Analysis of physicochemical properties of natural biofilm matrices formed in a sub-tropical region (Lake Biwa, Japan) and a tropical region (Karangkates Reservoir, Indonesia)

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Abstract. Aquatic ecosystems consist of various biotic and abiotic components. One of the biotic components that plays essential roles in the aquatic ecosystem are microbes. Most microbes live by forming a biofilm. The matrix of the biofilm provides a microhabitat that supports microbial life. Hence, the physicochemical properties of the biofilm matrix are essential to support the microbial activities in the aquatic ecosystems. The comprehension of the physicochemical characteristics of natural aquatic biofilms is critically urgent in order to fully understand the ecology of microbial biofilms providing specific habitats for microorganisms and to utilize the biofilms for the benefit of humans. The physicochemical properties of the biofilm are affected by the environmental factors that can differ by region. This study analyzed the physicochemical properties (electrical charge properties, functional groups, nutrient ions, dissolved organic substances) of the natural biofilm matrices by comparing the biofilms formed in lotic ecosystems. The biofilms were sampled from a sub-tropical region (Lake Biwa, Kyoto – Shiga, Japan) and a tropical region (Karangkates Reservoir, East Java, Indonesia). The following were observed as the results of this study: the similar kinds of functional groups, such as carboxyl and amino groups that exist in polymers of both biofilm matrices; the ionization of the functional groups resulting in the positively and negatively charged sites in the biofilm matrices; the fact that the biofilm matrices present similar kinds of nutrient ions and dissolved organic nutrients; inside the biofilm, the matrices are nutrient-rich microhabitats available for microbes in the aquatic ecosystems. According to the results of this study, the biofilm matrices analyzed (tropical and sub-tropical regions) share similar physicochemical properties.
Key Words: biofilm, nutrient ion, dissolved organic nutrient, tropic, sub-tropic.

Abstrak. Ekosistem akuatik terdiri dari berbagai komponen biotik dan abiotik. Salah satu komponen biotik yang memainkan peran penting di ekosistem akuatik adalah mikroba. Hampir seluruh mikroba di ekosistem akuatik hidup dengan membentuk biofilm. Matriks biofilm menyediakan mikrohabitat yang dapat mendukung kehidupan mikroba. Dalam konteks ini, properti fisika-kimia dari matriks biofilm esensial untuk mendukung aktivitas mikroba yang ada. Pemahaman terkait karakteristik fisika-kimia dari matriks biofilm yang tumbuh di lingkungan alami sangat penting untuk bisa memahami secara utuh ekologi dari biofilm mikroba yang menyediakan habitat spesifik untuk mikroorganisme dan memanfaatkan biofilm untuk kepentingan manusia. Properti fisika-kimia dari matriks biofilm akan dipengaruhi oleh faktor lingkungan yang bisa berbeda tergantung dari wilayah. Studi ini menganalisis properti fisika-kimia (properti muatan listrik, grup fungsional, ion nutrien, bahan organik terlarut) dari matriks biofilm yang tumbuh secara alami dengan mengkomparasikan biofilm yang tumbuh di perairan lentis. Sampel biofilm didapatkan dari daerah sub-tropis (Danau Biwa, Kyoto – Shiga, Jepang) dan daerah tropis (Waduk Karangkates, Jawa Timur, Indonesia). Berikut ini adalah hasil-hasil dari studi ini: 1) jenis fungsional grup yang sama seperti grup karbosil dan grup amino terdapat dalam polimer dari matriks biofilm; 2) ionisasi dari fungsional grup ini membuat biofilm matriks memiliki situs bermuatan listrik negatif dan positif; 3) kedua jenis biofilm memiliki jenis nutrien ion dan nutrien organik terlarut yang sama; dan 4) di dalam kedua biofilm terdapat mikrohabitat kaya nutrien yang tersedia untuk mikroba yang hidup di dalam biofilm. Berdasarkan hasil penelitian ini, biofilm mikroba yang diteliti dalam studi ini (tropis dan sub-tropis) menunjukkan properti fisika-kimia yang mirip.
Kata Kunci: biofilm, ion nutrien, nutrien organik terlarut, tropis, sub-tropis.

