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Adsorption of Nutrient Ions into Biofilm Matrices of Natural Microbial Consortium

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ABSTRACT

The presence of nutrient ions significantly affects the biotic components in the ecosystem, including the microbial consortium. Nutrient ions are an essential part of the aquatic ecosystem, which significantly affects microbial life. Most microbes live by forming biofilms that can uptake nutrient ions from the surrounding water. However, studies reporting the characteristics of uptake of nutrient ions by biofilms of the natural microbial consortium have been rarely reported. Understanding the uptake characteristics of cationic and anionic nutrients is an essential foundation for understanding biofilms as the predominant habitat of aquatic microbes. This study aims to analyze the adsorption characteristics of cationic nutrient (ammonium) and anionic nutrient (nitrate) by biofilm matrices. This study indicates that cationic nutrients can be adsorbed more into the biofilm matrix than anionic nutrients. The adsorption process of nutrient ions by biofilms is a fast process that allows the concentration of nutrient ions inside the biofilm matrix to be much higher than the surrounding water. According to the results of this study, the passive uptake of nutrient ions into the biofilm matrix is an important ecological factor providing nutrient ions for aquatic microbial life.

Key words : *Biofilm, Aquatic microbe, Microbial ecology, Nutrient ions, Aquatic ecosystem*

Introduction

Microbes are part of the biotic components whose ubiquitous in aquatic ecosystems. Despite their microscopic size, the abundance of microbes promotes their essential roles in ecosystems (Mahmoud, 2019; Smith, 2007). Microbes live by forming communities that attach to the substrate or float in water bodies (Kim *et al.*, 2016). The microbial community attached to the substrate and covered by a matrix of extracellular polymeric substances (EPS) is defined as a biofilm (Chen *et al.*, 2021).

Biofilms are the predominant microbial habitat in aquatic ecosystems (Ruhal and Kataria, 2021). Hence, comprehension of biofilms is fundamental to understanding the behavior of microbes in the environment. The micro environment in the biofilm matrix has different characteristics from the surrounding water, resulting in different microbial community structures inside the biofilm than in the surrounding water (Flemming, 2020). Biofilms play essential roles in aquatic ecosystems, such as microbial gene pools, supporting pollutant purification, and being actively involved in the nutrient cycles

(Kurniawan *et al.,* 2015). The biofilm matrix is a nutrient-rich microhabitat (Tsuchiya *et al.,* 2009) and can affect the dynamics of nutrients in aquatic ecosystems (Zhao *et al.,* 2019).

Biofilms may accumulate various ions, including nutrient ions, from the surrounding water (Geng *et al.,* 2019). The accumulation of nutrient ions has occurred from an early stage of the biofilm formation process (Hiraki *et al.,* 2009). The accumulation of these nutrient ions is the leading cause of biofilms becoming nutrient-rich habitats (Yuan and Oliver, 2019). These nutrient ions will affect microbial life (Li *et al.,* 2019) so that they become one of the main factors that encourage the formation of microbial communities in aquatic ecosystems (Kataky and Knowles, 2018).

Although the accumulation of nutrient ions by biofilms in the aquatic ecosystems is fundamental in the discussion of microbial ecology, studies regarding the uptake of nutrient ions by biofilms of the natural microbial consortium have been rarely reported. This study aims to analyze the adsorption characteristics of nutrient ions by a biofilm matrix that grows naturally in aquatic ecosystems. The nutrient ions that focus on this study are ammonium as a cationic nutrient and nitrate as an anionic nutrient. This study provides fundamental knowledge to understand the characteristics of the adsorption process of nutrient ions by the biofilm matrix in aquatic ecosystems. According to the results of this study, the passive uptake of nutrient ions into the biofilm matrix is an essential ecological factor providing nutrient ions for aquatic microbial life.

Materials and Methods

Kinetics of adsorption

The biofilms were washed six times with a 5 mM phosphate buffer salt solution (PBS) pH 7 by centrifugation. The biofilm pellets were stored at -40°C until ion adsorptions analyses were performed. 1 wet-g of the biofilm pellet was resuspended in 50 ml of 5 mM PBS of pH 7. The suspension was mixed vigorously with a vortex for 5 minutes, and then sonicated for 10 minutes, followed by the vortex for 30 seconds. Then, 5.0 mL of 20 mM of solution of reagent grade NH_4Cl or $NaNO_3$, prepared by dilute the chemical compound (Wako Pure Chemical Industries, Osaka, Japan) in 5 mM PBS of pH 7, was added to the suspension. The suspension was mixed well using magnetic stirrer. The aliquots of the suspension were taken after 1 min – 120 min, and then centrifuged (15,000×g at 4°C for 1 min) to separate the supernatant and the pellet. The ion concentration in the solution was measured using a capillary electrophoresis method (CAPI-3300, Otsuka electronics, Osaka, Japan). The adsorbed amounts of ion to biofilm weremeasured from the difference between ion concentration in the supernatant and in the control (only PBS and ion).

