THE GELATINOUS SHEATH OF MICROCYSTIS IN LAKE SUWA

Yumiko, Amemiya [1)]

1) Faculty of Environmental Engineering, Yamanashi University, Takeda 4, Kofu 400, Japan

ABSTRACT

Chemical characteristics of the gelatinous sheath produced by the three Microcystis species which constitute waterbloom in Lake suwa were investigated from an ecological standpoint.

The outlook and solubility of the gelatinous sheath were different among M. aeruginosa, M. viridis and M. wesenbergii, though the main component was similar for them all; heteropolysaccharides. The gelatinous sheath of the three species was composed of the same sugar constituents, but each proportion was different among them. The easily soluble part of the gelatinous sheath of M. aeruginosa and M. wesenbergii fluctuated considerably with time. The organic materials of the gelatinous sheath released to lakewater could depend on the dominant species. The sugar composition of carbohydrates in the cells changed according to environmental stresses such as water temperature, solar radiation and the nutrient concentration.

Key words···gelatinous sheath; heteropolysaccharides; Microcystis; slime; sugar composition;

INTRODUCTION

Microcystis produces gelatinous sheath surrounding the aggregate of cells. The gelatinous sheath could accelerate the constitute of waterbloom by adsorbing some metals and protecting the cells from microbial attack and low temperature. However, the knowledge on the gelatinous sheath of Microcystis is very limited yet. Shnyukova and Pirezhenko(3) reported that slime was composed of carbohydrates like hemicellulose and pectic acids. Nakagawa et al.(2) also reported chemical composition of slime of axenic M. aeruginosa K-3A isolated from Lake Kasumigaura.

It is also indicated that the extracellular products of phytoplankton are the important carbon source for heterotrophic bacteria in aquatic environment. Kato and Stabel (1) found that free-living bacteria took extracellular dissolved organic carbon released from phytoplankton and synthesized their cellular materials. Thus, to reveal the chemical nature of the gelatinous sheath of the extracellular products of Microcystis is essential in considering the function of Microcystis in an ecosystem.

In this paper, I briefly summarized some properties and sugar composition of the gelatinous sheath of the three species, and their behavior in Lake Suwa.

EXPERIMENTS

The cells of Microcystis were collected from Lake Suwa. They were isolated unialgally and cultured in B12 medium at 25°C and 2500 lux as the standard conditions. M. viridis NIES-102 was obtained from the National Institute for Environmental Studies.

The gelatinous sheath was extracted by shaking (Yamato shaker, Model SA-31, 8), heat treatment (18-121°C), sonication (Tomy Seiko ultradisruptor, UR-200P, 25W), addition of EDTA, and dissolution in alkaline solution. Then, the gelatinous sheath carbohydrates were separated from intracellular car-

bohydrates by using a column chromatography of DEAE-Sephadex A-25.

Lakewater samples with Microcystis were collected in the morning at around 9 a.m. from the surface at the lake center of Lake Suwa during the period of bloom. Sampled lakewater was fractionated into four fractions; Dissolved organic material fraction (DOM), ES-shake fraction which was obtained by shaking, ES-sonic fraction obtained through sonication, and residual cell fraction. The procedure of the preparation was summarized Fig. 1.

Carbohydrates and proteins were determined by phenol sulfuric method and Lowry method, respectively. Sugar constituents were determined by using a gas chromatography according to the method of Nakagawa et al. (2).

