and Harper (1969) to undertake appropriate observations using the acetylene procedure.

Brezonik and Harper (1969) have determined the intensity of nitrogen fixation in two dystrophic lakes, Mary and Mise, situated in two climatic zones. These lakes both have an extensive anaerobic zone. The meromictic Lake Mary, which is 1·2 hectares in area and 21·5 m in depth, is in the state of Wisconsin. Water colour varies from 150° to 300° on the platinum–cobalt scale, and water is anoxic below 5 m. The meromictic Lake Mise is in Florida and is similar to Lake Mary. It is 0·91 hectares in area and its maximum depth is 25 m. Water chromaticity varies from 30° to 300°. In winter there is slight water circulation and oxygen saturation. During a long period of stratification, oxygen disappears, and is absent from June to September at depths below 5 m. In the surface layer, down to 15 m, Lake Mary showed (Fig. 16) relatively weak free nitrogen fixation, 1·0 µg per litre per hour, which is only slightly in excess of the analytical error. Nitrogen fixation increased strongly in the bottom layers of the anaerobic zone. A relatively high activity of nitrogen fixation was recorded in Lake Mise. The maximum occurred in the oxygen-free zone at 9 m. Much lower fixation values were recorded in deep layers and no fixation occurred in the epilimnion down to the depth of 5 m. From their observations Keirn and Brezonik (1971) concluded that the total quantity of nitrogen fixed in Lake Mise was 39·2 kg in 1969 and 9·6 kg in 1970, or 1·14 and 0·28 kg per metre per year respectively.

It should be noted that Brezonik and Harper's data are very rough. The acetylene method was not then checked by the stable nitrogen
<table>
<thead>
<tr>
<th>Lake and month</th>
<th>Type of lake</th>
<th>Main types of blue-green algae</th>
<th>Total nitrogen fixed daily nitrogen</th>
<th>Nitrogen fixation nitrogen</th>
<th>Nitrogen % nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanctuary (August)</td>
<td>Eutrophic</td>
<td><em>Anabaena flos-aquae</em></td>
<td>3.6</td>
<td>125</td>
<td>3.5</td>
</tr>
<tr>
<td>Mendota (August)</td>
<td>Eutrophic</td>
<td><em>Gloeotrichia echinulata</em></td>
<td>0.72</td>
<td>8.5</td>
<td>1.18</td>
</tr>
<tr>
<td>Wingra (July)</td>
<td>Mesotrophic</td>
<td><em>Microcystis aeruginosa, Anabaena</em> sp.</td>
<td>2.1</td>
<td>12</td>
<td>0.55</td>
</tr>
<tr>
<td>Smith (June)</td>
<td>Subarctic, mesotrophic</td>
<td><em>Anabaena flos-aquae</em></td>
<td>0.41</td>
<td>2.88</td>
<td>0.07</td>
</tr>
<tr>
<td>Windermere, Northern basin (June)</td>
<td>Mesotrophic</td>
<td><em>Anabaena flos-aquae, Oscillatoria</em></td>
<td>0.164</td>
<td>0.098</td>
<td>0.060</td>
</tr>
<tr>
<td>Windermere, Southern basin (October)</td>
<td>Mesotrophic</td>
<td><em>A. solitaria, A. flos-aquae, Oscillatoria</em></td>
<td>0.47</td>
<td>2.82</td>
<td>0.060</td>
</tr>
<tr>
<td>Esthwaite Water (August)</td>
<td>Mesotrophic</td>
<td><em>Aphanizomenon flos-aquae, Anabaena</em> flos-aquae, A. circinalis</td>
<td>0.255</td>
<td>0.244</td>
<td>0.096</td>
</tr>
<tr>
<td>Loch Leven, Scotland (May, June, September)</td>
<td>Mesotrophic</td>
<td><em>Synechococcus sp., Oscillatoria</em> sp.</td>
<td>0.186</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tjeukemeer, Netherlands (September)</td>
<td>Eutrophic</td>
<td><em>Aphanizomenon sp., Oscillatoria</em> sp.</td>
<td>2.1</td>
<td>14.9</td>
<td>0.66</td>
</tr>
<tr>
<td>George, Uganda (March)</td>
<td>Tropical, eutrophic</td>
<td><em>Microcystis sp., Anabaena</em></td>
<td>2.2</td>
<td>4.1</td>
<td>0.19</td>
</tr>
<tr>
<td>McIlwaine, Rhodesia (March)</td>
<td>Tropical</td>
<td><em>Microcystis sp.</em></td>
<td>1.275</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kariba, Rhodesia (March)</td>
<td>Tropical</td>
<td><em>Oscillatoria sp.</em></td>
<td>0.30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
method. However, under conditions existing in nature there is no known nonenzymic reduction of acetylene to ethylene, which would appear as nitrogen fixation and thus interfere with the use of the method; all the more so that in the control tests involving binding of proteins with trichloroacetic acid, that reaction does not take place. For that reason, the authors ascribe the fixation of molecular nitrogen in the oxygen-free part of lake to microorganisms.