Introduction. Aquatic ecosystems consist of various biotic and abiotic components. The components interact with each other and play essential roles in the aquatic ecosystems. The ubiquitous biotic component in all aquatic ecosystems is microbes (Wu et al 2012). Microbes can use photosynthesis and release nutrients stored in organic tissues through their role in decomposition (Hiraki et al 2009). Hence, in order to understand the aquatic ecosystem, microbial life is one of the fundamental insights.

Microbes are a ubiquitous biotic component, and thus, contribute to the various processes in the aquatic ecosystems (Julien et al 2014; Meyers 2000). Microbes live in the aquatic ecosystem in a community structure called biofilm (Costerton et al 1995; Flemming & Wingender 2010). Biofilm is a predominant habitat of microbes in almost all aquatic ecosystems, including extreme environments (Rittmann 2018). Biofilms can accumulate nutrients from the water resulting in a nutrient-rich condition (Fomina & Gadd 2014). The accumulated nutrients are available to support the microbial growth inside the biofilms. Biofilms can also support purification (Pennafirme et al 2015) and become a microbial gene pool (Wu et al 2018) in aquatic ecosystems. In order to understand the aquatic microbial activities, biofilm matrices should be considered.

The matrix of the biofilms provides a specific microhabitat suitable for microbial life (Kurniawan et al 2015). Hence, the microhabitat inside the biofilm matrix produces different microbial community structures compared with that of the surrounding water (Tsuchiya et al 2011). One of the main characteristics of the biofilm matrix that drive the microbial activities inside the biofilms is the physicochemical properties of the biofilm matrix (Lewandowski & Beyenal 2013; Sutherland 2001). Thus, the understanding of the physicochemical properties of biofilm matrices formed in natural environments is critical in order to fully understand the ecology of microbes in the aquatic ecosystems and to utilize microbes in aquatic biotechnology.

Environmental factors influence the formation of the biofilm matrix in the aquatic ecosystems (Peng et al 2018). Different geographical regions, such as tropical and sub-tropical regions may produce different characteristics of the biofilm matrices, including their physicochemical properties. However, studies related to the natural biofilms, particularly the physicochemical properties of the biofilm matrix in various regions, has been rarely conducted. Most of the studies focus on biofilms produced in the laboratory or on monospecies biofilms.

The present study investigates the physicochemical properties (electrical charge properties, functional groups, nutrient ions, dissolved organic substances) of the microenvironment inside biofilms formed in the lentic ecosystems located in a tropical region (Karangkates Reservoir, Indonesia) and a sub-tropical region (Lake Biwa, Japan). The results demonstrate that the microenvironment inside the biofilm matrices show similar physicochemical properties that are not dependent on the latitude. To the best of our knowledge, this is the first study that compares the naturally formed biofilms in the tropical and sub-tropical regions in order to understand the physicochemical properties of the natural biofilm matrices in the aquatic ecosystems. The results of this study give fundamental insights to understand microbes as a component of aquatic ecosystems.

Material and Method

Sampling and study area. Biofilms in this study were sampled from the surface of stones collected from Karangkates Reservoir, Indonesia (tropical region) and Lake Biwa, Japan (sub-tropical region) (Figure 1). Biofilms from the tropical region were sampled in September 2014 (dry season), and biofilms from the sub-tropical region were sampled in June 2013 (summer). Stones were collected from a depth of 75 cm (30 stones from each sampling location) and brought back to the laboratory using a plastic container filled with surrounding water. The temperature in the containers was maintained at 4°C. The biofilms were removed from the surface of the stones using sterilized toothbrushes and suspended in distilled water. The filtrates and biofilm pellets, prepared by centrifuging(8000×g at 4°C for 10 min) the biofilm suspensions.

