

博士論文（要約）

Characterization of Low Molecular Weight Dissolved Organic Matter Contributing to COD_{Mn} in Lake Water by Orbitrap Mass Spectrometry

（湖水 COD_{Mn} に寄与する低分子溶存有機物の
Orbitrap 質量分析による特性評価）

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Water resources in Japan mostly rely on surface waters, especially lakes including natural lakes and artificial reservoirs. However, the deterioration of water quality in lakes causes various problems in drinking water. Chemical oxygen demand determined by the use of potassium permanganate (COD_{Mn}) as an oxidant has been used as an environmental water quality standard for lake water quality in Japan. Regardless of various countermeasures, the compliance rates of COD_{Mn} in lakes have not been improved for decades. COD_{Mn} is composed of particulate COD_{Mn} (PCOD_{Mn}) and dissolved COD_{Mn} (DCOD_{Mn}). Though PCOD_{Mn} is basically associated with algae in lakes, the composition of DCOD_{Mn} remains unknown. Bulk parameters such as DCOD_{Mn} or even dissolved organic carbon (DOC) does not show anything about the composition. Furthermore, it has been well known that oxidation power of potassium permanganate is highly dependent on the types of compounds. Therefore, it is difficult to clarify the exact targets contributing to DCOD_{Mn} . In order to implement the effective countermeasures to improve the compliance rates of COD_{Mn} in lakes, molecular-level characterization of organic matter is required to specify dissolved organic matter (DOM) responsible DCOD_{Mn} and understand the mechanism of organic pollution. Recent development of high resolution and high accuracy mass spectrometry coupled with soft ionization has shed a new light on the composition of DOM. With the power of new mass spectrometry, complex DOM can be resolved and molecular formula can be assigned to each component based on its accurate mass.

In this study, high resolution and high accuracy Orbitrap mass spectrometry (Orbitrap MS) coupled with negative electrospray ionization (ESI) was used to characterize DOM molecules in lakes which were extracted by solid phase extraction (SPE). Furthermore, DCOD_{Mn} molecules were screened by integrating the pretreatment by potassium permanganate oxidation and Orbitrap MS. DCOD_{Mn} molecules in this study were defined as molecules which completely disappeared from mass spectra after the oxidation by potassium permanganate. On the other hand, persistent molecules included molecules which were partially oxidized or were totally refractory. The objective of this study was to characterize DOM and DCOD_{Mn} molecules in lakes by making the best use of Orbitrap MS. Seasonal variations and potential sources of DOM and DCOD_{Mn} molecules in Lake Inba, a hypereutrophic lake in Japan, were intensively studied. In addition, DOM and DCOD_{Mn} molecules in different lakes with different water quality and geographical properties were compared to discuss common or unique molecules contributing to DCOD_{Mn} .

In Chapter 1, introduction and objectives of the thesis was described.

In Chapter 2, literature reviews related to the thesis was summarized.

In Chapter 3, all materials and methods in the thesis were described.

In Chapter 4, fundamental studies were discussed for applying Orbitrap MS to analyze DOM and screen DCOD_{Mn} molecules. While DOM consists of molecules with a wide range of molecular weights, the analytical window of Orbitrap MS is limited (<1 kDa). Therefore, the contribution of large molecular weight DOM larger than 1 kDa and low molecular weight DOM smaller than 1 kDa were investigated. Water samples collected from Lake Inba were fractionated with a 1 kDa molecular weight cutoff membrane to analyze DOC and DCOD_{Mn} concentrations in each size fraction. The fraction smaller than <1 kDa accounted for 52–63% and 51–65% of the collected DOM in terms of DOC and DCOD_{Mn}, respectively. Moreover, unfractionated samples, high molecular weight DOM larger than <1 kDa, and low molecular weight DOM smaller than <1 kDa were subjected to solid phase extraction and analyzed by Orbitrap MS. Peaks below 1 kDa were detected even from the > 1 kDa fraction as well as the < 1 kDa fraction and unfractionated sample. While these samples shared most of molecules, some differences were observed. Disintegration or fragmentation of large DOM by ESI, mitigation of ion suppression by size fractionation, or migration of low molecular weight DOM to > 1 kDa fraction during fractionation step could be the reasons for the observation. Unfractionated samples were used for further analysis.

Changes in molecular-level compositions of DOM during KMnO₄ oxidation was investigated by Orbitrap MS. Water sample collected from Lake Inba was pretreated with KMnO₄ oxidation by varying oxidation durations. It was found that the most of the changes occurred during preheating step before the temperature reached 95°C. Orbitrap MS could detect the dynamic changes of molecules. The molecules with high DBE-O (unsaturation index) were selectively oxidized and disappeared from the mass profile. These molecules were regarded as major molecules contributing to DCOD_{Mn}. Thereafter, little change was observed. Molecules which newly appeared during oxidation might be generated from larger molecules by oxidation. The current findings confirmed that Orbitrap MS was applicable to screen DCOD_{Mn} molecules from DOM pool.

