Biofilm as a bioindicator of Cr VI pollution in the Lotic Ecosystems

A Kurniawan1,2,* Sukandar3, C Satriya1,2 and Guntur2

1 Coastal and Marine Research Center, University of Brawijaya, Indonesia
2 Faculty of Fisheries and Marine Science, University of Brawijaya, Indonesia

E-mail: andi_k@ub.ac.id

Abstract. Biofilm is ubiquitous in aquatic ecosystems such as river. Biofilm have been reported to have high sorption capacities that promote the accumulation of nutrient ions inside biofilm matrix. The ion that can be accumulated inside the biofilm is not only nutrient ions but also other ions such as heavy metal ions. The pollution of heavy metal ions emerge as one of the biggest aquatic ecosystem problems. Thus, the effort to monitor the heavy metal pollution in the aquatic ecosystem in the aquatic ecosystems is needed. The difficulty to monitor the water pollution particularly in the lotic ecosystems is mainly related to the water flow. Therefore, the utilization of indicator of pollution in such ecosystem is fundamentally important. The present study investigated the accumulation of Cr VI inside biofilm matrices in the river ecosystems in order to develop biofilm as a bioindicator for pollution in the lotic ecosystems. The result indicates that biofilm can accumulate Cr VI from the surrounding water and reserve the ion. According to the result of this study, biofilm is a promising bioindicator to monitor the Cr VI pollution in the lotic ecosystems.

1. Introduction
Biofilm is found in most natural aquatic environments [1,2] and has been found to constitute the predominant mode of growth of microbe in streams and lakes in all parts of the world and to be both viable and metabolically active [3,4,5]. Various definitions of the term biofilm have been proposed. Generally, biofilm can be considered as an aggregate of microorganisms imbedded in a matrix composed of microbially produced extracellular polymer substances (EPS) and is attached to a surface [6].

The nutrient ion concentration in the interstitial water of biofilms formed on reed and stone surfaces were hundreds to thousands of times higher than those in lake waters regardless of the biofilm type and sampling site [7]. These particular nutrient-rich environments inside of these biofilm matrices are established from a very early stage of biofilm formation [5]. The adsorption of nutrient ions from the surroundings to the biofilm seems to form this nutrient-rich environment [8,9].

The ions that can be attracted and accumulated into biofilm are not only nutrient ions but also others such as heavy metal ions. Heavy metal pollution emerges as the one of the most serious problems in aquatic ecosystems [10,11]. One heavy metal that has become a pollutant is Cr VI [12]. Many efforts have been carried out to monitor water pollution. In the case of lotic ecosystems, the main problem of monitoring is related to water flow. Hence, the utilization of biological substances that can attract and retain Cr VI may be used as a bioindicator for the contamination. The present study investigated the accumulation of Cr VI inside biofilm matrix in order to develop biofilm as a bioindicator of pollution in lotic ecosystems. The result indicates that biofilm can accumulate Cr VI from the surrounding water and

Content from this work may be used under the terms of the CreativeCommons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd
reserve the ion. According to the results of this study, biofilm is a promising bioindicator to monitor Cr VI pollution in the lotic ecosystems.

2. Materials and Method

2.1. Sample preparation
Samples were collected from 3 sites at Badek River in Malang, Indonesia. Stones were taken from a depth of about 20 cm and brought back to the laboratory in a plastic container filled with surrounding river water; the container was maintained at 4°C. The biofilm on the surfaces of the stones were removed using a toothbrush and suspended in sterilized distilled water. The filtrates and biofilm pellets were prepared by centrifuging (8,000 × g at 4°C for 10 min) the biofilm suspensions. The pellets of the biofilms (approximately 1 wet-g) were taken and then dehydrated for 3 days until the weight was stable to give a dry weight. This data was used to convert the dry weight per the wet weight of biofilm polymer.

2.2. Electrophoretic mobility
The pellets of biofilm were washed three times as follows. The pellets were resuspended in 40 mL of 10 mM NaCl aqueous solution. The suspension was centrifuged (8,000 × g at 4°C for 10 min), and the supernatant was discarded. The obtained biofilm pellets (ca. 0.03 g) were 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 a vortex for 10 s. The detailed method of electrophoretic mobility measurement is described by Kurniawan and Fukuda [13].