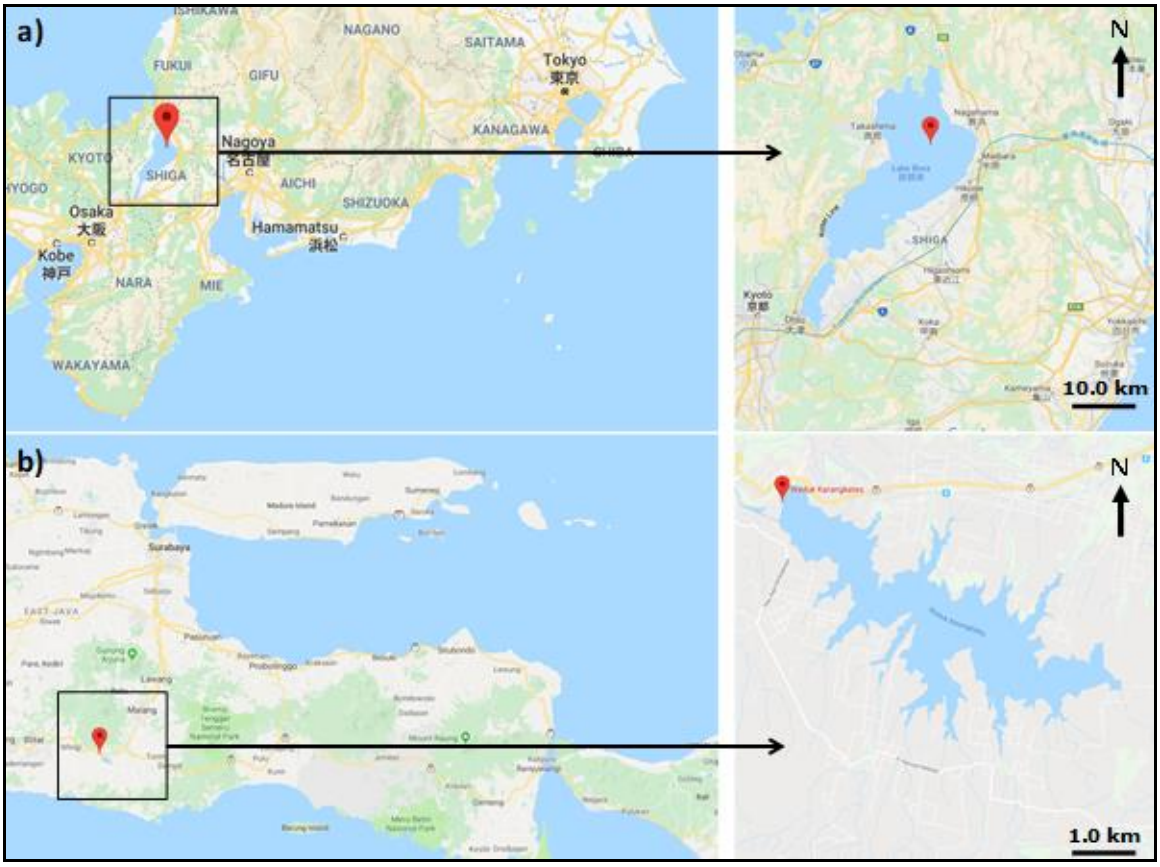

Figure 1. The sampling area in the present study. a) Lake Biwa, Japan; b) Karangkates Reservoir, Indonesia.