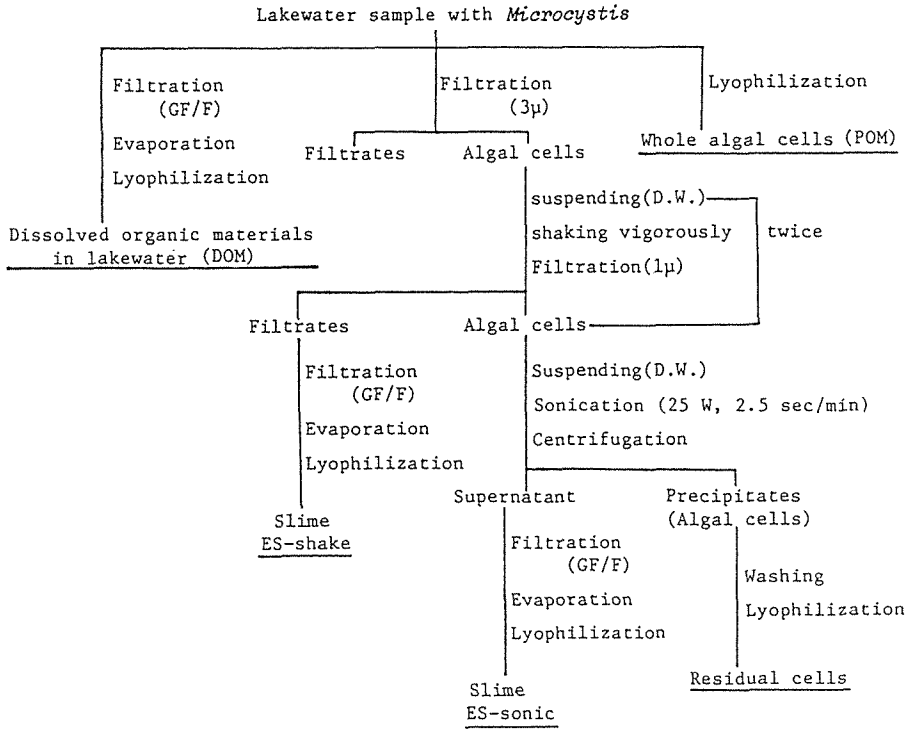

Fig. 1 Preparation of extracellular products

RESULTS AND DISCUSSIONS

Microscopic observations of the gelatinous sheath of Microcystis

The gelatinous sheath of the three Microcystis species collected in Lake Suwa was examined through microscopy. The gelatinous sheath of M. aeruginosa appeared clearly when stained with an India ink (Photo 1a). Some gelatinous sheath surrounding cells was stained light blue with alcian blue stain containing 0.1 M $MgCl_2$ (Photo 1b), while the others were not stained. This gelatinous sheath is also called slime, because it is undefined and unstructured. Photo 1c shows M. viridis stained with an India ink. This gelatinous sheath is also grouped into slime. This slime was stained blue with alcian blue containing 0.1 M $MgCl_2$. The slime was smooth with solid surface and somewhat waving. When a cell is released from the slime, a trace of the cell was clealy seen. Photo 1d shows M. wesenbergii stained with a nigrosin. There observed slime in the most outer layer, and the sheath exists

as a structured layer inside the slime. In addition, the sheath surrounds the cells in sol type slime. When the sheath is cut, cells come out slowly. The gelatinous sheath of three Microcystis species showed different aspects under a microscope.

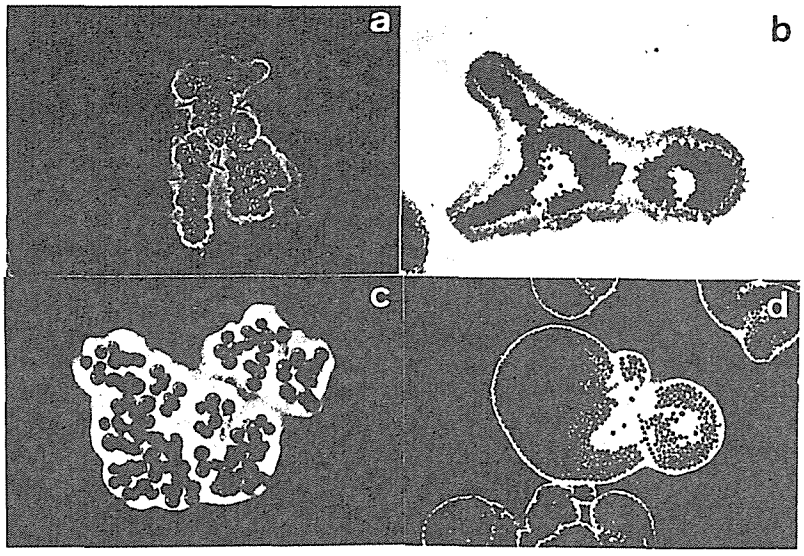

Photo 1 Three species of *Microcystis*
a. b. *Microcystis aeruginosa*
c. *Microcystis viridis*
d. *Microcystis wesenbergii*

