Poll Res. 41 (2) : 411-415 (2022)

ISSN 0257–8050
DOI No.: http://doi.org/10.53550/PR.2022.v41i02.006

BIOSORPTION CHARACTERISTICS OF HG(II) INTO BIOFILM

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(Received 7 September, 2021; Accepted 7 October, 2021)

ABSTRACT

Biosorption is an alternative technology developed to overcome pollution, including heavy metal contamination in aquatic ecosystems. The accuracy of choosing a biosorbent strongly influences the success of biosorption. The organisms that are widely developed as biosorbents are microbes. Microbes live in ecosystems by forming biofilms. Therefore, knowledge related to the mechanism of heavy metal adsorption, such as Hg(II) by biofilms, will contribute to the development of biofilm-based biosorption. This study aims to analyze the biosorption characteristics of Hg(II) by biofilms taken from aquatic ecosystems. The methods used are kinetic adsorption analysis, isotherm adsorption analysis, and FTIR spectra analysis of biofilms. This study indicates that the adsorption process of Hg(II) into the biofilm is a physicochemical process. The adsorption sites that bind Hg(II) are negatively charged sites resulting from the ionization of functional groups such as carboxyl groups present in polymeric biofilms. According to this study, biofilm is an excellent biosorbent to adsorb pollutants such as Hg(II) from aquatic ecosystems.

KEY WORDS : Heavy metal, Water pollution, Adsorption, Biofilms, Microbial ecology

INTRODUCTION

Water pollution is one of the main problems being facing the world today. Pollution can be caused by many types of pollutants, such as heavy metals (Briffa *et al.*, 2020). Heavy metals are often produced by industrial activities such as the manufacturing industry. One of the pollutants produced by this industry is Hg(II) (Driscoll, 2013). This metal is widely used as a catalyst in the manufacturing industry (Liu *et al.,* 2020). Hg(II) waste may also come from the chlorine-alkali industry, electrical equipment, paints, thermometers, tensimeters, agricultural industries, and detonator factories. Besides, Hg(II) is present in amalgams which are used as dental fillings (Litfak *et al*., 2003). Hg(II) is one of the most reactive pollutants (Salem *et al.*, 2000) and can accumulate through the food chain (Ayangberno and Babalola, 2017; Arne and Hans, 1971; Hosseini *et al*., 2013). When accumulated in the body, Hg(II) can cause neurological disorders, prevent DNA replication, and is a carcinogen (Dwayana and Fahruddin, 2012; Jaishankar, 2014).

Various technologies have been developed to overcome heavy metal pollution, such as Hg(II). One technology that has great potential is biosorption (Paula *et al.,* 2018). In principle, biosorption is the process of adsorption of pollutants by utilizing biomass (Torres, 2020). The abundant biomass in aquatic ecosystems is microbes. Most microbes live in aquatic ecosystems by forming biofilms (Carla *et al.*, 2015; Balcázar *et al.*, 2015; Kurniawan and Yamamoto, 2019). Biofilm is a collection of microorganisms attached to the surface (Donlan, 2002) and encased in a matrix of Extracellular Polymeric Substances (EPS) (Chew and Yang, 2016; Karygianni *et al.*, 2020). In their natural environment, streamer biofilms show a diversity of microbial communities consisting of various organisms such as bacteria, archaea, algae, yeasts, fungi, and protists (Lear, 2016; Kurniawan and Yamamoto, 2019; Davey, 2000).

Although biofilms are reported to have the ability to accumulate heavy metals, the use of biofilms that grow naturally in nature for the biosorption of Hg(II) is still rarely reported. Understanding the biosorption of Hg(II) by biofilms will open up opportunities to develop effective and efficient water treatment technologies. This study aims to analyze the biosorption characteristics of Hg(II) by biofilms by investigating the kinetics of adsorption and adsorption isotherms. FTIR spectra analysis was also carried out to analyze the adsorption sites used to bind Hg(II). According to the results of this study, the biofilm matrix is a biosorbent that can be developed in the biosorption of Hg(II) from aquatic ecosystems.

MATERIALS AND METHODS

Sample preparation

The biofilm used in this study was sampled from the Bulak aqueduct, Surabaya, Indonesia. The biofilms grew on stones that had been placed two months before sampling. The biofilm was taken from the stone surface using a toothbrush and suspended in 80 ml distilled water. The biofilm suspension was then brought to the laboratory at 4 °C. The biofilm suspension was then centrifuged (6500 rpm for 5 minutes), the biofilm pellets were taken and washed with distilled water through centrifugation (6500 rpm for 5 minutes) 3 times. The biofilm pellets were stored at -20 °C before being used in the experiments.