Adsorption Isotherm

Two milliliters of a 3 mM NH_4Cl aqueous solution or $NaNO_3$ aqueous solution prepared using the method described above was added to 8-mL samples of the biofilm suspension (0.5 wet-g of biofilm in PBS or distilled water for NH_4^+ or NO_3^- adsorption, respectively) and mixed well using a magnetic stirrer (approximately 700 rpm). After 5 min, 3 mL of the suspension was subsampled and centrifuged (8,000 × *g* at 4°C for 3 min) to separate the centrifuged pellet and the supernatant. Two milliliters of the obtained supernatant were used to measure the ion concentration using the capillary electrophoresis method (CAPI-3300; Otsuka Electronics). The remaining 1 mL of the subsample (leftover supernatant and centrifuged pellet) was resuspended in the original sample suspension.

To increase the ion concentration stepwise, the above procedure was repeated 6 times using a 3-mM concentration of the ion solution, 6 times using a 20-mM concentration, and finally 3 times using a 100-mM concentration. The amount of ions adsorbed to the biofilm was calculated from the difference between the amount of ions added to the biofilm suspension and the actual amount of ions in the biofilm suspension after each addition of ion solution.

The maximum adsorption amount and the adsorption equilibrium constant for ion adsorption were calculated using a variant of the Langmuir isotherm equation (Fomina and Gadd, 2014) as described below:

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

This equation is based on the assumption that a dynamic equilibrium exists between the adsorbed ion (N; mmol dry-g–1) and the free ion in solution, the concentration of which is given as an equilibrium concentration (C; mM). The adsorption equilibrium constant (b) was defined as the ratio of the adsorption and desorption rates. The value of b increases as the adsorption rate exceeds the desorption rate. The plot of C/N against C yields a straight line with a slope of $1/N_{max}$ and a y-axis intercept of $1/(N_{max})b$; thus, the values of N_{max} (the maximum amount of adsorbed ion; mmol dry-g–1) and b can be calculated. To prevent ion uptake by microbes in the biofilm, all adsorption isotherm experiments were conducted in an ice bath (approximately 0°C).

Results and Discussion

Kinetics of adsorption

The effect of contact time on the rate of adsorption of ions by biofilm was analyzed in this study. The studied ions were ammonium and nitrate which are nutrient ions that affect the dynamics of microbial communities and water quality in aquatic ecosystems (Hui *et al.,* 2018; Li *et al.,* 2015). Ammonium was analyzed as a representative of the cationic nutrients, while nitrate represents the anionic nutrients. Both of these ions were adsorbed into the biofilm rapidly, where the number of ions adsorbed after 5 minutes was the same as after 120 minutes (Figure 1). The uptake of ions that occur quickly is a characteristic of passive uptake, with physicochemical mechanisms being the main driving force (Kurniawan *et al.,* 2020). The uptake of ammonium and nitrate by the biofilm in this study also seems to occur through this mechanism.

The amount of ammonium and nitrate adsorbed by the biofilm was approximately 0.073 mmol/wet-g and 0.045 mmol/wet-g, respectively. Hence, more cations can be adsorbed than anions in the biofilm matrix (Kurniawan and Tatsuya, 2019). It seems that the biofilm, which has a net negative charge at pH 7 (pH in this study), allows the biofilm to adsorb more cations than anions. These results indicate that the uptake of ions by the biofilm is strongly influenced by electrostatic interactions between electrically charged sites in the biofilm matrix and the adsorbates.

Adsorption isotherm

To examine the characteristics of ion adsorption in the biofilm in more detail, the adsorption isotherms of the examined ions to the biofilm were analyzed (Figure 2). Ions were adsorbed to the biofilm even at low ion concentrations and then leveled off at higher ion concentrations. The adsorption of ions could be fitted well using the Langmuir isotherm model (r^2e”0.95) (Figure 3 and 4 for ammonium and nitrate, respectively). The maximum adsorption amount (N_{max}) and the adsorption equilibrium constant (b) values of the ion adsorption were calculated using a variant of the Langmuir isotherm model (Table 1).

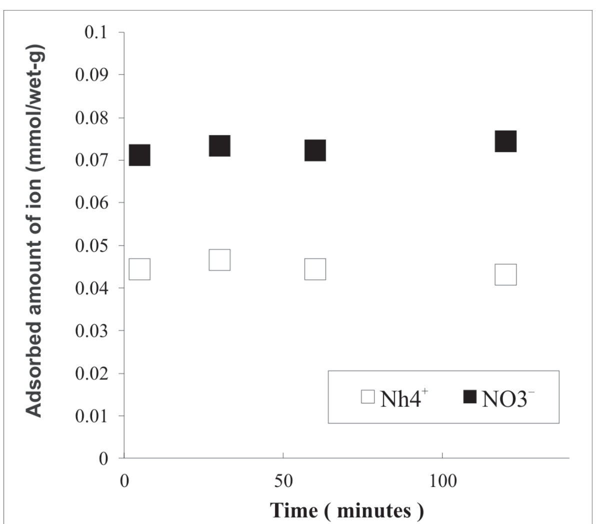

Fig. 1. Kinetics of Adsorption of nutrient ions on the biofilm matrices. Ammonium and nitrate are indicated by open (□) and solid (■) symbols, respectively.