Solubility of the gelatinous sheath in water

Solubilization of the gelatinous sheath was attempted by a shaking method, heat treatment, sonication, addition of EDTA, and dissolution in alkaline solution (Table 1). Microcystis cells collected in Lake Suwa on August 6 1986 were employed. The slime of M. aeruginosa and M. wesenbergii could be partly solubilized by all of the treatment methods. However, the sheath of M. wesenbergii was not solubilized by these mild treatments. With M. viridis, the slime could not be solubilized at all by these mild physical treatments. It could be solubilized with sodium hydroxide at the concentration of 0.25 N. The solubility of the gelatinous sheath also differed among the three species.

Table 1 Methods for gelatinous sheath extraction

1. Shaking	Part of slime of *M. aeruginosa* and *M. wesenbergii*
2. Continuous centrifugation (16,000 x g)	Part of slime of *M. aeruginosa* and *M. wesenbergii*
3. Sonication (25 W)	Part of slime of *M. aeruginosa* and *M. wesenbergii*
4. Heating at 75°C	Part of slime of *M. aeruginosa* and *M. wesenbergii*
more than 100°C	Slime of *M. aeruginosa, M. wesenbergii* and *M. viridis*
5. EDTA extraction	Part of slime of *M. aeruginosa* and *M. wesenbergii*
6. Sodium hydroxide	
0.025 N or more	Slime of *M. aeruginosa and M. wesenbergii*
0.25 N or more	Slime of *M. viridis*

Chemical composition of the gelatinous sheath

The chemical composition of the gelatinous sheath was determined in different times. The gelatinous sheath from Microcystis cells collected from Lake Suwa was continuously centrifuged by 1.6 x 10^4 G. The gelatinous sheath was consisted of carbohydrates with uronic acids by 35 to 47%. Some 18 to 24% was proteins (Table 2). The ash content ranged from 10 to 20%. Little amounts of lipids and nucleic acids were also detected.

Table 2 Chemical composition of the gelatinous sheath of *Microcystis*

Component	5 Sep. 1980 %	22 July 1981 %	22 Aug. 1981 %	9 Aug. 1982 %
Carbohydrates	46.3	45.3	47.0	35.4
(Uronic acids)	(14.0)	(14.2)	(14.8)	(12.2)
Proteins	18.2	24.5	24.3	23.4
Fats	3.0	2.3	4.1	2.7
Nucleic acids	2.2	1.4	1.6	3.0
Ash	18.6	9.7	14.6	20.1
Others	11.7	16.8	8.4	15.4

Sugar composition of the gelatinous sheath of the three cultured species

The sugar composition was determined and compared for the three species. The gelatinous sheath was prepared through the sonication of cells cultured in B12 medium and a column chromatography of DEAE-Sephadex A-25. Figure 2 shows the carbohydrate fractions separated into three peaks. Fractions A was eluted with Tris buffer at pH 8.0 without sodium chloride. A buffer containing sodium chloride at a concentration of 0.1 M for peak B, and a buffer with a linear gradient from 0.1 to 0.8 M sodium chloride for peak C, were employed respectively. It was found that intracellular carbohydrates appeared in fraction A and carbohydrates of the gelatinous sheath appeared in fraction C. Figure 3 shows the sugar composition of the gelatinous sheath. Xylose or glucose was the most abundant for two strains of M. aeruginosa, while for M. wesenbergii glucose, galactose, and xylose were the dominant constituents. For M. viridis, mannose was the main constituent. There found different proportions of sugars in the different strains and species even under culture condition.