![Figure 1. The electrophoretic mobility of biofilm measured in various pH](image)

2.3. Cr VI concentration
The sample used to measure the concentration of Cr VI inside the biofilm was a biofilm suspension that was prepared by brushing the biofilm from the surface of the stones in sterilized distilled water. The concentration of Cr VI in the surrounding river water was measured from the surrounding river water of the stones (about 15 cm). The concentration of Cr VI was measured using the Atomic absorption spectroscopy method.

3. Result and Discussion

3.1. Characterization of biofilm polymers
The accumulation of various ions into biofilm matrix is promoted by the ability of biofilm to attract the ions [14,15,16]. This ability is mainly promoted by the electrostatic interaction between the biofilm
polymers and the ions. In order to analyse the electrostatic characterization of biofilm polymers, the electrophoretic mobility measurement was conducted in the present study.

Electrophoretic mobility (EPM) of biofilm polymers were measured in various pH (pH 2 until pH 9) (figure. 1). The biofilm showed a net negative charge at around neutral pH, which is typically the pH of aquatic ecosystem. This indicates that biofilm carries negative charge sites in a natural aquatic ecosystems. The EPM of biofilm moves to the positive charge when the pH is down to the acid condition, which suggests that the decrease of negative charge is due to the protonation of functional group carrying negative charge. The great change of the EPM value around pH 4 indicates the presence of a functional group with pKa around pH 4 such as carboxylic groups [17]. The EPM of biofilm shows a positive value around pH 2 indicating the presence of positively charged sites such as amino groups [18]. The result of EPM measurement indicates that biofilm carries both negative and positive charge sites in aquatic ecosystems.

The presence of positively and negatively charged sites becomes a key factor in promoting the ability of biofilm to attract cationic and anionic substances from the surrounding water including heavy metals such as Cr VI. Hence, biofilm should accumulate Cr VI if the ion exists in the river water. Thus, to analyse the presence of Cr VI in the aquatic ecosystem, the measurement of the concentration of Cr VI inside the biofilm matrix may be used as an indicator.

### 3.2. Cr VI accumulation

The concentrations of Cr VI inside the biofilm and in the surrounding river water were measured (table 1). The concentrations of Cr VI inside the biofilm ranges from 1.8 µmol/wet-g to 2.3 µmol/wet-g. These concentrations were much higher than those in surrounding river water (0.03 µmol/wet-g until 0.4 µmol/wet-g). Interestingly, although the concentration of Cr VI in the river water became lower due to the input of rain water or the cessation of pollutant input, the concentration of the ions inside the biofilm was still much higher. The attraction and retention of the Cr VI in the biofilm is mostly due to the electrostatic interaction [14] and ion exchange mechanism [8]. The Cr VI can be retained on the charge sites of biofilm polymers and in the regions between the biofilm polymers [8].

| Sample | Cr VI concentration (ppm) |
|--------|---------------------------|
|        | Site 1 | Site 2 | Site 3 |
| Biofilm | 2.3±0.3 | 1.9±0.6 | 1.9±0.3 |
| Water  | 0.032±0.002 | 0.036±0.005 | 0.042±0.007 |

### 3.3. Biofilm as an bioindicator of water pollution

Water pollution promotes various problems not only to aquatic organisms but also to humans [12,17]. Many technologies have been suggested to solve these problems [18]. Moreover, to monitor the pollution and hinder more negative effects from it, the monitoring of pollution is critically important. For the case of water pollution in aquatic ecosystems, the pollution monitoring of lotic ecosystems such as rivers is much more difficult to monitor than lentic ecosystems such as lakes. The difficulty is mainly related to the presence of water flow. Hence, the contamination will be not detected if the measurement uses only the river water as a sample and is conducted during an unsuitable time such as after rainfall or when pollution sources such as factory plants do not operate temporarily. Therefore, the utilization of bioindicators such as aquatic organisms to monitor the pollution in lentic ecosystems is fundamentally required.

