Responses of Algae and Bacteria to Nutrient Additions in Bottled Lake Water*

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Abstract

Surface water of Lake Yunoko, a small eutrophic lake in central Japan, was bottled, enriched with glucose and/or mineral nutrients, and incubated for five days in the surface layer of that lake.

Bacteria reacted promptly to the addition of glucose. When, however, an ample amount of glucose was added to the lake water, certain mineral nutrients, possibly nitrogen, became secondarily limiting to the growth of bacteria. Although mineral nutrients alone stimulated the growth of algae, the stimulatory effect was far greater when both glucose and mineral nutrients were enriched.

Introduction

One of the prominent phenomena linked with eutrophication of aquatic environments is the outburst of algal growth. Phytoplankton ecologists have long accumulated the data to show that the growth or standing crop of phytoplankton is primarily limited by mineral nutrients such as nitrogen and phosphorus. However, since the report of KUENTZEL (1969), who insisted the importance of carbon dioxide supplied by bacterial decomposition of organic matter derived from inflowing sewage or industrial wastes, much controversy about the causes of eutrophication has arisen in North America (see, e.g., Proceedings of the Symposium on Nutrients and Eutrophication (ASLO, 1972)). Some investigators (KING, 1970; LANGE, 1971; KERR et al., 1973) supported KUENTZEL’s theory, but others (SHAPIRO, 1970; VALLENTYNE et al., 1970) opposed. More recently, the results of long-term experiments carried out in the Experimental Lakes Area, Canada, have shown the primary importance of mineral nutrients, especially phosphorus, to the eutrophication of lake (SCHINDLER et al., 1971, 1972, 1973; SCHINDLER and FEE, 1973, 1974).

Whatever the causes of eutrophication are, algae and bacteria are co-existing in aquatic environments, and thus they might have certain interactions in connection with various nutrients. Although much information about the effect of nutrients on the growth and production of phytoplankton is accumulating, the investigations into the interaction between algae and bacteria are relatively few.

In this paper, we report the results of an enrichment experiment carried out in Lake Yunoko, a small eutrophic lake in montane area of central Japan.

Methods

Lake Yunoko (area, 33ha; maximum depth, 12m; mean depth, 7.3m) with an altitude of 1,478m is located in the Nikko National Park, central Japan. Its biological productivity has been explored intensively.

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A field experiment was performed at the end of July 1972, when the water body completely stratified thermally. Surface water near lake center was poured into four clear 10l glass-bottles leaving air phase, and the bottled samples were enriched with nutrients as shown in Table 1. The bottles were then stoppered and incubated in the surface layer of the lake for five days. Since the bottles contained air and were agitated gently by wave, no precipitation of plankton was observed during the incubation period. Subsamples were withdrawn periodically and subjected to chemical and biological analyses.

Direct microscopic count of bacteria was made by the method of LUMPKINS and ARVESON (1968), using a membrane filter (SARTORIUS Co.; pore size, 0.2μm). Viable heterotrophic bacteria were counted by the agar plate method, in which nutrient broth diluted to 1/10 of the original concentration was used. Plates were incubated at 20°C for 15 days. Some microscopic observation on the abundance of phyto- and zoo-plankton was attempted, using plankton concentrates fixed with LUGOL solution, but the plankters were identified only at genus level.

The methods of chemical analyses employed were as follows: chlorophyll, UNESCO (1969); total carbon dioxide, SATAKE et al. (1972), using an infra-red gas analyzer (HITACHI-HORIBA Co.); dissolved oxygen, WINKLER method; sugar, anthrone method; ammonium, with the Nessler reagent after distillation; nitrite, with the GRIESS-ROMIJN reagent; nitrate, cadmium-copper reduction method described by STRICKLAND and PARSONS (1972); reactive phosphorus, MURPHY and RILEY (1962); particulate organic carbon (POC) and nitrogen (PON), with a CHN corder (YANAGIMOTO Co.). Except chlorophyll, dissolved oxygen, total carbon dioxide, POC, and PON, analyses were all made on samples filtered through a glass-fiber filter (MILLIPORE Co.) previously washed and combusted.

**Results**

Some physico-chemical and biological features of the surface water at the commencement of the enrichment experiment are shown in Table 2, where it can be seen that the water was fairly rich in inorganic carbon and nitrogen, but poor in reactive phosphorus. Both total and viable heterotrophic bacteria were abundant, reflecting a eutrophic nature of the lake. Microscopic observation of the plankton revealed that the predominant phytoplankters were *Asterionella*, *Fragilaria*, *Dinobryon*, *Cryptomonas*, and *Rhodomonas*, and the pre-

<table>
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<th>Experimental plot</th>
<th>Nutrients added</th>
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<td>1. Control (no addition)</td>
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<tr>
<td>2. Minerals (5mg N/l as (NH₄)₂SO₄, 2mg P/l as KH₂PO₄, 2.5mg K/l as KH₂PO₄)</td>
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<tr>
<td>3. Glucose (30mg/l=12mg C/l)</td>
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<tr>
<td>4. Minerals and glucose (same concentrations as above)</td>
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dominant zooplankters were *Tintinnidium, Keratella, Polyarthra, Asplanchna,* and *Filinia.* Crustacean zooplankters were few.

