BIOFILM MATRICES AS BIOMONITORING AGENT AND BIOSORBENT FOR CR(VI) POLLUTION IN AQUATIC ECOSYSTEMS

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ABSTRACT

Heavy metal pollution in aquatic ecosystems has become one of the primary environmental problems. Cr(VI) is one of the most toxic and carcinogenic heavy metal pollutants. The degradation of aquatic ecosystems due to the Cr(VI) pollution usually occurs slowly, but the impact is accumulative. Hence, an awareness of the pollution often occurs when the impacts have already in the acute or chronic level. Therefore, the technologies to monitor and to solve the Cr(VI) pollution are critically important. The application of biological resources emerges as an alternative technology to solve the problems. This study analyzes the utilization of biofilm as a biomonitoring agent and a biosorbent to monitor and to immobilize Cr(VI) in the aquatic ecosystems, respectively. The development of the biofilm as a biomonitoring agent is conducted through the investigation of Cr(VI) concentration in the biofilm and the surrounding river water, while the utilization of the biofilm as a biosorbent is developed through the analysis of Cr(VI) adsorption characteristics to the biofilm. The results of this study reveal that the concentrations of Cr(VI) inside the biofilms are hundreds of times higher than the surrounding river water. The biofilms seem to accumulate the Cr(VI) from the surrounding river water through a physicochemical process. According to the result of this study, biofilms can become a promising biological agent to monitor and to immobilize Cr(VI) in the aquatic ecosystems.

Keywords: Biofilm; Biomonitoring; Biosorption; Cr(VI); Water Pollution.

1. INTRODUCTION

Aquatic ecosystems have been contaminated by various pollutants such as heavy metal including Cr(VI) (Kyzas and Matis, 2018; Hea, et al., 2018). The pollutants lead to the degradation of aquatic ecosystems which usually occurs gradually, but its effect is accumulative. Hence, the awareness about the degradation appears when the impacts have been already in the chronic level (Bland, et al., 2018; Singh, 2015).

Heavy metals such as Cr(VI) have become a serious environmental problem because it's characteristics that persistent and may be easily accumulated and magnified through food webs (Sukandar and Kurniawan, 2017). Cr(VI) is not only toxic but also may lead to various human health problems such as cancer and kidney dysfunctionality (Chen, et al., 2017). Cr(VI) largely exists in wastewater of various industrial activities such as a leather tanning process (Jobby, et al., 2018). The contamination of the ion will increase along with the increase of its utilization. Thus, the existence of the Cr(VI) in the aquatic ecosystems should be continuously monitored.

Various technologies to monitor the presence of heavy metals such as Cr(VI) in the aquatic ecosystems have been proposed (Fomina and Gadd, 2014; Chojnacka, 2010). One of the promising technologies is biomonitoring. Biomonitoring is the utilization of a living organism or a part of a living organism as a biological agent to monitor the pollutant in the ecosystems (García-Seoane, et al., 2018; Kirman, et al., 2018). The primary requirements of the biological agents are ubiquitous, easily grown and able to accumulate the pollutants.

The efforts to remove the excess of Cr(VI) from the aquatic ecosystems is critically important. Various technologies have been proposed to solve heavy metals pollution including Cr(VI). Biosorption is one of the proposed technologies which have been reported to become inexpensive and environmentally safe. This technology uses living organisms or a part of the living...
organisms to immobilize the pollutant from the ecosystems. The primary key to success in the biosorption is the ability of biosorbent to adsorb the pollutant. The biological agents used as a biosorbent should be easy to be obtained and having a high capacity to remove the pollutant.

Biofilm as a predominant habitat of microbes (Flemming and Wingender, 2010; Costerton, et al., 1995; Fabbri, et al., 2018) can become a biomonitoring agent and biosorbent for Cr(VI) in the aquatic ecosystem. Biofilm is a matrix-enclosed microbial community that attached on the almost every substrate in the aquatic ecosystems (Tsuchiya, et al., 2009). Previous studies have shown that the biofilm can attract various ions including heavy metals (Kurniawan, 2012; Kurniawan, 2015). In this study, the Cr(VI) concentration in the biofilm and the surrounding water of the biofilm in the river ecosystems were investigated in order to analyze the biofilm utilization as a biomonitoring agent. Moreover, the characteristics of Cr(VI) adsorption to the biofilm were also analyzed to develop the biofilm as a biosorbent for Cr(VI). The results of this study suggest that the biofilm can become promising biomonitoring agent and biosorbent to monitor and remove the heavy metal such as Cr(VI) from the aquatic ecosystems.