Using the same method, Keirn and Brezonik have determined nitrogen fixation in silt deposits of 25 lakes, in 7 of which fixation proved to be fairly intensive (Table 8). Thus, nitrogen fixation in Florida lakes varied from 0.33 to 59 µg N₂ per kg of silt per hour in the surface layer of silt deposits and from 0.02 to 1.1 µg of N₂ per kg per hour of silt at 30–50 cm. The authors isolated 7 bacterial species from

<table>
<thead>
<tr>
<th>Lake</th>
<th>Type of sediment</th>
<th>1969</th>
<th>1970</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beavens Arm</td>
<td>Brown, lumpy</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>Kanapacha</td>
<td>Brown, lumpy</td>
<td>36</td>
<td>59</td>
</tr>
<tr>
<td>Orange</td>
<td>Brown, lumpy</td>
<td>9.5</td>
<td>28</td>
</tr>
<tr>
<td>Moss Lee</td>
<td>Black, jelly-like</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>Apoka</td>
<td>Black, jelly-like</td>
<td>1.7</td>
<td>—</td>
</tr>
<tr>
<td>Alice</td>
<td>Brown</td>
<td>1.2</td>
<td>0</td>
</tr>
<tr>
<td>Unnamed (20)</td>
<td>Sandy</td>
<td>0</td>
<td>16</td>
</tr>
</tbody>
</table>

Fig. 16. Fixation of molecular nitrogen in Lake Mary (A) and Lake Mise (b) (after Keirn and Brezonik, 1971.)
those deposits, including Clostridium sp., capable of reducing acetylene to ethylene in the laboratory. Keirn and Brezonik consider these forms capable of nitrogen fixation.

When Winogradsky studied in detail the ecology of Azotobacter we concluded that nitrogen fixation in nature occurs due to substances present in the soil in minimal concentrations. The above methods have enabled us to approach the problem from a quantitative aspect, to assess the magnitude of nitrogen fixation in reservoirs, and to find out which organisms are responsible for replenishing lakes with fixed nitrogen under given conditions.

7 Degradation of organic matter

Organic matter is produced in lakes as a result of photosynthesis by phytoplankton and higher aquatic plants, but is also supplied externally along with water from the catchment area. If we assume that the bulk of organic substances is accounted for by carbohydrates, then the mineralization of organic matter may be represented schematically as follows:

\[ \text{CH}_2\text{O} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} \]

In other words, the intensity of mineralization can be judged from the quantity of consumed oxygen.

As a rule, processes of mineralization of organic matter in a lake are carried out by heterotrophic bacteria and are associated with the latter's energy and synthetic metabolism. Energy metabolism involves the formation of reducing agents, NAD-H_2, and of high-energy compounds like ATP, owing to the energy released upon the oxidation of organic matter by molecular oxygen. Synthetic metabolism involves, in addition to the utilization of ATP and NAD-H_2, both the assimilation of formed organic compounds and the heterotrophic fixation of carbon dioxide, which latter accounts for some 6 per cent of the carbon contained in the microbial biomass.