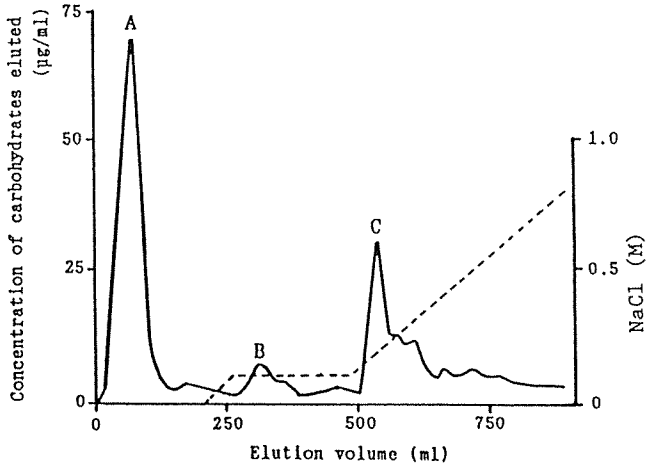

Fig. 2 Separation of carbohydrates in colonies of *M. aeruginosa* No. 1 by anion-exchange column chromatography

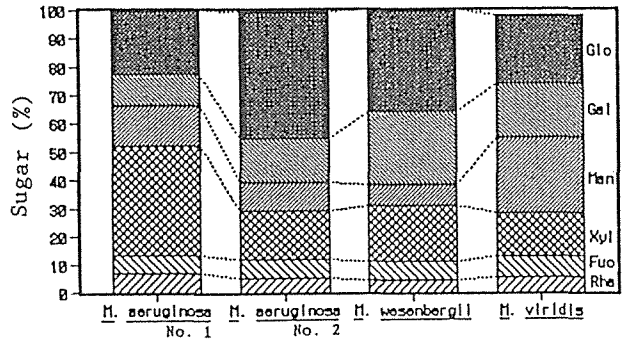

Fig. 3 Sugar composition of gelatinous sheath carbohydrates of each species

Behavior of the gelatinous sheath of Microcystis collected from Lake Suwa

The behavior of the gelatinous sheath in Lake Suwa was examined during a bloom of Microcystis in 1985, when cyanobacteria occupied from 93.8 to 99.7% of the total algal cell numbers from August 1 to October 4. Then it decreased to 79.6% on October 13. Microcystis species accounted for above 90% in cell volume among cyanobacteria between August 13 and October 13. The change in the main components of the gelatinous sheath was determined quantitatively and qualitatively for the fractionated lakewater. Table 3 shows the percentage of the ES-shake and the ES-sonic fractions relative to the total algal cell fraction. The gelatinous sheath of M. aeruginosa and M. wesenbergii was extracted in the ES-shake and the ES-sonic fractions, while that of M. viridis remained in residual cell fraction. In July to September, the percentage of gelatinous sheath in the ES-shake and the ES-sonic fractions was 7 to 22%. The low percentage was obtained on August 31 and September 23, when M. viridis predominated.

Table 3 Percentage of ES-shake and ES-sonic to POM (algal cells) (%)

	Slime		
	ES-shake	ES-sonic	Total
20 July	5.7	16.0	21.7
1 Aug.	3.9	8.2	12.1
13 Aug.	3.3	11.1	14.4
31 Aug.	3.4	5.0	8.4
23 Sept.	2.3	4.7	7.0
4 Oct.	9.8	14.5	24.3
13 Oct.	2.0	6.7	8.7

Dissolved organic carbon ranged from 3.9 to 5.2 mg/l with small fluctuations, and slime layer of the ES-shake and ES-sonic fractions ranged from 0.35 to 5.2 mg/l. POM fraction chiefly consisted of algal cells, and fluctuated from 2.4 to 11.4 mg C/l. The DOC contains not only algal extracellular products but other sources as products of decomposition.