2. MATERIALS AND METHODS

2.1. Sampling Site And Sample Preparation

Samples in this study were biofilms formed on the stones and the surrounding river water of the biofilms (ca. 15 cm from the biofilm surface) collected from the Badek River in Malang City, East Java, Indonesia. In the around riverbank area of the Badek River, there are leather tanning plants. The plants often discard the wastewater from the leather tanning process to the Badek River. The samples of the biofilms were collected from 3 sampling sites. Site 1 is the location near the outlet of the tannery plants, Site 2 is in the middle of the watershed area between the Site 1 and the end of the watershed of Badek River, and Site 3 is at the end of the Badek River watershed before the river joined with the Brantas River.

The samples of biofilms and the surrounding water were collected in 2 times. The first sampling time is when the tannery plants operated, and the second sampling times is when the tannery plants did not operate. The samples were collected from the middle and the both sides of the river.

The stones covered by biofilm and the surrounding river waters were brought back to the laboratory in plastic containers. The temperature of the container is maintained at around 4 °C. The biofilms were removed from the stones using a sterilized toothbrush in 80 mL of distilled water. The Cr(VI) concentration in the suspension was assumed as the diluted Cr(VI) concentration inside the biofilms. The Cr(VI) concentrations were measured using an Atomic Absorption Spectroscopy Shimadzu AA-6800 (Shimadzu Corporation, Japan). All the samples were stored in -25 °C before used in the experiment.

2.2. Kinetics Of Adsorption

Eighty milliliters of 50 mg·L⁻¹ K₂Cr₂O₇ aqueous solutions were prepared by diluted reagent grade K₂Cr₂O₇ to the distilled water. 0.5 gram of biofilm was added to the suspension and mixed well using a magnetic stirrer. The biofilm suspensions were sub-sampled after 5, 15, 30, 60, and 180 minutes. The sub-sample was centrifuged (8,000 × g at 4°C for 10 min) and the supernatant was collected. The concentration of Cr(VI) in the supernatants were measured using an Atomic Absorption Spectroscopy Shimadzu AA-6800 (Shimadzu Corporation, Japan). The same K₂Cr₂O₇ aqueous solutions without the biofilm were used as a control in the kinetics of adsorption experiment. The adsorbed amounts of Cr(VI) were calculated from the difference of the Cr(VI) concentration in the control and the sub-samples. All the kinetics experiments were repeated three times independently.

2.3. Adsorption Isotherm

A half gram of biofilm pellet was added to 80 mL of K₂Cr₂O₇ aqueous solution (5, 25, 50, 300, 600, 1200 mg·L⁻¹) prepared with the same method described above. After 15 minutes, the suspensions were centrifuged (8,000 × g at 4°C for 10 min), and then, the supernatants were collected. As the control, the same concentration of K₂Cr₂O₇ aqueous
solutions without the addition of the biofilm was prepared. The adsorbed amounts of Cr(VI) to the biofilms were calculated from the difference of concentration between the control and the supernatants. The concentration of Cr(VI) was measured using an Atomic Absorption Spectroscopy Shimadzu AA-6800 (Shimadzu Corporation, Japan). All the adsorption isotherm experiments were repeated three times independently.

The adsorption isotherm of biofilm is analyzed using the variant of the Langmuir Adsorption Equation as described below (Vijayaraghavan and Balasubramanian, 2018; Freifelder, 1985)

\[
\frac{C}{N} = \frac{1}{(N_{\text{max}})b} + \frac{C}{N_{\text{max}}}
\]

(1)

The Langmuir adsorption equation assumes that a dynamic equilibrium exists between the adsorbed Cr(VI) (N; mg·g⁻¹) and the free Cr(VI) in solution (C; mg·L⁻¹). The adsorption equilibrium constant (b) is the ratio of the adsorption and desorption rates. The plot of C/N against C shows a straight line with a slope of 1/Nmax and a y-axis intercept of 1/(Nmax)b. Then, the values of Nmax (the maximum amount of adsorbed ion; mg·g⁻¹) and b (L·mg⁻¹) were calculated (Kurniawan, 2012).