Upon addition of glucose and/or mineral nutrients to the lake water, bacteria reacted rapidly as shown in Fig. 1. Total bacteria increased significantly only in plot 4. Neither glucose nor minerals alone promoted conspicuously the growth of total bacteria. A similar result was obtained for the heterotrophic bacteria. However, glucose or mineral nutrients alone promoted more intensively the growth of heterotrophic bacteria than that of total bacteria. In plot 3, a peak of heterotrophic bacteria appeared after one day of incubation (the number of bacteria at this peak was ten times larger than that at the start of the experiment), whereas, in plot 2, it appeared after four days. This delayed growth of heterotrophic bacteria in plot 2 is suggested to be due to the effect of promoted algal growth. Above results indicate clearly that available organic carbon was the principal limiting factor for the growth of heterotrophic bacteria in the surface water of the lake, at least at the moment of sample collection.

Fig. 2 shows the changes in chlorophyll *a* concentration. In contrast with bacteria, phytoplankton reacted slowly to the enrich-
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Substantial differences in chlorophyll concentration among four plots appeared after three days of incubation. Interestingly, the final standing crop of phytoplankton was far larger when both glucose and mineral nutrients were added than when mineral nutrients alone were added. Although no detailed observation on the phytoplankton composition was made in the course of incubation, the density of *Asterionella* increased fairly in plots 2 and 4 compared with plot 1 (control). However, the major part of the increase in phytoplankton biomass in plot 4 seemed to be due to a significant increase of small green algae such as *Scenedesmus*, which was poor in the original water.

Sugar concentrations in the media enriched with glucose (plots 3 and 4) changed as shown in Fig. 3, where it is apparent that glucose disappeared within two days in plot 4, while a large amount of it remained in plot 3 at the end of the experiment. The remaining of glucose in plot 3 might be due to the lack of certain mineral nutrients, as will be described later.

The changes in concentrations of inorganic nitrogen and phosphorus are given in Fig. 4, from which the data on nitrite are omitted, because no appreciable amount of nitrite was detected in every plot. Ammonium was not detectable in plots 1 and 3 during the whole course of incubation. In the media enriched with ammonium (plots 2 and 4), however, it decreased more quickly in plot 4 than in plot 2. On the other hand, there was no significant decrease in the concentration of nitrate in plots 1, 2, and 4, whereas it was almost exhausted in the first two days in plot 3. These results exemplify that ammonium is more assimilable for microbes than nitrate. No significant decrease in phosphorus concentration was found in plots 2 and 4. In addition, the levels of phosphorus concentration in plots 1 and 3 were always low but detectable at a concentration of about 14 μg/l.

In this enrichment experiment, high levels of dissolved oxygen were kept in all plots.
Similarly, ample amounts of total carbon dioxide were present in all plots during the whole course of incubation (Fig. 5). These facts might suggest that neither dissolved oxygen nor inorganic carbon was limiting to the growth of algae and bacteria in this study.

Fig. 6 shows the changes in C/N ratio of particulate matter in four plots. (Fig. 5). These facts might suggest that neither dissolved oxygen nor inorganic carbon was limiting to the growth of algae and bacteria in this study.

Fig. 6 shows the changes in C/N ratio of particulate matter in each plot. The ratio was almost constant in plot 1, while it increased significantly in plot 3 and decreased in plots 2 and 4. These results indicate that microbial populations in plot 3
were deficient in nitrogen, suggesting that the most limiting mineral nutrient in the lake water was nitrogen.

**Discussion**

Our data presented here have shown that the primarily important factor in controlling the biomass of heterotrophic bacteria was available organic carbon in Lake Yunoko, at least at the moment of the experiment. When, however, an ample amount of organic carbon was supplied, certain mineral nutrients, possibly nitrogen, became secondarily limiting to the growth of heterotrophic bacteria. On the other hand, when only mineral nutrients were added to the lake water, heterotrophic bacteria increased slowly and reached a peak after four days of incubation. This delayed increase of heterotrophic bacteria progressed in parallel with the increase of algae. This fact suggests that the delayed growth of heterotrophic bacteria was caused by the supply of available organic carbon by algae. This supposition is consistent with an observation that a peak of heterotrophic bacteria appears at near chlorophyll maximum layer in the stagnation period in Lake Yunoko (Tezuka, 1970). More recently, Tanaka et al. (1974, 1975) reported that the peak of heterotrophic bacteria was closely related to the peak of the algal activity to produce extracellular products, principally glycollate, in Lake Biwa.

The most interesting result obtained by our study was the finding that the growth of algae was more accelerated by the enrichment of both glucose and mineral nutrients than by that of mineral nutrients alone. In this connection, Kuentzel's theory can be suggestive. With respect to our study, however, it is difficult to conclude that the theory is correct, because ample amounts of carbon dioxide were detected in all plots during the whole course of incubation, as already shown in Fig. 5. Another possible explanation for the stimulatory effect of organic carbon on the growth of algae might be sought either in the direct utilization of added organic carbon by algae or in the supply by bacteria of certain growth-stimulating factors to algae. The former is not likely to be the case, since in plot 4, glucose was already exhausted within two days of incubation, and an active algal growth began after three days. An additional support to this view is provided by the fact that glucose alone (plot 3) had almost no stimulatory effect on the growth of algae. On the contrary, the latter mechanism seems more likely to have been operative in this experiment. A number of algae have been shown to require accessory growth factors or to be stimulated by them (Saunders, 1957; Droop, 1962). Gordon et al. (1969) exemplified an interaction between algae and bacteria mediated by growth factors in a laboratory microcosm. More extensive studies should be carried out in future to clarify the effect of organic substances on the growth of algae.

Finally, it must be stressed that no matter how complicated the interrelations among organic matter, bacteria, and algae are, our data do not necessarily deny the primary importance of mineral nutrients to the eutrophication of aquatic environments. Our data have only shown the fact that algal growth in short term can be stimulated by the supply of organic carbon. Eutrophication is a long-term phenomenon taking place in aquatic environments.
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References


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