

# Feature Article

Application

## Analysis of the Chromophoric Dissolved Organic Matter in Process Water by EEMs and PARAFAC

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In sewage and industrial wastewater treatment facilities, monitoring the concentration of organic material is important for operation and maintenance. However, even if the Total Organic Carbon (TOC) in the wastewater is the same, it is known that there will be differences in the water quality after the process. For that reason, it is important to understand the component groups that make up the Dissolved Organic Matter (DOM), as well as their prevalence. In this study, we measured the Chromophoric Dissolved Organic Matter (CDOM) in each sample using three-dimensional Excitation-Emission Matrices (EEM) and the Parallel Factor Analysis Method (PARAFAC), and analyzed the behavior of each component group in wastewater treatment processes using a membrane bioreactor method. We then compared our results with Specific Ultraviolet Absorption (SUVA) and PARAFAC analysis results, which are used as relative indicators of recalcitrant organic matter.

### Introduction

The behavior of Dissolved Organic Matter (DOM) in lakes, marshes, and basins is important for preventing water pollution in the environment. Regardless of the fact that the load of pollutants flowing from basins into lakes and marshes has been on a decreasing trend in recent years, a paper reported that the organic matter concentrations in water in lakes and marshes are somewhat on an increasing trend.<sup>[1]</sup> In Japan, there are reports that recalcitrant organic matter is increasing and accumulating in lakes, marshes, and closed inner bays.<sup>[2]</sup> In Japan, factories and offices are some of the sources of organic matter, and concentration regulations have been enacted and the total volume of pollutant load discharged into public water is being controlled based on the Water Pollution Control Law. Sewage and factory wastewater treatment plants remove pollutants by coagulation sedimentation processes and biological treatments in order to meet the requirements in these regulations. It is important to monitor the organic matter concentration in each process in order to perform the process efficiently. Membrane Bioreactor (MBR) are also used for sewage and factory wastewater treatment plants, and it has been reported that MBRs can remove the Biological Oxygen Demand (BOD) and suspended solids more efficiently

than the conventional method.<sup>[3]</sup> However, as operating time increases, the filtration performance decreases markedly and causes membrane fouling, which is a serious problem. Membrane fouling is thought to be caused by natural organic matter represented by humic substances in the water and extracellular polymers secreted by microorganisms in activated sludge.

BOD<sub>5</sub> (5-day BOD), COD (Chemical Oxygen Demand) and TOC (Total Organic Carbon) have been used as indicators of DOM. But BOD<sub>5</sub> only detects materials that can be broken down by microorganisms, and takes 5 days for analysis. COD has a large margin of error by analysis workers, and also has other problems such as not quantitatively oxidizing organic matter in water.<sup>[4]</sup> It is possible to use carbon conversion to quantitatively express TOC as the total amount of DOM, and this has been used as an indicator for organic matter in environmental water and in various types of wastewater.<sup>[5]</sup> Therefore, TOC is becoming a major water quality item in organic matter analysis. However, even though TOC can be used to find out the quantity of organic matter, it cannot be used to understand the quality. For example, different sets of wastewater that have the same TOC values show clear differences in processing performance. DOM information is very important for checking how



Figure 1 Aqualog

much recalcitrant COD components are included in the DOM in the process water, or to check the processing process to classify the substance groups and types of DOM. It is possible to obtain detailed information about DOM by analyzing the DOM using a chromatographic method, but it is not practical to change the processing for each dissolved substance. Understanding the groups of components that make up the DOM and their ratios, and clarifying the differences in processing capability by component group makes it possible to propose optimized solutions for treatment. DOM in sewage and industrial wastewater contains humic substances and proteins.

Components contained in the DOM that fluoresce by absorbing light in the ultraviolet and visible ranges are called CDOM (Chromophoric DOM). Three-dimensional excitation-emission fluorescence spectroscopy (excitation-emission matrix: EEM) are used as techniques for simple analysis using the optical characteristics of CDOM from natural and man-made organic compounds.<sup>[6, 7]</sup> Fluorescence spectra differ from the ultraviolet visible absorption spectra because fluorescence spectra have several emission peaks, and are advantageous because it is possible to obtain more information on DOM origins and composition. EEM can be measured quickly with a small amount of samples, and simple pre-treatment such as filtration. We measured the water quality of each processing step in the wastewater treatment process using a three-dimensional fluorescence measurement device. (Aqualog; Figure 1) We applied the multivariate analysis method (Parallel Factor Analysis: PARAFAC) and evaluated the behavior of the component spectra. For information about Aqualog equipment calibration and PARAFAC details, please see the previous report.<sup>[8]</sup>