Electrophoretic mobilities. The pellet of the biofilm was washed three times, as follows. The pellet was resuspended in 40 mL of 10 mM NaCl aqueous solution. The suspension was centrifuged (8000×g at 4°C for 10 min), and the supernatant was discarded. The obtained biofilm pellet (0.03 g) was suspended in 1 mL of 10 mM NaCl aqueous solution. The suspension was mixed vigorously with a vortex for 5 min, then sonicated (2510J-MT, Yamato Scientific, Tokyo, Japan; 42 kHz, 125 W) for 10 min, followed by the vortex for 10 s. The obtained suspension was mixed with 10 mM of PBS at a ratio of 1:19 and used to analyze the electric charge of the BF polymer. The electrophoretic mobility (EPM) of the biofilm was measured on a ZETASIZER Nano-Z (Malvern Instruments, Ltd., Worcestershire, England) in phosphate buffered saline (PBS) varying in pH values from 2.0 to 9.0. The pH of the buffer was adjusted with 20 mM of HCl or NaOH.

FTIR spectra. The biofilm pellet was dried at 60°C until the constant weight was reached. 0.01 g of the dry biofilm pellet was mixed with KBr. The FTIR spectra of biofilms were measured using a Shimadzu FTIR Spectrometer 84002 (Shimadzu Corporation, Japan). A pellet of KBr without biofilm was used for the background measurement.

Nutrient ion concentrations. Part of the filtrates of the biofilm suspension (prepared by centrifugation) was filtered through a glass fiber filter (Whatman GF/F, Whatman International, Ltd., Maidstone, England) that was preheated at 630°C. The filtrates were regarded as the diluted interstitial water of biofilm and analyzed for the ion concentrations of the interstitial water of biofilm. The ion concentration was measured using a capillary electrophoresis method (CAPI 3300, Otsuka Electronics).

Dissolved organic substances. The filtrates of biofilm were analyzed on a Prominence series HPLC (Shimadzu, Kyoto, Japan) equipped with a RID, using a Cosmosil Sugar-D column (4 mm I.D. x 250 mm, Nacalai Tesque, Kyoto, Japan). The flow rate was 1 mL/min and the mobile phase was a mixture of acetonitrile and phosphate buffer (7:3). The injection volumes of mixed monosaccharide standards and biofilm filtrates were 10 μL. The temperatures of the column and RID were 25°C and 40°C, respectively. Monosaccharides in the interstitial water were identified by comparing their retention times with those of the authentic saccharides under the same HPLC conditions.

Results and Discussion

Biofilm polymer characteristics. The electrophoretic mobilities (EPM) of the biofilms formed in the tropical and sub-tropical regions were investigated in order to analyze the electrical charge properties of the biofilms (Figure 2). EPM values of the biofilms range from negative to positive values, depending on pH. It seems that both positively and negatively charged sites exist in the biofilm matrices.