In studying the relationship between oxygen uptake by microorganisms in energy metabolism and the heterotrophic assimilation of carbon dioxide in synthetic metabolism, Romanenko (1965, 1971) has found that most heterotrophs take up some 7 μg of CO_2 carbon per 1000 μg of oxygen used in oxidizing organic matter. Similar values have been obtained by Romanenko for natural microbiocenoses in lakes and artificial reservoirs.
It is thus possible to determine the quantity of oxygen taken up by microorganisms for the breakdown of organic matter by measuring heterotrophic assimilation of carbon dioxide. Such a determination is particularly important in those cases when the lake under study is oligotrophic and the extent of daily consumption of oxygen in a closed volume of water, which characterizes the magnitude of breakdown, approaches the limit of analytical sensitivity of dissolved oxygen analyses by the Winkler method. Under those circumstances, it is necessary to employ a more sensitive, though indirect, radiocarbon method for estimating the heterotrophic assimilation of carbon dioxide.

In most studies, the major problem of ecological microbiology, i.e. the mineralization of organic matter by total microflora in the water of lakes, has been tackled by estimating the absorption of oxygen. Short-term experiments have been staged with an isolated volume of water under conditions as close as possible to the natural ones, and the radiocarbon method has been used only for oligotrophic lakes.

As known, the intensity of phytoplankton photosynthesis falls sharply with depth because of the decreasing light flux, and is absent altogether below the photic zone. Degradation of organic matter, on the other hand, occurs throughout the water mass.

During spring floods and rains, enormous quantities of allochthonous organic substances enter continental lakes with the surface run-off. These substances undergo decomposition in the water body and partly pass down to silt deposits.

Determinations of the breakdown of organic matter have been made by Romanenko (1967) in various types of artificial lakes. Table 9 contains some data for the summer period. It will be seen from the Table that the degradation of organic matter usually predominates over its primary production. This fact is of fundamental importance because it shows the dominating role of bacteria in the degradation of incoming organic matter in natural and artificial lakes.

All organic suspensions, both those formed in the lake itself and those that have entered it from the catchment area, pass through the water mass before getting to the bottom. A large part of them undergoes mineralization.

Figure 17 shows changes in composition of suspended matter with depth in Lake Beloje as recorded in an analysis carried out in mid-summer when strong development of phytoplankton was observed in the lake (Kuznetsov, 1949).
<table>
<thead>
<tr>
<th>Name of lake</th>
<th>Type of lake</th>
<th>Ratio of breakdown to photosynthesis under 1 m²</th>
<th>Photosynthesis in 11 of surface water (mg C per day)</th>
<th>Breakdown in 11 of surface water (mg C per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tashkeprinskoje</td>
<td>Mountain, oligotrophic</td>
<td>2000</td>
<td>0.017</td>
<td>0.2</td>
</tr>
<tr>
<td>Syrojazynskoje</td>
<td>River bed, mesotrophic</td>
<td>1530</td>
<td>0.039</td>
<td>26</td>
</tr>
<tr>
<td>Ghirjurtskoje</td>
<td>River bed, mesotrophic</td>
<td>1344</td>
<td>0.050</td>
<td>21</td>
</tr>
<tr>
<td>Kamskoje</td>
<td>Lakes of the Northern Dvina Canal</td>
<td>3634</td>
<td>0.042</td>
<td>1344</td>
</tr>
<tr>
<td>Beloje, Novgorod region</td>
<td>Eutrophic</td>
<td>400</td>
<td>0.087</td>
<td>20</td>
</tr>
<tr>
<td>Kovzhskoje</td>
<td>Oligotrophic</td>
<td>59</td>
<td>0.087</td>
<td>0.95</td>
</tr>
<tr>
<td>Siverskoje</td>
<td>Dystrophic</td>
<td>54</td>
<td>0.079</td>
<td>0.104</td>
</tr>
<tr>
<td>Pokrovskoje</td>
<td>Mesotrophic</td>
<td>31</td>
<td>0.098</td>
<td>0.104</td>
</tr>
<tr>
<td>Blagoveshchenskoje</td>
<td>Eutrophic</td>
<td>28</td>
<td>0.134</td>
<td>0.134</td>
</tr>
<tr>
<td>Kubenskoje</td>
<td>Oligotrophic</td>
<td>23</td>
<td>0.143</td>
<td>0.143</td>
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<tr>
<td>Rybinskoje</td>
<td>Dystrophic</td>
<td>0.92</td>
<td>0.131</td>
<td>0.034</td>
</tr>
<tr>
<td>Gorlovskoje</td>
<td>Mesotrophic</td>
<td>0.92</td>
<td>0.158</td>
<td>0.2</td>
</tr>
<tr>
<td>Kuybyshevskoje</td>
<td>Eutrophic</td>
<td>0.92</td>
<td>0.158</td>
<td>0.2</td>
</tr>
<tr>
<td>Volgogradskoje</td>
<td>Oligotrophic</td>
<td>0.92</td>
<td>0.158</td>
<td>0.2</td>
</tr>
<tr>
<td>Tsimlianskoje</td>
<td>Eutrophic</td>
<td>0.92</td>
<td>0.158</td>
<td>0.2</td>
</tr>
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<td>Onezhskoje</td>
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<td>0.92</td>
<td>0.158</td>
<td>0.2</td>
</tr>
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<td>0.158</td>
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</tr>
<tr>
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<td>0.158</td>
<td>0.2</td>
</tr>
<tr>
<td>Vygozerskoje</td>
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<td>0.158</td>
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<tr>
<td>Matka</td>
<td>Mesotrophic</td>
<td>0.92</td>
<td>0.158</td>
<td>0.2</td>
</tr>
<tr>
<td>Alaukstas</td>
<td>Eutrophic</td>
<td>0.92</td>
<td>0.158</td>
<td>0.2</td>
</tr>
<tr>
<td>Doktas</td>
<td>Eutrophic</td>
<td>0.92</td>
<td>0.158</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Fig. 17. Changes in the composition of suspended matter in Lake Beloje in the stratification period. Plankton (1), organic detritus (2) (as percentage of total surface solids). (Kuznetsov, 1949.)