## EEM and PARAFAC

The fluorescence spectroscopy method applies excitation light to the substance to be measured, and measures the fluorescence spectrum emitted. Fluorescence analysis has high sensitivity and can be done quickly with a small amount of samples and simple pre-treatment. It is known

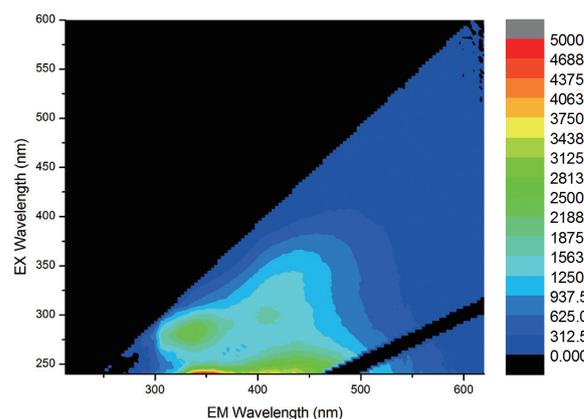


Figure 2 EEM of a Point Where the Kamo River and Katsura River Intersect

that can be obtained details, dynamics and structure of DOM from ultraviolet absorption spectra.<sup>[8]</sup> On the other hand, components that do not produce fluorescence when the excitation light is applied cannot be measured. EEM is a technique for continuously measuring the fluorescence wavelengths emitted for various excitation light wavelengths. EEM is an analytical method that can get detailed information about DOM from the intensity and peak position. Figure 2 is an EEM of river water that was collected at the confluence of the Kamo River and Katsura River in Kyoto.

Operation is simple. Put a liquid sample in a 1-cm-long cell for measuring fluorescence, anchor it in the sample room, and start taking measurements. In general when suspended solids are included, samples are analyzed after being filtered through a filter with a pore size of 0.45-0.70  $\mu\text{m}$ . (models such as GF/F made by Whatman and GB-140 made by Advantec are often used). In addition, it is necessary to take care because sometimes fluorescence components in EEM change greatly before and after filtration through a filter. EEM spectra need to be corrected to get an accurate fluorescence from CDOM. Some of these corrections are using Raman units or quinine sulfate unit, Rayleigh scattering masking, removing Raman light scattering, Inner Filter Effects (IFE), and device spectra corrections on the obtained EEM analysis results.<sup>[9]</sup> Aqualog has functions for easily doing these operations. EEMs include much information, so it is difficult to obtain detailed information due to the overlap of the spectra. It is also difficult to quantify the information on the individual spectra. The CDOM components can be identified by applying PARAFAC for EEM and separating the individual peaks. One of the multivariate analysis methods, PARAFAC uses a number of EEM to statistically break down the overlapping peaks into fluorescence components that have the same behavior.