3. RESULTS AND DISCUSSION

The concentration of Cr(VI) inside the biofilms and the surrounding water were investigated in this study. The characteristics of Cr(VI) adsorption to the biofilms were also analyzed. The results are used to examine the possibility to utilize the biofilm matrices as a biomonitoring agent and a biosorbent for Cr(VI) contamination in the aquatic ecosystems.

3.1. Cr(VI) Concentration In The Biofilm And The Surrounding Water

The concentrations of Cr(VI) in the biofilms and the surrounding river waters were measured when the leather tanning plants did not operate and operated (Table 1). The results indicate that the concentration of Cr(VI) inside the biofilms were hundred times higher compared to those of in the surrounding river waters. This results indicated that biofilms might accumulate the Cr(VI) from the surrounding waters [16]. Biofilms in the aquatic ecosystems have been reported to have the ability to attract and reserve ions including heavy metal ions such as Cr(VI) (Kurniawan, 2015; Cheng, et al., 2018, Julien, et al., 2014).

When the tannery plants operated, concentrations of Cr(VI) in the water of Badek River were 0.078-0.13 mg·L⁻¹. These concentrations are higher than the threshold for human health (0.05 mg·L⁻¹). These facts suggested that the Badek River seems to be contaminated by the Cr(VI). However, the concentrations of Cr(VI) in the river water when the tannery plants did not operate were only 0.022-0.041 mg·L⁻¹ which are lower than the threshold of human health. These results mean that if the measurement to investigate the Cr(VI) contamination in the river conducted in the time when the tannery plants did not operate, the contamination of Cr(VI) in the Badek River will not be revealed. These conditions may lead to the misjudgment on the river pollution status. This fact revealed the limitation of the utilization of Cr(VI) concentration in the river water as a standard to monitor the water pollution.
Table 1. The concentration of Cr(VI) inside the biofilm and in the surrounding river waters. Experiment repeated 3 times. ± values represent the standard deviation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time</th>
<th>Cr(VI) concentration (mg·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Site 1</td>
<td>Site 2</td>
</tr>
<tr>
<td>Biofilm</td>
<td>Not operating</td>
<td>3.2 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Operating</td>
<td>4.02 ± 0.33</td>
</tr>
<tr>
<td>River Water</td>
<td>Not operating</td>
<td>0.041 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Operating</td>
<td>0.13 ± 0.01</td>
</tr>
</tbody>
</table>

The monitoring of heavy metals such as Cr(VI) contamination in the river requires the more suitable and accurate indicators than the concentration of the heavy metals in the river water. Hence, the concentration of Cr(VI) in the indicators should represent and indicate the existence of the heavy metals in the river not only in the sampling day but also in the extended periods. Heavy metals such as Cr(VI) are also can be accumulated in the living organism, and then, through the food web can be magnified. Therefore, the indicators should also represent the potentiality of this dangerous cycles. In this case, the utilization of biological agents to monitor the heavy metal pollution may appear as a promising alternative.

Biofilm is the predominant habitat of microbes and ubiquitous in the aquatic ecosystems. The biofilm easily forms on the all substrates in the aquatic ecosystems [13]. Biofilm also directly involves with the food chain because it is a feed for many fishes, and thus, the heavy metals accumulated inside the biofilm may be magnified through the food webs. The results of this study show that the biofilms may accumulate the heavy metals such as Cr(VI) from the surrounding waters. If there is a heavy metal input into the river at a particular time, the heavy metal will be attracted and retained in the biofilm matrix for an extended period. Hence, the presence of heavy metals in the biofilm is very likely to be used as an indicator of heavy metal input into the river.

Moreover, the heavy metal concentrations inside the biofilm will dynamically change along with the change of the ion concentrations in the surrounding waters [17]. When the concentration of the heavy metals in the surrounding water decrease, the biofilm may release the heavy metal to the surrounding water. The results of this study suggest that the biofilm may be used as a promising biological agent to monitor heavy metal pollution in the aquatic ecosystems.