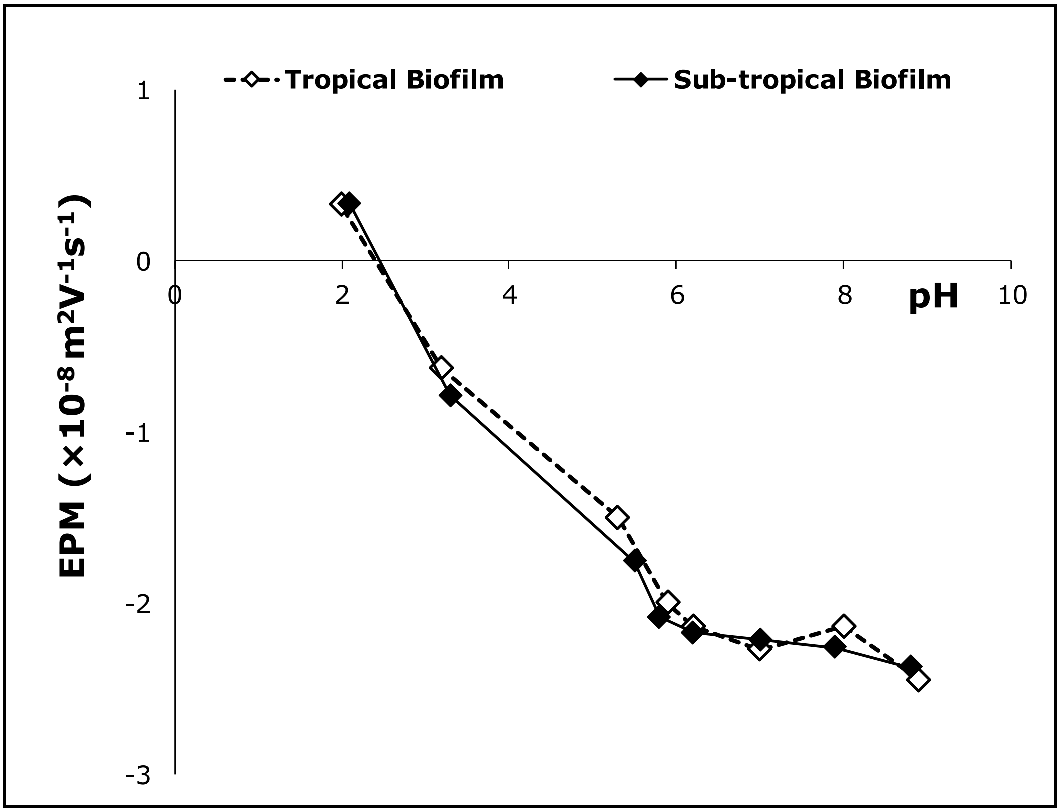

Figure 2. Electrophoretic mobility (EPM) of biofilms formed in tropical and sub-tropical regions.

Around neutral pH values, the EPM values of the biofilms show negative values. This result indicates that in the natural aquatic ecosystems with pH around 7, the biofilms carry a net negative charge. The EPM values of the biofilms change significantly around pH 4. These results suggest the presence of functional groups in the biofilm polymers that are actively protonated around pH 4 (Hiraki et al 2009). This result may correspond to the activity of the carboxyl groups having PKa around pH 4 (Freifelder 1985).

The EPM values of the biofilms shift to lower negative values along with the decrease of pH, and then, show positive values around pH 2. The negatively charged sites of the biofilm polymers seem to decrease due to the protonation of functional groups carrying negative charges. In pH 2, where the protonation of functional groups occurs in a considerable amount, the negatively charged sites of biofilm become electrically neutral sites. This condition promotes the higher ratio of positively charged sites compared to the negatively charged sites, and thus, the EPM value of the biofilms show a net positively charge around pH 2. The positive EPM values seem to correspond to the presence of positively charged functional groups, such as amino groups in the biofilm polymers (Kurniawan & Fukuda 2016).

The presence of functional groups in the biofilms was clarified by measuring the FTIR spectra of the biofilm polymers (Figure 3). FTIR spectra analysis of the biofilms shows that different functional groups exists in the biofilms (Hiraki et al 2009). The biofilms formed in tropical and sub-tropical regions have similar peak characteristics. Peaks related to main functional groups of these biofilms appeared at the following ranges: 400–1000 cm^{-1}, 1300–1600 cm^{-1} and 2800–3400 cm^{-1}.