Figure 4 shows the carbohydrate and protein contents in each fraction in different months. These components in the DOM fraction comprised only about 30% and little fluctuated. During the bloom, the carbohydrate content was higher than the protein content. The largest fluctuation in the percentages of the two components was in the ES-shake fraction. The total of the two components amounted to about 80% on the average. The fluctuation of the ES-sonic frac-

tion was smaller than the ES-shake fraction, and the carbohydrate content was generally higher than in the POM fraction. In the POM fraction, the content of each component was fairly constant, containing with much protein and less carbohydrate. The amounts of carbohydrates and proteins in the gelatinous sheath of M. aeruginosa and M. wesenbergii, which are readily soluble in water, fluctuated very significantly.

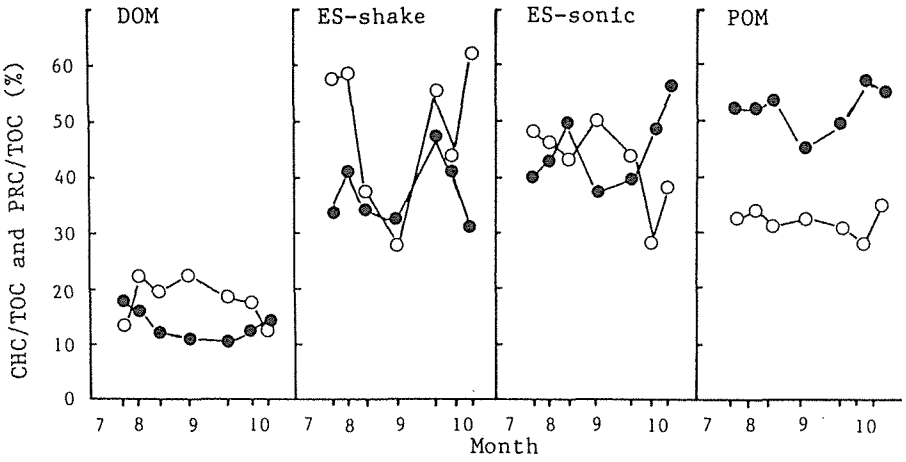

Fig. 4 Changes with time in the carbon content of carbohydrates (CHC) and proteins (PRC) in DOM, two fractions, and POM

—○— CHC/TOC —●— PRC/TOC

The physical treatments of shaking and sonication to extract slime fraction cannot be an absolute separation of the slime. I, thus applied a chemical procedure using 0.5 N sodium hydroxide with EDTA at 4°C. About 15 to 19% of the POM was extracted (Fig. 5). This content is higher than that of the physical treatment. The high contents in October are probably caused by solubilization of intracellular materials by chemical extract.

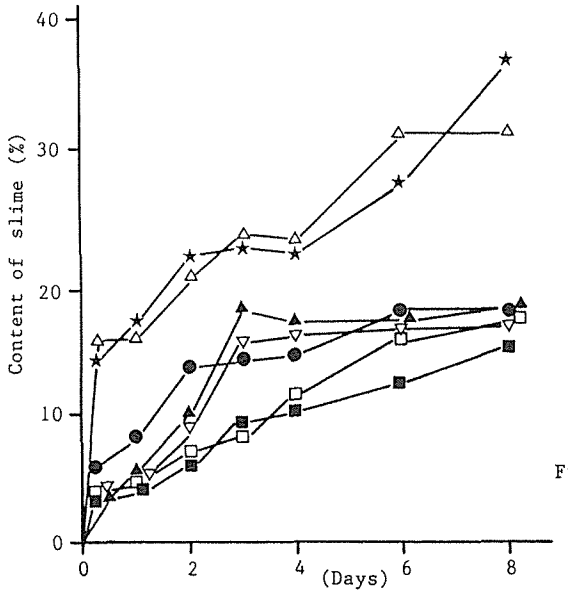

Fig. 5 Solubilization of the slime from the algal cells using 0.5N NaOH with 2% EDTA

—●— 7/20 —□— 8/ 1 —■— 8/13 —▲— 8/31
—▽— 9/23 —△—10/ 4 —★— 10/13

The amount of carbon of the physically extracted slime fraction was compared to that of the chemically extracted fraction. The recovery efficiency of the gelatinous sheath by the physical treatment compared to the chemical treatment decreased apparantly, when M. viridis became dominant in cell number among the three species (Fig. 6). The nature of the slime layer of M. viridis could differ from other two species. The organic materials of the gelatinous sheath released to lakewater might depend on the dominant species.