Kinetics of Adsorption

The chemical solutions used in this study were prepared by dissolving the reagent grade $HgSO_4$ in distilled water. 0.8 grams of pelleted biofilm was put into 50 ml of 50 mg/l of $HgSO_4$ solutions. The resulting suspension was homogenized with a magnetic stirrer. Then 4 ml of biofilm suspension was subsampled after 5, 15, 30, and 60 minutes. Each subsample was then centrifuged (6500 rpm for 3 minutes). The concentration of Hg(II) in the supernatant was then measured using Atomic Absorption Spectroscopy. The experiment is repeated three times, independently.

Adsorption Isotherm

Biofilm pellets (0.8 g) were added to 80 ml of $HgSO_4$ solution with varying initial concentrations (10, 30, 62.5, 500, 1,000 mg/l). The suspension biofilm obtained was homogenized with a magnetic stirrer. Ten minutes later, the biofilm suspension was centrifuged (6500 rpm for 3 minutes), then the supernatant was stored for use as a sample for measuring the concentration of Hg(II). The experiment was repeated three times independently. The adsorption isotherm data were then analyzed using the following variants of the Langmuir isotherm adsorption:

$$\frac{C}{N} = \frac{1}{(Nmax)b} + \frac{C}{Nmax}$$

C is the equilibrium concentration (mg/L), N is the amount of Hg(II) adsorbed (mg/wet-g), Nmax is the maximum adsorption amount (mg/wet-g), and b is the adsorption equilibrium constant (L/ mg).

FTIR Spectra Analysis

Dry biofilm pellets (0.01 g) were mixed with KBr. The resulting pellet was put into a sample holder and used as a sample for FTIR measurements using a Shimadzu FTIR Spectrometer 84002 (Shimadzu Corporation, Japan). The biofilms sampled in the FTIR spectra analysis were before and after Hg(II) adsorption in the kinetic of adsorption experiment.

RESULTS AND DISCUSSION

Kinetics of Adsorption

The adsorption of Hg(II) by biofilm was analyzed at contact times of 5, 15, 30, and 60 minutes (Figure 1).

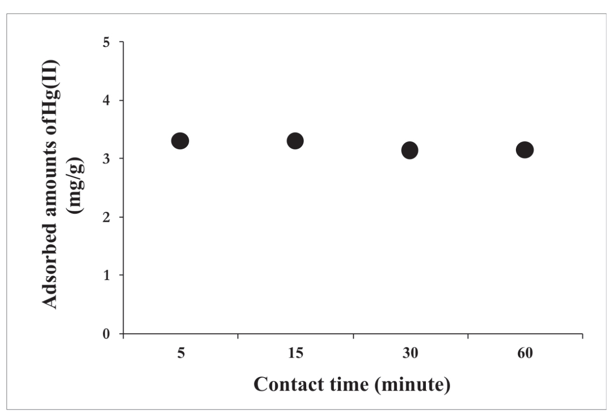

Fig. 1. Time course of Hg(II) adsorption to the biofilm.

The amount of Hg(II) adsorbed by the biofilm after 5 minutes was 3.2 g/wet-g, and this amount was relatively stable until the end of the experiment. These results indicate that the adsorption by the biofilm matrix takes place in a fast process. These characteristics are similar to the results shown in the adsorption of other heavy metals by biofilms (Cloirec *et al.,* 2003). The fast adsorption process is one of the main characteristics of the physicochemical (Kurniawan and Yamamoto, 2019) adsorption process. It seems that the adsorption of Hg(II) by the biofilm analyzed in this study also goes through the same process.

The physicochemical process in the adsorption of ions such as Hg(II) by biofilms can take place through an electrostatic interaction mechanism (Torres, 2020) or an ion-exchange mechanism (Bashir *et al.,* 2020). Hence, Hg(II) can be bound by negatively charged sites present in the biofilm polymers (Kurniawan *et al.,* 2012). On the other hand, adsorption through an ion-exchange mechanism can occur because Hg(II) ions replace ions that have been previously adsorbed in the polymer biofilm. The replaced ions may be the ions abundant in the biofilm matrix, such as Na, K, Mg, and Ca (Song and Laura, 2006).