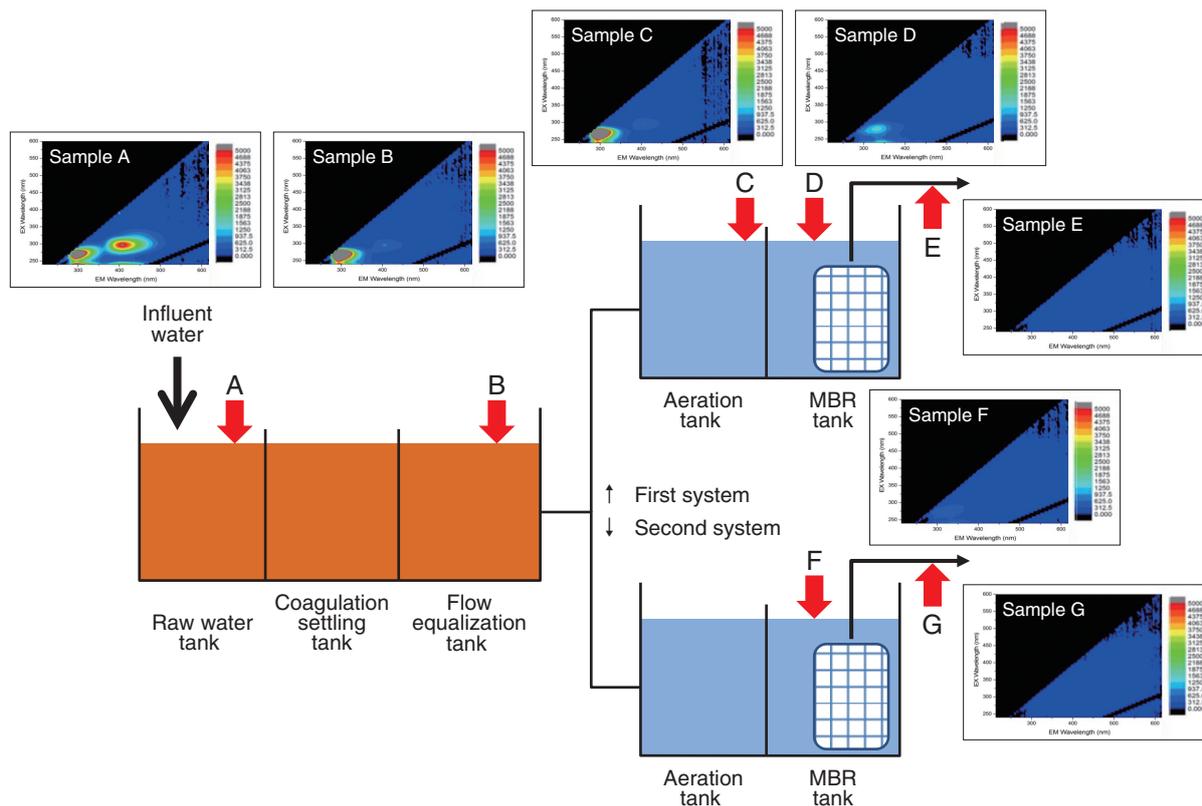


Figure 3 Schematic of Test Plant and Sampling Points (Samples A-G) (EEM of Each Sample)

### Specific Ultraviolet Absorption (SUVA)

The Aqualog simultaneously analyzes fluorescence and absorbance. It is known that the absorbance of the sample increases as the concentration of organic matter increases. In particular, the absorbance from humic substances increases exponentially as the wavelength becomes short. In particular, SUVA is defined using the DOC concentration in the sample and the absorbance at 254 nm. SUVA is calculated using the following equation.

$$SUVA = 100 \times A_{254} / (L \times C)$$

A is the absorbance at 254 nm, L is the cell length [cm], and C is the DOC concentration in the sample [mg/L]. In environmental water analysis, the SUVA value indicates the relative ratios of aromatic compounds contained in the DOM. In particular, there have been reports that in cases where the SUVA value exceeds 0.02-0.04, the water contains high levels of biologically recalcitrant organic matter.<sup>[10]</sup> In this evaluation, we compared the SUVA values and water quality analysis items for each type of process water.

### Analysis of Water in Industrial Wastewater Treatment Processes

As previously explained, fluorescence analysis methods are widely used for analyzing DOM in water in the environment. But there are few examples of applying the fluorescence analysis method to industrial wastewater treatment processes. Therefore, we collected samples from each wastewater treatment process at a chemical plant in Japan between September 2013 and January 2014 and measured TOC, COD<sub>Cr</sub>, and EEM. We found the EEM by scanning excitation wavelengths from 220 to 600 nm and detecting emission wavelengths from 220 to 600 nm. The integration time is 0.1 second. After taking measurements, we did blank correction, Rayleigh masking, and IFE correction. Figure 3 is an overview of the sampling points in the wastewater treatment processes used in this paper.

This process is consisted of a coagulating sedimentation process, biological treatments, and a Membrane Bio-reactor (MBR) process. The process was divided into two different steps after the flow equalization tank. In this paper, these are called the first system and second system. Each process has a different air diffuser installed in the aeration tank. Samples collected at Sampling Points A-G were stored in a plastic container at 10°C or below and

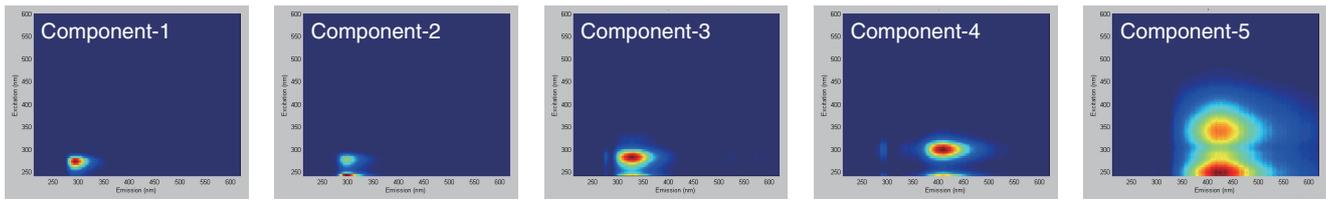


Figure 4 Results of Parallel Factor Analysis

transported shielded from light. Figure 3 shows an EEM of the samples.