It can readily be seen that the bulk of suspended matter in the surface water at the time of blooming consisted of planktonic organisms and only a small part of it was accounted for by formless detritus. If this value is taken as 100 per cent, then over 90 per cent of suspended matter is decomposed during the descent to 7 m, and some of it becomes formless detritus. Still less suspended matter is found at 12 m. These results agree well with the data from chemical assays of the composition of plankton and of surface layers of silt in Lake Beloje at a depth of 12 m (Speranskaja, 1935). Comparison of those two analyses has enabled us to calculate (Kuznetsov, 1959) that 90 per cent of dead plankton is mineralized in the water body of the lake. The same has been reported by Ohle (1958, 1962) who studied plankton breakdown in Lake Plensee and other lakes of Schleswig-Holstein.

Determination of the extent of mineralization is of great importance for assessing the balance of ingress and breakdown of organic matter in a lake (Kuznetsov and Bezler, 1971).

In the Rybinsk artificial lake the extent of organic breakdown has been determined for a number of years since 1965, from the decrease in oxygen content upon 24-hour exposure of isolated water samples taken from different sites of the lake. Breakdown usually has two maxima in the annual cycle: in June when water warms up and the organic substances
supplied with melt water are decomposed, and in August, during the period of maximum production of organic matter in photosynthesis.

Some of the organic matter sediments and undergoes further decomposition. Thus in 1967, over a period of 164 days of the growing period, the amount of decomposed organic matter expressed in terms of carbon was 139 mg C per m² per day or 23 g C per m² throughout the growing period. Comparison of the values of aerobic breakdown in a water column 1 m² in area with that in the surface layer of silt of the same area has shown the quantity of organic matter mineralized in the water mass to be about 7-fold that in the surface layer of silt deposits (Table 10). Thus, while photosynthesis fluctuated in Lake Rybinsk from 180 to 330 thousand tons of C in the different years, mineralization of organic carbon in the water mass varied from 270 to 950 thousand tons; also, some 15 per cent of the organic matter underwent aerobic mineralization in the silt deposits.

A general characterization of the cycle of organic substances on the basis of principles of ecological microbiology has been made by Kuznetsov and Bezler (1971) for Lake Rybinsk in 1965 (Table 11).

The above data indicate that mineralization had accounted for some 500 thousand of the 800 thousand tons of organic carbon entering the lake while the net change to the system during the period was an accretion of a mere 58 thousand tons. These data, of course, imply a number of assumptions and show only the order of magnitude for a lake having an area of 4500 square kilometres and a total volume of 25 cubic kilometres. They do, however, clearly show that the lake represents an enormous purifying plant.