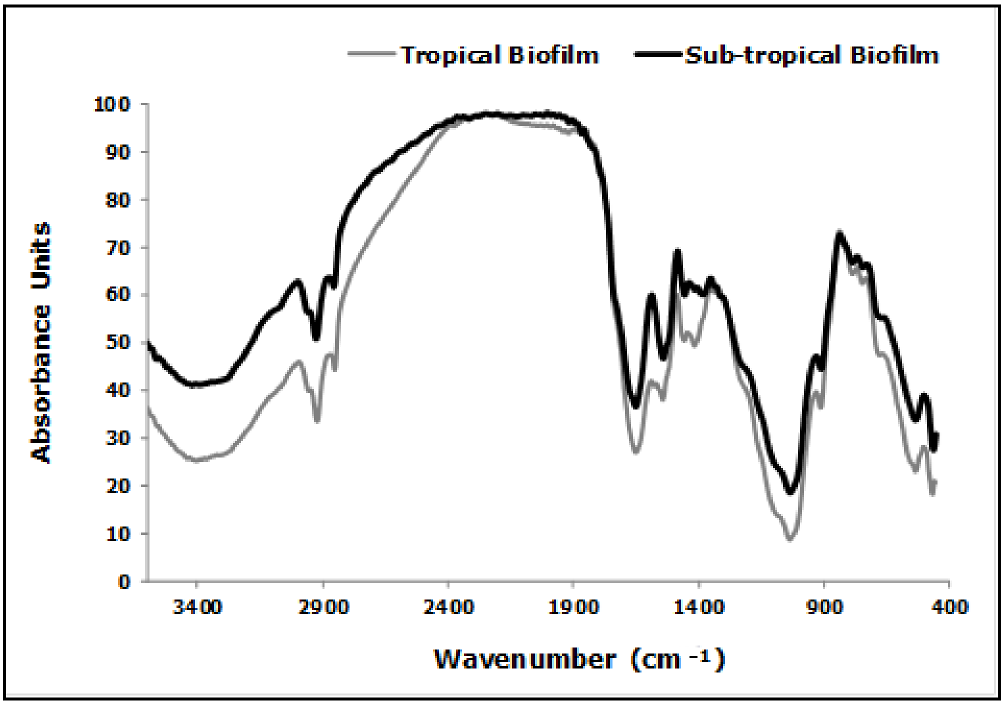

Figure 3. FTIR spectra of biofilms formed in tropical and sub-tropical regions.

The peaks between 1300 and 1600 cm^{-1} indicate the presence of the carboxyl groups in the biofilm (Freifelder 1985). A broad and strong band in the region of 2800 – 3400 cm^{-1} could suggest the presence of hydroxyl groups (Tugarova et al 2016). The band at 2847 cm^{-1} and 2948 cm^{-1} may ascribe to the carboxyl groups. The range of 400 – 1000 cm^{-1} may indicate the presence of phosphate groups. C-N bending is observed in 1414 cm^{-1}, indicated the existing of amino or amide framework (Freifelder 1985).

The results of the EPM measurements and FTIR spectra suggest that the biofilms formed in tropical and sub-tropical regions seems to have similar characteristics of electric charge properties. The electrically charged sites in the biofilm matrices seem to be a result of ionization of various functional groups in the biofilm polymers, such as carboxyl and amino groups. The electrical charged sites in the biofilm polymers may attract and retain various ions, including nutrient ions, from the surrounding water into the biofilm matrix. The presence of the electrically charged sites in the biofilm matrix (promoted by the ionization of functional groups in the biofilm polymers) is vital for the accumulation of nutrient ions inside the biofilms. The electrically charged sites become binding sites for the nutrient ions. The main mechanisms of the accumulation process are reported to be an ion exchange mechanism and electrostatic interactions (Kurniawan & Fukuda 2016).

Nutrient ion microhabitat. The nutrient ions inside the biofilms formed in tropical and sub-tropical regions and the surrounding water of the biofilms were analyzed. The same kinds of nutrient ions (NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}) were observed in the biofilms and in the surrounding water (Table 1). The concentrations of nutrient ions inside the biofilms were much higher than those of surrounding waters. In the case of the biofilm formed in the tropical region, the order of the ions from the highest concentration to the lowest was NH_4^+, NO_3^-, NO_2^- and PO_4^{3-}, while for the case of the biofilm formed in the sub-tropical regions it was NH_4^+, NO_3^-, PO_4^{3-} and NO_2^-.

The order of nutrient ions, by concentration, for each region in the surrounding waters was the same with that inside the biofilms. These results indicate that the nutrient ion concentrations inside the biofilms were firmly related to the nutrient ion concentrations in the water environments (Vijayaraghavan & Balasubramanian 2015; Wang & Chen 2008). The biofilm matrix adsorbs the nutrient ions from the surrounding water (Chojnacka 2008). Thus, when the concentration of the nutrient ions in the surrounding water is high, the same condition will occur inside the biofilm.