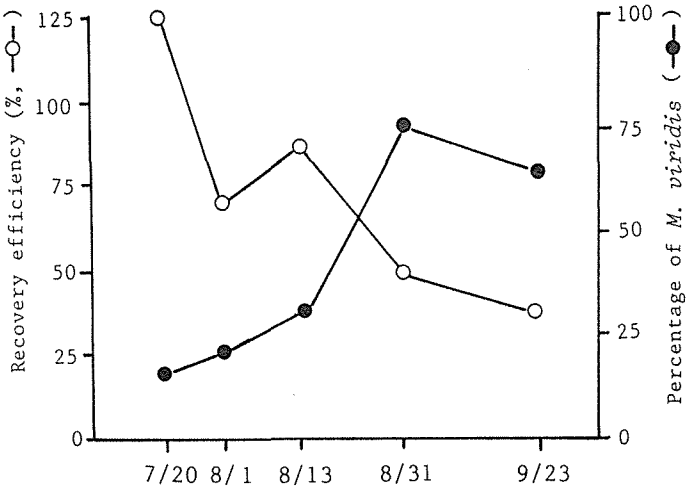

Fig. 6 The relation between the prevalence of *M. viridis* in *Microcystis* cells and the recovery efficiency of the slime by the physical method compared to the chemical analysis

Figure 7 shows the seasonal changes in the composition of neutral sugar for four fractions. In the DOM fraction, there observed little change in the percentage of the different constituents. In the ES-shake fraction, the percentage of glucose fluctuated largely. The other constituents appeared at about equal percentages and changed little in percentage. However, the percentage of mannose increased from the end of August to September. Mannose and fucose also increased at this time in both the ES-sonic fraction and the residual cell fraction. Thus, the intracellular sugar composition changed slightly. Because the dominant species changed in the middle of August, the changes in the intracellular sugar composition seemed not to correspond to the change in the dominant species.

I studied the environmental conditions that might affect the changes of chemical constituents of the gelatinous sheath. The photosynthetic activity of Microcystis drops significantly at water temperatures below 20°C. After September 21, the water temperature decreased below 20°C, and the gross primary productivity decreased sharply (Fig. 8). Solar radiation also affected the synthesis of carbohydrates. The decreased solar radiation on September 22 may also explain the changes in sugar composition (Fig. 8). Though the inorganic phosphorus and nitrogen concentrations of the lakewater are not shown, the amount of inorganic phosphorus decreased and that of inorganic nitrogen increased between September 21 and 30. This means that changes of environmental stress such as water temperature, solar radiation and the concentration of nutrients may result in the changes in the sugar composition of the cells.