Based on the results in Figure 3, we found that the fluorescence peak (fluorescence excited 300 nm, and emitted 410 nm) intensity decreased significantly before and after the coagulation sedimentation tank (Samples A and B). There was no major change in the fluorescence peak position and intensity before and after the aeration tank (Samples B and C) in the first system. When we compared the EEM of aeration tank water (Sample C) and MBR tank water (Sample D), we found that the fluorescence peak at Ex/Em=300/410 nm was almost quenched, and the fluorescence peak intensity at Ex/Em=280/330 nm also decreased significantly. The area from the aeration tank to the MBR tank is a biological treatment, and we believe that the DOM was broken down as the process progressed. The fluorescence peak in the membrane filtration water (Sample E) was almost quenched. There is reason to believe that DOM was trapped by the membrane.

On the other hand, when we compared the EEM for the water in each process MBR tank (Samples D and F), the fluorescence peak intensity was lower in second system. For the results of these measurements, we applied PARAFAC. There were 159 samples. The PARAFAC analysis results showed that there are 5 types of component spectra included in EEM. (Figure 4) Comparing the separated component spectra with the earlier literature,<sup>[11-13]</sup> Component 1 is a tyrosine-like component, Component 2 is tryptophan-like component,

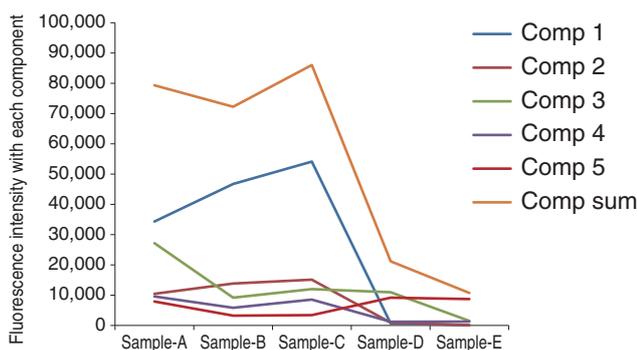


Figure 5 Comparison of Contributing Rates at Each Process (First System)

and Components 4 and 5 are humic-substance-like components. The results of a separate study by the authors on Component 2 suggested that it has a phenylalanine-like fluorescence peak.

Using PARAFAC to combine these component spectra makes it possible to numerically express the intensity of the component spectra included in each EEM obtained from the samples. We calculated the intensity of the component spectra of each EEM from the raw water tank to the MBR-treated water in first system. (Figure 5) As a result, tyrosine-like components increased gradually until the aeration tank water, and were quenched in the MBR tank water. Phenylalanine-like fluorescence components were greatly reduced between the aeration tank and the MBR tank. We consider that tyrosine-like and phenylalanine-like components were probably degraded by biological treatments. Tryptophan-like fluorescence components were greatly reduced before and after the coagulation sedimentation tank, but the residual components could not be removed with biological treatment, and were completely quenched after membrane treatment. We consider that the components stuck to the membrane. Out of the humic acid and fulvic acid-type components, Component 4 was quenched in the MBR tank, and Component 5 ended up with the inverse effect, increasing due to biological treatment.

Next, we found the SUVA value from the absorbance measured at the same time using the Aqualog and the TOC values of the samples. Figure 6 shows the semi-logarithmic graph of COD<sub>Cr</sub> and SUVA of each sample.