The previous studies reported that the concentrations of nutrient ions inside the biofilm are synchronizing with those in the surrounding environment (Costerton et al 1995; Flemming & Wingender 2010; Kurniawan & Yamamoto 2019). Nutrient ions accumulating in the biofilm matrix becomes one of the primary nutrient cycling processes in water environments, such as ponds or aquariums. Hence, the microbial activities related to the accumulation of nutrient ions into the biofilm matrix are essential processes that should be considered when trying to gain insight into nutrient cycling in aquatic ecosystems.

Table 1

The concentrations of nutrient ions in the biofilm matrices and surrounding water in the tropical and sub-tropical regions

No	*Ion*	*Tropical (mM)*		*Sub-tropical (mM)*	
		Biofilm	Water	Biofilm	Water
1	NH_4^+	4.2	0.0108	0.45	0.0013
2	NO_3^-	12.5	0.09	1.4	0.0049
3	NO_2^-	0.41	0.006	0.055	0.0001
4	PO_4^{3-}	0.25	0.0013	0.072	0.0001

Dissolved organic nutrient microhabitat. The microenvironment inside the biofilm is a nutrient-rich microhabitat available for microbes (Liu & Tang 2018; Tsuchiya et al 2009; Zhang et al 2018). The nutrients inside promote the microbial life resulting in the different community structures compared with those in the surrounding water (Gul et al 2018; Jimoh & Cowan 2017). The other nutrient sources for the microbial growth besides the nutrient ions adsorbed from the surrounding water, as described before, are dissolved organic nutrients (Gadd 2009; Hiraki et al 2009).

In this study, the dissolved organic nutrients inside the biofilms formed in the tropical and sub-tropical regions were investigated by an HPLC with a Sugar-D column (Figure 4). Several peaks of chromatograms were detected for the biofilms formed in tropical and sub-tropical regions. The detected peaks have the same detection times as ribose, mannose, glucose, sucrose, maltose and maltotriose (Cheng et al 2018; Jobby et al 2018). The similar chromatograms of both biofilms indicate a similarity of the dissolved organic nutrients from the biofilms. The saccharides inside the biofilms may be easily used by microbes in various water environments (Sutherland 2001). The microhabitat inside the biofilms formed in the tropical and sub-tropical regions seems to be formed by nutrient-rich dissolved organic substances that may promote the microbial growth.