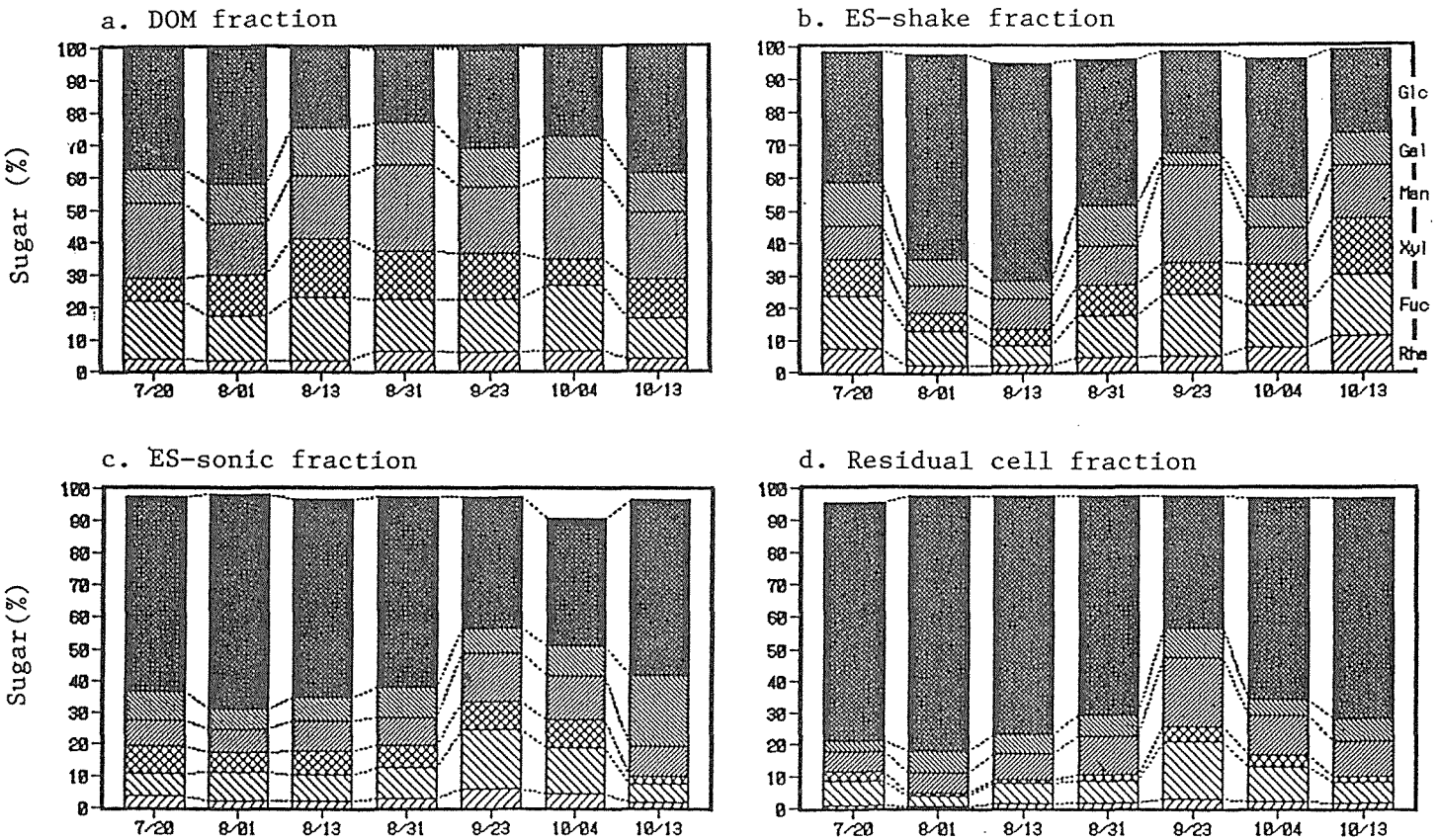

Fig. 7 Changes in sugar composition of four fractions

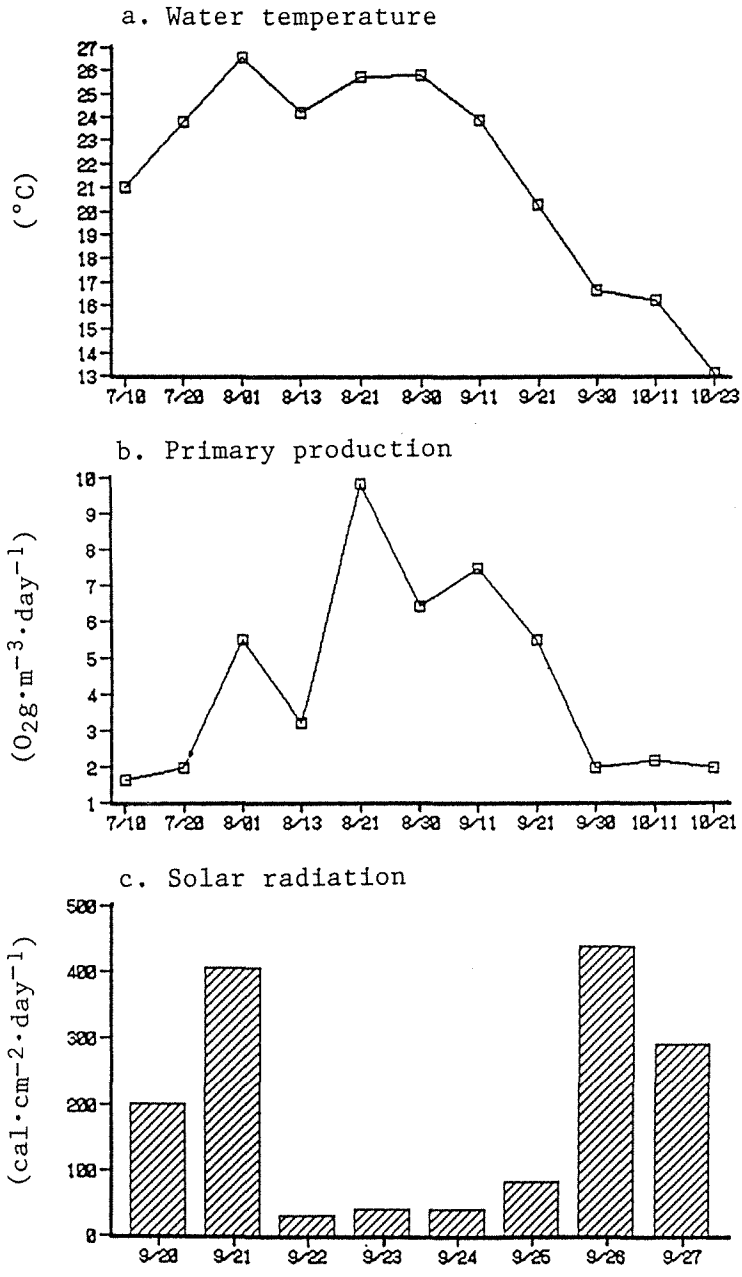

Fig. 8 Water temperature, primary production and solar radiation during the observation period in Lake Suwa

In contrast to the carbohydrates, the amino acids composition of protein which was produced during the bloom of Microcystis showed small fluctuation. This may suggest that the proportion of structural substance as protein is not so easily affected by environmental stress than the reserved one of carbohydrates.

The features of the gelatinous sheath of the three species are distinguished under the microscope. The gelatinous sheath also differed in solubility and sugar composition. The difference in features of gelatinous sheath among the three species may be caused by the difference in sugar composition, because the properties of polysaccharides are known to depend on the nature of their constituent sugars, the mode of their arrangement, and linkage configuration and position. The repeating unit of the main polysaccharides of gelatinous sheath might differ to a great extent among the three species.

In lakewater, the contents of carbohydrates and proteins of gelatinous sheath fluctuated and its sugar composition also changed from July to October. The changes in chemical composition of gelatinous sheath were probably resulted from a shift of dominant species, and production and solubilization of parts of gelatinous sheath which were affected by environmental conditions. In order to elucidate the behavior and role of the gelatinous sheath in lakewater, knowledges on chemical structure of gelatinous sheath are strongly required. There are at least three steps. First, the chemical structures specific to each species are to be found after the gelatinous sheath is solubilized and purified. Second, the chemical structures of the gelatinous sheath produced in relation to changing environmental conditions are revealed through the study of chemical structures of gelatinous sheath produced under the various culture conditions. Third, the chemical structure of the gelatinous sheath solubilized in water is to be also revealed, because this part dissolves in lakewater as a form of DOC. Thereafter, the function of the gelatinous sheath of Microcystis will be understood more clearly in ecosystems.

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