In Chapter 5, seasonal variations of DOM and DCOD_{Mn} molecules in Lake Inba (the western Lake Inba) were studied. Lake Inba is the most polluted lake which is used for water supply in Japan. The lake is severely deteriorated due to high organic concentrations and eutrophication. Water samples were collected from Lake Inba bimonthly from May in 2016 to March in 2017. They were concentrated by SPE and analyzed by Orbitrap MS in negative ESI mode to characterize DOM molecules. Approximately 50% of the assigned molecular formulas consisted of only carbon, hydrogen and oxygen atoms in all samples, followed by molecules additionally containing

nitrogen and sulfur. Carboxylic group was mainly suspected as a possible form of oxygen in the CHO formulas. Based on the van Krevelen diagram analysis, the major components of DOM formulas in all samples had similar elemental fingerprints to lignin and tannin. According to cluster analysis, DOM composition were clustered as 1) May, July and September, 3) November, January, March.

The water samples were treated by potassium permanganate before SPE. Comparison of mass spectra before and after the oxidation pretreatment were evaluated to screen DCOD_{Mn} molecules. In this study, DCOD_{Mn} molecules were defined as molecules which completely disappeared after the oxidation, while persistent molecules were defined as molecules which remained even after the oxidation. Small portions of DOM molecules were categorized to DCOD_{Mn} molecules according to the definition of this study, indicating that the limited range of compounds in DOM were easily oxidized by potassium permanganate. The percentages of CHON molecules in DCOD_{Mn} molecules were larger compared to those in persistent molecules. The cluster analysis of DCOD_{Mn} molecules was not consistent with the clustering pattern of DOM molecules. DBE-O of DCOD_{Mn} molecules were relatively higher than that of persistent ones, indicating that DBE-O could be the useful parameter to estimate whether molecules are mainly responsible for DCOD_{Mn} or not.

In Chapter 6, DOM molecules in major rivers (Kashima River and Kanzaki River) inflowing to Lake Inba were matched with DOM and DCOD_{Mn} molecules in Lake Inba to discuss the potential sources of these molecules. Molecular-level characterization of DOM indicated that DOM in Lake Inba was largely contributed from the rivers. Cluster analysis indicated that DOM compositions of Lake Inba was highly dependent on those in the rivers in different seasons. Contribution of the rivers to DCOD_{Mn} in Lake Inba was discussed by comparing DOM molecules in the river samples and DCOD_{Mn} molecules in Lake Inba. 62–96% of the total DCOD_{Mn} molecules in the lake could be matched with molecules in the rivers. This indicated that the rivers were one of the important sources of DCOD_{Mn} in the lake.

In Chapter 7, DOM and DCOD_{Mn} molecules in eight different lakes were analyzed. In addition to Lake Inba collected in September, 2016, seven lake water samples were collected from Lake Hachiro, Lake Kasumigaura, Lake Tega, Lake Tsukui, Lake Miyagase, Lake Ogochi, and Lake Biwa during August to October in 2016. These lakes were classified into high DOM lakes and low DOM lakes according to the levels of DOC and DCOD_{Mn}. CHON, CHOS and CHONS formulas in DOM molecules were more abundant in high DOM lakes. CHO formulas were more dominant in low DOM lakes. As observed in Lake Inba in Chapter 5, only the limited number of

DOM molecules were recognized as DCOD_{Mn} molecules in different lakes. While this is partly due to the limitation of current analytical condition, it could be consistent with the poor coverage of COD_{Mn}. The compositions of DCOD_{Mn} molecules were significantly different from persistent molecules as well as original DOM molecules. CHON, CHOS and CHONS were more dominant in DCOD_{Mn} molecules in high DOM lakes and Lake Biwa. On the other hand, CHO molecules were always dominant in persistent molecules. The cluster analysis demonstrated that compositions of DCOD_{Mn} molecules in high DOM lakes were different from those in low DOM lakes.

In Chapter 8, the overall conclusions and recommendations were described. This study showed that Orbitrap MS combined with potassium permanganate pretreatment was able to screen DCOD_{Mn} molecules from DOM in lake water. This is the first study to reveal the composition of DCOD_{Mn} molecules and their seasonal variations in lakes. The results of the thesis are informative to deeply understand DCOD_{Mn} molecules and effective countermeasures to achieve the COD standard in lakes. However, even Orbitrap MS can evaluate only some portions of DOM within the analytical window. DOM molecules which cannot be extracted by SPE nor ionized by ESI are not detected. Further study is necessary to accumulate the scientific knowledge about DCOD_{Mn} molecules as well as DOM molecules in lake water.