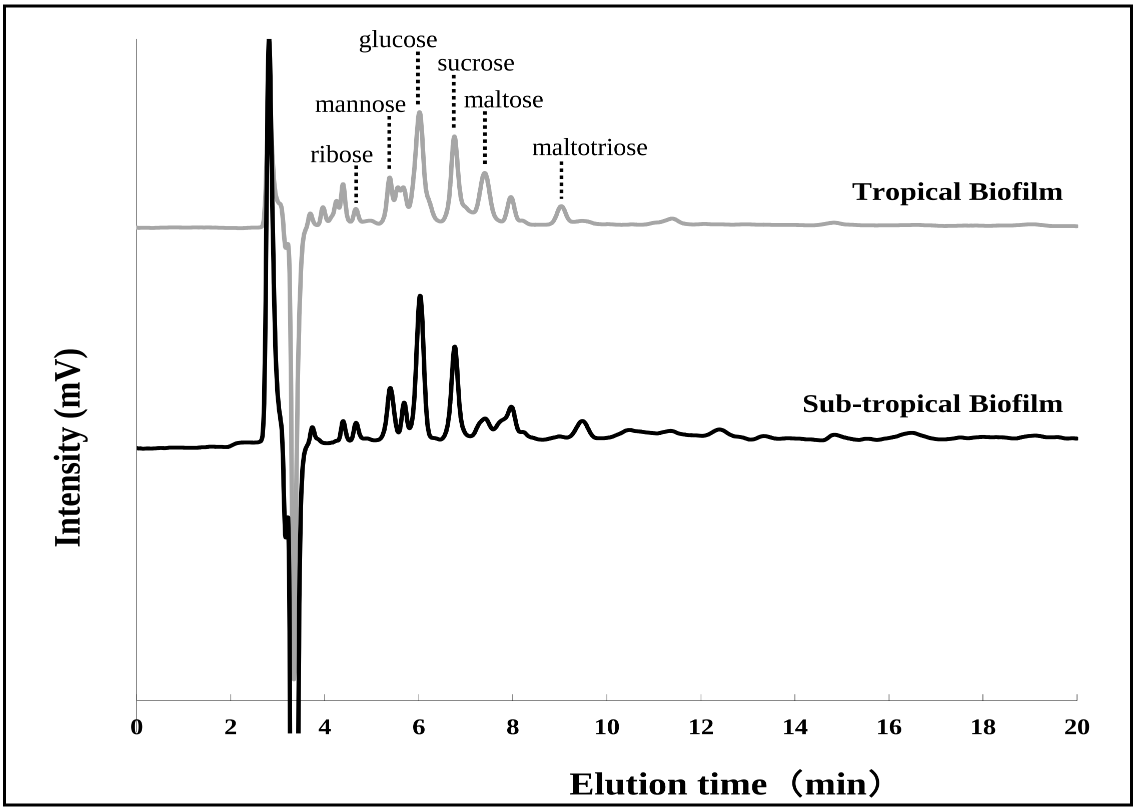

Figure 4. HPLC chromatograms of the biofilms formed in the tropical and sub-tropical regions.

Microbes have essential contributions to the ecological processes in water environments, such as decomposition, mineralization and purification of pollutants by forming biofilms. The physicochemical properties of the biofilm matrix perform essential functions to support the microbial activities in water environments. The results of this study indicate that the physicochemical properties of the matrices of biofilms seem not to be depended on the geographical latitude.

The physicochemical properties of the biofilm matrices promote the various functions of the biofilms as a microhabitat for microbes, including mechanical stability and water binding (Flemming & Wingender 2010). These properties can lead the biofilm matrix to impound various nutrients from the lake water or the reservoir water. The accumulation of the nutrients is a vital process in the oligotrophic environment (Decho 1990). The physicochemical properties of the biofilm matrix will also facilitate the microbial activities related to the degradation of various materials in the water. The degradation of the materials is carried out by colonization of the materials and the excretion of extracellular enzymes (Flemming & Wingender 2010). In this case, the physicochemical properties of the biofilm matrix may avert the enzyme loss from the microbial habitats (Li et al 2019). This function supports an establishment of the necessary enzymatic processes in the water environments especially related to the degradation of particulate matter.

The biofilm matrix can also induce various negative impacts to the benefit of humans. The presence of a biofilm matrix produces a decrease of production efficiency through physical deterioration and chemical interference (Lewandowski & Beyenal 2013). In aquaculture or aquarium environments, the biofilm matrix can become the microhabitat that protects pathogen microbes from the various treatments carried out for the sake of water quality management.

Conclusions. This study analyzed the physicochemical properties of natural biofilm matrices, by comparing the biofilms formed in the sub-tropical region (Lake Biwa, Kyoto

– Shiga, Japan) and the tropical region (Karangkates Reservoir, East Java, Indonesia). The following were observed as the results. First, the similar kinds of functional groups, such as carboxyl and amino groups, exist in polymers of both biofilm matrices. Secondly, the ionization of the functional groups resulting in the positively and negatively charged sites in the biofilm matrices was observed. Next, the biofilm matrices have similar kinds of nutrient ions and dissolved organic nutrients. Lastly, inside the biofilm matrices are nutrient-rich microhabitats available for microbes in the aquatic ecosystems. According to the results of this study, the biofilm matrices formed in different latitudes share similar physicochemical properties. The results of this study contribute to developing a holistic view of recent understanding of biofilms in aquatic ecosystems affecting water and processes in water ecosystems.

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