3.2. Kinetics Of Adsorption

The time course of Cr(VI) adsorption to the biofilm was investigated (Fig. 1). The adsorbed amounts of the Cr(VI) to the biofilm were relatively stable from 5 min until the end of the experiment (± 1.8 mg·g⁻¹). This result suggested that the adsorption of Cr(VI) to the biofilm is a speedy process. This process is a characteristic of the passive uptake mechanism (Kyzas, et al., 2014). It seems that the accumulation of Cr(VI) to the biofilm in this study occurred through a physiochemical process (Kurniawan, 2015; Ifelebuegu, et al., 2018). Our previous studies suggested that the biofilms may attract and retain ions including heavy metals such as Cr(VI) through the ion exchange mechanism and the electrostatic interactions (Kurniawan, 2012).
3.3. Adsorption Isotherm

In order to analyze the Cr(VI) adsorption mechanism to the biofilm in more detail, the adsorption isotherm of Cr(VI) to the biofilm collected from the Badek River was investigated. The initial concentrations used in the experiment were 5-1200 mg·L⁻¹. The adsorbed amounts of Cr(VI) to the biofilm increase along with the increase of the Cr(VI) concentration in the surrounding waters, and then, leveled off in the high concentration (Fig. 2).

The adsorption isotherm of Cr(VI) to the biofilm shows the L type of adsorption. This type of curve suggested the adsorption may be described using the Langmuir Adsorption Equation. The variant of the Langmuir adsorption equation was used to analyze the adsorption of Cr(VI) to the biofilms. The result showed that the adsorption of Cr(VI) to the biofilm fit well with the Langmuir adsorption model (R² = 0.95) (Fig. 3). The Nmax of the Cr(VI) to the biofilm collected from the Badek River in this experiment is 9.09 mg·g⁻¹. The adsorption equilibrium constant is 0.01 mg·L⁻¹. The adsorption of Cr(VI) to the biofilm seems to occur in a monolayer form where the retained mechanisms are solely due to the interaction between the Cr(VI) and the biofilm. In this case, the binding sites in the biofilm have an equal affinity to the Cr(VI). The adsorption of Cr(VI) to the biofilm increase linearly in the low concentration, then slightly increase along with the increase of the concentration and finally reached the saturation point where the adsorption amount becomes constant. The results support the suggestion that Cr(VI) seems to be adsorbed to the biofilm through the physicochemical process such as an ion exchange mechanisms and an electrostatic interaction ([Kurniawan, 2012]).
surrounding waters. The high effectiveness in the low concentration it seems due to the high ratio of the available binding sites in the biofilms compared to the amount of ions in the surrounding waters. The effective level of adsorption ability (the effectiveness is more than 50 %) is reached until 50 mg·L⁻¹ of Cr(VI). This concentration should be taken as one of the primary considerations to develop a biofilm reactor to remove Cr(VI) from the aquatic ecosystems.

Figure 4. Time The effectiveness of Cr(VI) adsorption to the biofilm

4. CONCLUSIONS
This study analyzes the utilization of biofilm as a biomonitoring agent and a biosorbent to monitor and immobilize Cr(VI) in the river ecosystems, respectively. The results show that the Cr(VI) concentration inside the biofilm is hundred times higher compared to the Cr(VI) concentration in the river water. The Cr(VI) concentration inside the biofilms can represent the input of the ions to the river ecosystems for an extended period, and thus, represent the history of Cr(VI) input to the ecosystems. This study revealed that the biofilm matrix might become a promising biological agent to monitor water pollutant such as Cr(VI) in the aquatic ecosystems. The biofilms of River Badek seems to adsorb Cr(VI) through physicochemical interactions. The maximum Cr(VI) adsorption ability of the biofilm is 9.09 mg·g⁻¹ and the adsorption equilibrium constant is 0.01 mg·L⁻¹. According to the results of this study, the biofilm may become a promising biomonitoring agent and a biosorbent for water pollutant such as Cr(VI) in the aquatic ecosystems.

5. REFERENCES
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