X-ray imaging of Picoplankton in Lake Biwa by Soft X-ray Microscope at Ritsumeikan University SR Center

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Abstract. Lake Biwa is the largest lake in Japan. Recently, its chemical oxygen demand (COD) index is increasing in spite of a decrease in the values of biochemical oxygen demand (BOD) index. In order to elucidate the mechanism, the first X-ray of a microstructure of picoplanktons inhabiting Lake Biwa was taken. After chemical fixation treatment, the laboratory-cultured Synechococcus cells were observed by soft X-ray microscope (BL12) of Ritsumeikan University SR Center. Each cell shows a dark sub-micron core. The low contrast region around the core can be interpreted as agar layers. The cells were estimated as 0.7 μm in diameter and agar layers were estimate as 1.2 μm in diameter. Namely the agar layer increases the quantification of organic compounds by factor of 1.7.

1. Introduction
Lake Biwa, located in the centre of Shiga prefecture, is the largest lake in Japan. It has a surface of 674 km² and is 103m deep (at the deepest point). It was divided into two parts, northern basin (NB) and southern basin (SB) at the narrowest point as boundary. The NB has less population than the SB, and NB is filled with nature. The lake Biwa has 460 inflowing rivers, but there are only two outflows, the Seta River and the Biwako Canal, and provides water for 14 million people in the vicinity and downstream urban regions including Kyoto and Osaka. In the past, Lake Biwa was a typical oligotrophic lake. The quality of the ecosystem has been rapidly reduced. In accordance with the guidelines issued by the OECD, the NB is classified as mesotrophic and the SB has gradually shifted from mesotrophic to eutrophic.

The water quality of Lake Biwa has been regularly surveyed since 1966. In early summer of 1989, a dense bloom of picophytoplankton occurred in the NB. During this bloom, the transparency of the lake decreased to less than half (1.2 m) of that observed during the same period in 1988 [1]. In addition, recently, the chemical oxygen demand (COD) index is increasing in spite of a decrease in the values of biochemical oxygen demand (BOD) index. These results suggest that an organic matter which is hard to decompose underwater has been increasing. Picophytoplankton is considered as an important source of the organic matter. We have started imaging of the picophytoplankton for investigating the microstructures of the picophytoplankton inhabiting Lake Biwa by X-ray microscope.
2. Picophytoplankton
Plankton consists of any drifting organisms that inhabit the pelagic zone of oceans or bodies of fresh water. They are defined by their ecological niche rather than their genetic classification. They are also often described in terms of size. Photosynthetic picoplankton (picophytoplankton) is the fraction of the plankton performing photosynthesis composed by cells between 0.2 and 2 µm. It is especially important in the central oligotrophic regions. Because of its very small size, it is difficult to study by classical methods such as optical microscopy. Since the 1970s, the spread of the epifluorescence microscopy has allowed to detect picophytoplankton such as Synechococcus which possess phycoerythrin.

3. Materials and methods
Laboratory-cultured Synechococcus cells, photosynthetic picoplankton, were isolated by centrifugation at 6,200 rpm for 3 min at room temperature. The cells were fixed with 1 % glutaraldehyde for 30 min at room temperature. After fixation, Synechococcus cells suspension was placed on a copper mesh with polyvinyl formbar (PVF) membrane and air-dried. When approaching a wet sample observation, a wet sample holder was used [2]. It consists of 2 plates and each plate consists of a thin polyimide film (thickness < 300 nm) and a metal support thin metal plate. Synechococcus cells suspension was sandwiched between them. X-ray observation was performed at soft X-ray microscope beam line BL12 of Ritsumeikan University SR Center. Observing wavelength is 2.3 nm and exposure time is 5 min.

4. Results and Discussion
Figure 1 shows air-dried and wet Synechococcus cells. In all micrographs, each cell is clearly distinguishable. Spherical cells and cocoon form cells were observed. The cell of the cocoon form is on cell division. Each cell has a dark sub-micron core. Since a Synechococcus cell is covered with agar layer, the low contrast region around the core can be interpreted as agar layer. From figure 1 (b), the cell was estimated as 0.7 µm in diameter and agar content was estimated as 1.2 µm in diameter.

Using the image contrast, fraction of carbon was estimated under the following assumptions: (1) during the drying process, a cell size maintains. Although agar size on the grid plane also maintains, thickness of agar layer is reduced. Therefore the rate of volume contraction of agar is K. (2) Cell consists of protein. (C_{94}H_{139}N_{24}O_{31}S, \mu_{cell} = 1.81 / \mu m at 2.3 nm, \rho = 1.35 g/cm^3) (3) Agar consists of agarose. (C_{12}H_{22}O_9, \mu_{agar} = 0.95 / \mu m at 2.3 nm, \rho = 1.54 g/cm^3) Figure 2 shows X-ray image intensity profile of PP cells. The X-ray transparency ratio is obtained as A: B = 2:7. Using these values, rate of volume contraction of agar layer, K, is estimated as about 80 %. Quantity of organic matter is converted into that of carbon. In case of protein, carbon is 94 per mol and in case of agarose, carbon is 12 per mol. Agar layer makes the amount of carbon of a cell increases 4.5 - 7 times. Namely the agar layer increases the quantity of organic compounds by maximum factor of 7. Quantification of agar content is required for assessing the water quality in Lake Biwa. Improving a sample preparation and observation technique, agar content will be able to be quantified more correctly.

5. Conclusions
In Lake Biwa, COD index is increasing in spite of a decrease in the values of BOD index. Picophytoplankton is considered as an important source of non-biodegradable organic compounds. Picophytoplankton was observed by the soft X-ray microscope.
1. Each Synechococcus cells were successfully observed with high resolution. In addition, agar content around the cell was also observed.
2. The cell size was estimated as 0.7 µm in diameter and agar layer was estimated as 1.2 µm in diameter. The agar layer increases the quantification of organic compounds by maximum factor of 7.
Figure 1. X-ray microscopic images of laboratory-cultured *Synechococcus* PGS and PP cells. (a-1) Dried and (a-2) wet *Synechococcus* PGS cells, (b) dried *Synechococcus* PP cells.

Figure 2. X-ray image intensity profile of PP cells. Digitally enlarged image of figure 1 (b) left side is used for the estimation. A is a cell with agar content. B is agar only.

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References
[1] Eguchi M, Oketa T, Miyamoto N, Maeda H and Kawai A 1996 *J. Plankton Res* 18 539-550
[2] Takemoto K, Watanabe N, Hirai A, Nakayama Y and Kihara H 1998 in "X-ray Microscopy and Spectromicroscopy (eds. J. Thieme, G. Schmahl, D. Rudolph, & E. Umbach)", Springer-Verlag, Berlin, pp.I-129 - I-134