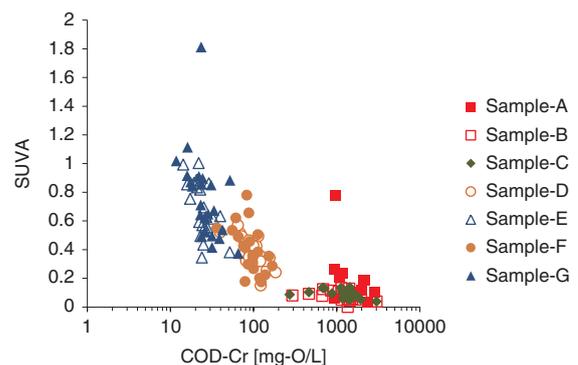


Figure 6 Relationship Between COD<sub>Cr</sub> and SUVA

When the wastewater treatment progresses, the COD component decreases significantly, even though SUVA increases. (Figure 6) The PARAFAC results showed that the MBR tank water (Samples D and F) included tryptophan-like components and humic substances. The MBR treated water (Samples E and G) only included humic substances. It is known that proteins generally contribute to SUVA, so it is important to take care. However, it is believed that increased SUVA in membrane filtration water with decreased protein components indicates that the ratio of the aromatic compounds in DOC increased in the membrane filtration water.

## Conclusion

In this paper, we have examined the possibility of applying EEM to wastewater treatment processes. As a result, we were able to use EEM-PARAFAC to get an understanding of changes in the composition (quality) of the CDOM in each process step sample. Information about the quality of these samples cannot be obtained only with conventional TOC and COD<sub>Cr</sub> values. In particular, using PARAFAC to separate the components in the samples and understand their behavior can be expected to improve the operating conditions of treatment processes. We believe that understanding the raw water quality and differences in processing characteristics for each type of wastewater in advance will be useful for optimizing the design of the processes and managing operation. Understanding the composition and behavior of CDOM to prevent membrane fouling, reduce chemicals used, and improve water quality makes it possible to achieve a better operation level than control using conventional water quality items as indicators. EEM analysis is expected to play an important role in water analysis in academic research fields and monitoring water quality in national and public institutions. Ensuring water resource in Asia and a stable supply is an important issue. We hope to widely use this analytical method for future water treatment processes.

## References

- [1] Ministry of the Environment (2014). FY2014 Public Water Quality Measurement Results (in Japanese)
- [2] A. Imai et al. (2001). Studies on the Origin and Dynamics of Recalcitrant Organic Matter in Lakes and its Effects on Lacustrine Ecosystems and Water Quality Special Research Report from the National Institute for Environmental Studies, Japan (in Japanese). SR-36-2001
- [3] Y. Hitoshi et al. (2014). Summary of Operating Conditions of the Sewage Treatment Membrane Bioreactor Plant in Sanpo in the city of Sakai (in Japanese). Collection of Guidelines from the Japan Sewage Works Association
- [4] Ministry of Health, Labor, and Welfare (2003). Board of Health Water Authority, Report from FY2002 Seventh Health Science Council Water Quality Management Specialist Committee, "Organic Substance Indicators (Draft of TOC Reference Values)"
- [5] T. Yahashi et al., Analysis of Water Quality Characteristics in Domestic Waste Water Treatment Facilities (in Japanese). *Journal of Japan Society on Water Environment*, **23**, 584(2000)
- [6] Hudson, et al., *River Research and Applications*, **23**, 631 (2007)
- [7] K. Komatsu et al. (2008). Characterization of Dissolved Organic Matter (DOM) in Lake Kasumigaura and Several DOM Sources Using Three-Dimensional Excitation-Emission Matrix Fluorescence Spectra (in Japanese). *Journal of Japan Society on Water Environment*, **31**, 261-267
- [8] N. Maie et al. (2009). Expansion of Methods for Analyzing Humic Substances in Recent Years (in Japanese). *Journal of Japanese Society of Soil Science and Plant Nutrition*, **80**, 419-426
- [9] Adam M. Gilmore et al. (2013). Analysis of the chromophoric dissolved organic matter in water by EEMs with HORIBA Jobin Yvon fluorescence instrument called "Aqualog", *Readout*, **41**, 19(2013)
- [10] T. Fukushima. Characteristics of Ultraviolet Absorbance: Dissolved Organic Carbon Ratios in Lake Kasumigaura, Lake Biwa, and Lake Nojiri, and Their Application to Lake Environmental Management (in Japanese). *Journal of Japan Society on Water Environment*, **20**(6), 397-403.
- [11] C.A. Stedmon et al. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry* **82**, 239(2003)
- [12] C.A. Stedmon et al. Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. *Limnol. Oceanogr.* **50**(2), 286(2005)
- [13] P. Kowalczyk et al. Characterization of dissolved organic matter fluorescence in the South Atlantic Bight with use of PARAFAC model: Interannual variability. *Marine Chemistry* **113**, 182(2009)



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