### Table 10

Production and breakdown of organic matter in Lake Rybinsk during growing period 1964-1969 (1000 tons C per lake)

<table>
<thead>
<tr>
<th>Years</th>
<th>Phytoplankton photosynthesis</th>
<th>Production of bacterial biomass</th>
<th>Breakdown in water mass</th>
<th>Aerobic breakdown in surface layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1964</td>
<td>100</td>
<td>117</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1965</td>
<td>183</td>
<td>291</td>
<td>490</td>
<td>—</td>
</tr>
<tr>
<td>1966</td>
<td>330</td>
<td>174</td>
<td>950</td>
<td>—</td>
</tr>
<tr>
<td>1967</td>
<td>280</td>
<td>136</td>
<td>633</td>
<td>95</td>
</tr>
<tr>
<td>1968</td>
<td>167</td>
<td>86</td>
<td>270</td>
<td>74</td>
</tr>
<tr>
<td>1969</td>
<td>261</td>
<td>143</td>
<td>456</td>
<td>113</td>
</tr>
</tbody>
</table>
TABLE 11
Characteristics of processes involved in the transformation of organic matter in Lake Rybinsk

<table>
<thead>
<tr>
<th>Source</th>
<th>Input of organic substances</th>
<th>Breakdown and output of organic substances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In tons of C for entire lake</td>
<td>In g of C m⁻²</td>
</tr>
<tr>
<td>With river waters and meteoritic sediments</td>
<td>474590</td>
<td>116-0</td>
</tr>
<tr>
<td>Photosynthesis of plankton and higher aquatic plants</td>
<td>167460</td>
<td>40-0</td>
</tr>
<tr>
<td>Bacterial assimilation of CO₂</td>
<td>17580</td>
<td>4-2</td>
</tr>
<tr>
<td>Remains of land vegetation</td>
<td>19000</td>
<td>4-5</td>
</tr>
<tr>
<td>Washing out of shores and peat bogs</td>
<td>54500</td>
<td>13-0</td>
</tr>
<tr>
<td>Nonrecorded sources of organic matter supply</td>
<td>24570</td>
<td>5-7</td>
</tr>
<tr>
<td>Positive balance for the water mass</td>
<td>57820</td>
<td></td>
</tr>
</tbody>
</table>

8 Conclusions

Winogradsky wrote that "a direct impact of environmental factors on the form and function of living beings is a law that admits no exceptions".

Despite this, bacteriologists did not pay much attention to creating conditions approaching the natural ones in their experimental studies of the forms and functions of naturally occurring microorganisms. This aspect seemed to be of secondary importance. Indeed, this question is of no interest to those who are concerned with the study of fermentation processes of industrial raw materials where specially bred species are often involved, adapted to those conditions for which they have been selected by the experimenters themselves.

The situation becomes entirely different if one is concerned with the
species that make up the microflora of natural habitats such as soil and water. In this case, ignorance of the conditions of life and activity of organisms under natural conditions is fraught with the danger of making erroneous conclusions regarding their roles in a non-laboratory environment. For that reason, it is always necessary to have a clear idea of the chemistry and physical characteristics of the medium in which these organisms developed in nature.

In studying soil microorganisms, Winogradsky took precisely that path of study. In contrast to the classical methods, isolating bacterial cultures from rich nutrient media, he advanced the principle of elective media, which enabled him at once to isolate a number of new organisms and to study their physiology wherever necessary.

Furthermore, basing himself on the study of the external environment, he developed a whole series of methods for observing the behaviour of microorganisms in natural substrates, which enabled him to discover the phenomenon of chemosynthesis and to understand, to a large extent, the processes occurring in soils.

Thus, while warning against pedantism in science, Winogradsky at the same time warned against limiting methods of investigation to the classical. It was necessary to make a quantitative assessment of the intensity of natural biological processes.

All these principles of Winogradsky are valid today. Science, however, is rapidly advancing and, despite a large quantity of scientific ballast, puts forward new ideas, improves its research methods and gains deeper insight into the nature of things. Today the time is ripe to pass to the study of biological processes directly in natural environments.

Evidently, problems of ecology can be resolved only by conducting a totality of physicochemical studies of the environment, in conjunction with studies of the physiology, biochemistry, morphology and fine structure of organisms and with experiments using radioactive labels or careful chemical assays to follow changes occurring in the environment under the effect of the natural microflora.

This is the road of present-day development of ecological microbiology whose firm foundations were laid down by Winogradsky.
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