Adsorption isotherm

Hg(II) adsorption characteristics into the biofilm were further analyzed by conducting an isotherm adsorption experiment. In this study, Hg(II) adsorption by biofilm was observed using various Hg(II) concentrations (Figure 2). The amount of Hg(II) adsorbed by the biofilm increased along with the increase in the equilibrium concentration and tended to be stable at high concentrations. At low concentrations (below 100 mg/l), the ratio between available adsorption sites and the number of Hg(II) ions is still relatively large. This large ratio causes Hg(II) ions to be readily adsorbed by the biofilm matrix (Kurniawan *et al.,* 2015).

The ratio of free adsorption sites to the number of Hg(II) ions in the water decreases with increasing adsorption sites that bind Hg(II) ions. At high concentrations (above 400 mg/l), almost all ionizable adsorption sites have bound Hg(II) so that the amount of Hg(II) adsorbed does not increase even though the equilibrium concentration is increased. The trend of adsorption of the Hg(II) isotherm into the biofilm forms an L-type adsorption isotherm. This result suggests that the adsorption shows the adsorption characteristics of the Langmuir mode (Zhu *et al.,* 2020).

Hg(II) adsorption characteristics by biofilm were further analyzed using a variant of the Langmuir adsorption model (equation 1). In this model, the maximum adsorption amount (Nmax; mg/wet-g) and the adsorption equilibrium constant (b; L/mg) are calculated assuming that a dynamic equilibrium occurs between the adsorbed ions (N) and free ions in solution, which then results in the equilibrium concentration. (C). The adsorption equilibrium constant is defined as the ratio of the rate of adsorption and desorption where the value of b will increase if the rate of adsorption exceeds the rate of desorption. Plotting C/N against C produces a straight line with a slope of 1/Nmax, and the y-axis intercept is 1/(Nmax)b (Figure 3), and thus the values of Nmax and b can be calculated.

Data plotting of Hg(II) adsorption into biofilms based on the Langmuir isotherm adsorption model has a value of R= 0.97. This result indicates that Hg(II) adsorption by the biofilm shows the characteristics of the Langmuir adsorption type.

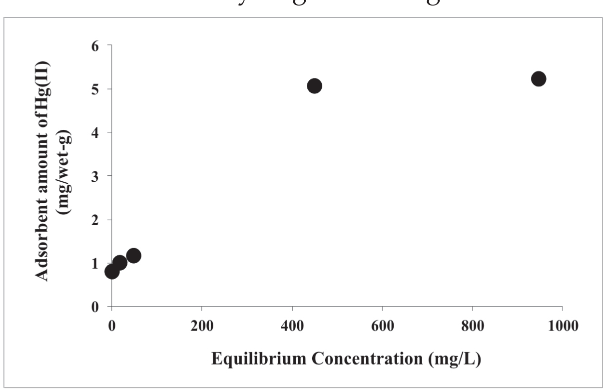

Fig. 2. Adsorption isotherm of Hg(II) to biofilms

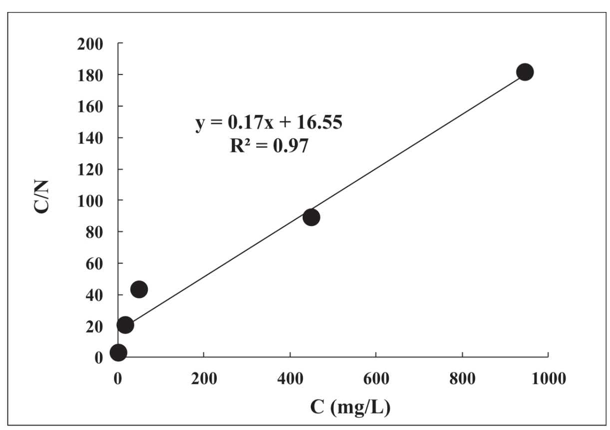

Fig. 3. The overlay of the Hg(II) adsorption to the biofilm using the variant of the Langmuir Isotherm Model. C is the equilibrium concentration and N is the adsorbed amount.

Based on the slope and intercept values obtained, the biofilm's Nmax Hg(II) in this study was 5.9 mg/ wet-g, and the b value was 0.01 L/mg. These results indicate that biofilms can be used as good biosorbent in Hg(II) adsorption from aquatic ecosystems. The results of this study can be the foundation for developing biofilms as biosorbent in water treatment of various pollutants in aquatic ecosystems.