The utilization of aquatic organisms as a bioindicator of water pollution is believed to be an effective alternative to other technologies and is considered environmentally safe [19]. In particular case, the biofilm that can attract and retain pollutants such as Cr VI may serve as an alternative. This current study indicates that biofilm can attract and retain Cr VI in the lotic ecosystems. This toxic substance
will be accumulated continuously if the contamination of Cr VI to the river is not stopped. Even if the discharge of Cr VI is stopped temporarily to the river or there is dilution due to the input of rain water, the Cr VI will still exist in the biofilm. By monitoring the concentration of Cr VI inside the biofilm formed in the river, the level of pollution of Cr VI in the river can be monitored. Moreover, the biofilm is directly connected to the food web of the aquatic ecosystems. If the contaminated biofilm is consumed by higher trophic level organisms such as fish, the Cr VI may be accumulated in the fish. Hence, if the fish is consumed by a human, the Cr VI may also accumulated in the human. The ability to attract and retain heavy metal ions such as Cr VI makes the biofilm a promising biosorbent and bioindicator for water pollution in lotic ecosystems [20, 21].

4. Conclusion
Biofilm carries electric charge sites which can attract and retain heavy metal ions such as Cr VI. These mechanisms cause the concentration of Cr VI inside the biofilm to be much higher than the surrounding river water. The results of the present study indicates that biofilm may accumulate Cr VI from surrounding river water and reserve the ions. Cr VI will be accumulated continuously if the input of Cr VI to the river is not stopped. Even if the discharge of Cr VI is stopped temporarily to the river or there is dilution due to the input of rain water, the Cr VI will still exist in the biofilm. Hence, even if the river water in one site changes due to water flow that makes it difficult to detect the Cr VI in the site, the history of Cr VI pollution can still be tracked through the measurement of Cr VI in the biofilm. According to the results of this study, biofilm is a promising bioindicator to monitor the Cr VI pollution in lotic ecosystems such as rivers.

5. References
[1] Costerton J W, Lewandowski Z, Caldwell D E, Korber D R, Lappin S H M 1995 Ann. u Rev. Microbiol. 49 711–745
[2] Kurniawan A, Yamamoto T, Tsuchiya Y, Morisaki H 2012 Microbes. Environ. 27 399–406
[3] Lappin-Scott and Costerton JW 1995 Microbial biofilms (Cambridge: Cambridge University Press)
[4] Hall-Stoodley L, Costerton J W, Stoodley P 2004 Nat. Rev. Microbiol. 2 95–108
[5] Hiraki A, Tsuchiya Y, Fukuda Y, Yamamoto T, Kurniawan A, Morisaki H 2009 Microbes. Environ. 24 265–272
[6] Lewandowski Z, Beyenal H 2007 Fundamental of biofilm research (New York: CRC press)
[7] Tsuchiya Y, Hiraki A, Kiriyama C, Arakawa T, Kusakabe R, Morisaki H 2011 Microbes. Environ. 26 113–119
[8] Kurniawan A, Tsuchiya Y, Eda S, Morisaki H 2015 Colloids. Surf. B. Biointerfaces. 136 22–26.
[9] Watnick P, Kolter R 2000 J. Bacteriol. 182 2675–2679
[10] Quintelas C, Rocha Z, Silva B, Fonseca B, Figueiredo H, Tavares T 2009 Chem. Eng. J. 152 110–115
[11] Okabe S, Oshiki M, Kamagata Y 2010 Microbes Environ. 25 230–240
[12] Sukandar, Kurniawan A 2017 Biosorption of Cr VI using Rice Straw Waste Ponte 73 185–190.
[13] Kurniawan A, Fukuda Y 2016 Electric charge characteristics of biofilms formed on various surfaces J.Pure App. Chem. Res. 5 95–100
[14] Kurniawan A, Yamamoto T, Tsuchiya Y, Morisaki H 2012 Microbes. Environ. 27 399–406
[15] Kurniawan A, Fukuda Y 2015 Microbiol. Indones. 9 106–112
[16] Gadd, G.M 2009 J. Chem. Technol. Biotechnol. 84 13–28
[17] Freifelder D 1985 Principles of physical chemistry with application to the biological sciences (Boston: Jones and Bartlett Publisher) 2nd ed.
[18] Walton H F, Rocklin R D 1990 Ion exchange in analytical chemistry (Florida: CRC Press)
[19] Volesky B 2007 Water Res. 41 4017–4029.
[20] Kurniawan A, Yamamoto T 2013 Biofilm polymer for biosorption of pollutant ions Procedia Environ. Sci. 17 179–187
[21] Kjelleberg S, Givskov M 2007 The biofilm mode of life. In: Kjelleberg S, Givskov M (eds) the biofilm mode of life: mechanisms and adaptations, original, horizon bioscience, norfolk UK, p 5–21