FTIR spectra

The negative electrical charge on the polymer biofilm is a binding site for heavy metals, including Hg(II) in this study (Ayangberno and Babalola, 2018). This electrically charged site results from the ionization of functional groups present in the biofilm polymers (Kurniawan *et al*., 2012). In order to analyze this functional group, the FTIR spectra of the biofilm before and after Hg(II) adsorption were analyzed in this study (Figure 4).

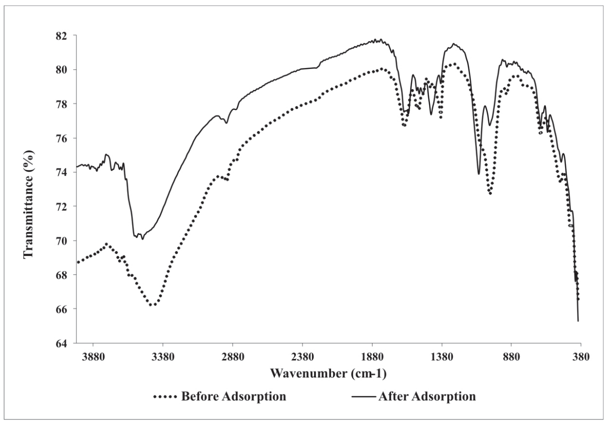

Fig. 4. FITR spectra of matrix biofilm before (a) and after (b) adsorption of Hg(II).

The results of the FTIR spectra analysis showed that several functional groups exist in the biofilm. The FTIR spectra of the biofilm before adsorption of Hg(II) showed the following results: peaks at 3695.48 cm^{-1} and 3451.59 cm^{-1} indicated the presence of O-H, peaks at 2924.67 cm^{-1} indicated C-H, peaks at 1650.11 cm^{-1} and 1542.12 cm^{-1} indicates C=C, peaks at 385.72 cm^{-1} indicate C-H, peaks at 1033.50 cm^{-1} indicate C-O, and peaks at 668.87 cm^{-1} and 542.15 cm^{-1} indicate the presence of C=O.

The results of the FTIR spectra analysis of the biofilm after adsorption of Hg(II) showed the presence of peaks at 3856.25 cm^{-1}, 3749.35 cm^{-1}, and 3569.98 cm^{-1} which indicated the presence of O-H, peaks at 2925.73 cm^{-1} and 1455.33 cm^{-1} indicates the presence of C-H, the peak at 1618.60 cm^{-1} and 1540.81 cm^{-1} indicates the presence of C=C, the peak at 1112.79 cm^{-1} indicates C-O, the peak at 1034.45 cm^{-1} and 618.84 cm^{-1} indicates the COOH, peaks at 669.29 cm^{-1} and 521.05 cm^{-1} indicate the C=O.

The comparison between the FTIR spectra of the biofilm before and after Hg(II) adsorption indicated that a significant change occurred in the COOH, which is characteristic of the carboxyl group. The hydroxyl functional group is reported to bind heavy metal ions present in the waters (Ibbet *et al.*, 2006; Mao *et al.*, 2009). The biosorption process involves ionic interactions, polar interactions, combined interactions, and mineralization between metals and biopolymers (macromolecules) as a source of functional groups, which play an essential role in binding metal ions.

CONCLUSION

This study analyzed the adsorption of Hg(II) by biofilms. The results show that the adsorption process of Hg(II) into the biofilm is fast and takes place physicochemically. The adsorption sites that bind Hg(II) are negatively charged sites resulting from the ionization of functional groups such as carboxyl groups present in polymer biofilms. The adsorption of Hg(II) to the biofilm follows the Langmuir adsorption model, which indicates that the adsorption of Hg(II) to the biofilm matrix forms a monolayer in which the affinity of Hg(II) with each adsorption site in the biofilm has the same strength. The results of this study indicate that biofilms can be used as good biosorbent to adsorb pollutants such as Hg(II) from aquatic ecosystems.

ACKNOWLEDGEMENT

This study was supported by the Research Group Research Grant Program, Interdisciplinary Postgraduate Program, University of Brawijaya. The author would like to thank Mr. Adi Tiya Yanuar, Mr. Zulkisam Pramudia, and Ms. Yogita Dwi Susanti for the assistance provided during the implementation of this research.

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