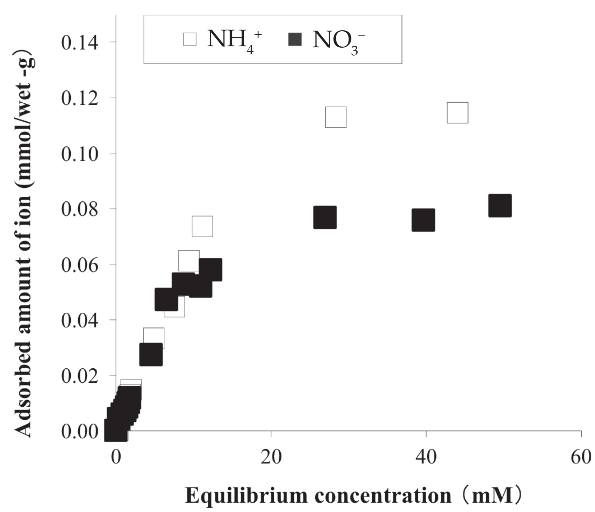

Fig. 2. Adsorption isotherm for nutrient ions on the biofilm matrices. Ammonium and nitrate are indicated by open (□) and solid (■) symbols, respectively.

Table 1. Maximum adsorption amount (N*max*) and adsorption equilibrium constant (b) of nutrient ions on the biofilm formed on the stones calculated using the Langmuir Isotherm model.

Adsorbent	Adsorbate	Nmax (mmol/wet-g)	b (L/mmol)
Biofilm	NH^{4+}	0.2	0.04
	NO3–	0.1	0.08

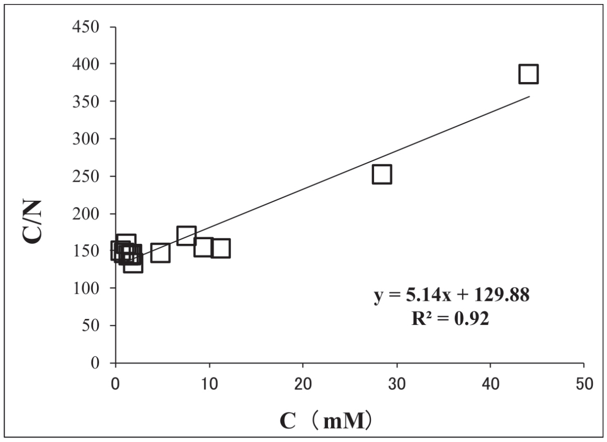

Fig. 3. The plot of C/N against C yields a straight line from adsorption isotherm of ammonium

The Nmax for ammonium and nitrate to the biofilm were 0.2 mmol/wet-g and 0.1 mmol/wet-g, respectively. The biofilm showed a greater Nmax value for cation adsorption than for anion adsorption. Thus, the biofilm seemed to have a higher adsorption capacity for cations than for anions, possibly because the biofilm carried more negatively charged sites than positively charged sites at a pH of approximately 7 (the pH of lake water), as revealed in the electric charge measurement (Fig. 4). The adsorption equilibrium constant values (b), which corresponded to the ratio of the adsorption and the desorption rates for each ion, were also calculated. The b values for ammonium and nitrate were 0.04 Ll/mmol and 0.08 l/mmol, respectively. These adsorption equilibrium constant values were much lower than the values of ion exchange resins reported in a previous study (Kurniawan *et al.*, 2013). The lower b value for the biofilm may indicate that ions are more loosely bound to and more easily desorbed from the biofilm polymer (Vijayaraghavan and Yun, 2008; Chubar *et al.*, 2008; Ozdemir *et al.*, 2009; Johnson *et al.*, 2006). In this case, microbes would be able to use these ions as nutrients more efficiently (Battin *et al.*, 2016; Kurniawan *et al.*, 2012; Sutherland, 2001).

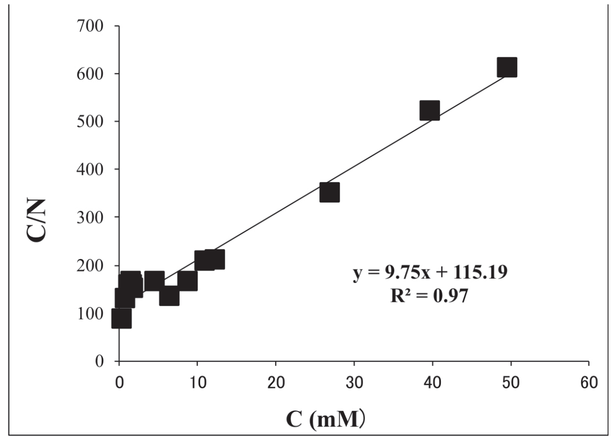

Fig. 4. The plot of C/N against C yields a straight line from adsorption isotherm of nitrate

Conclusion

This study indicates that both cationic nutrient (ammonium) and anionic nutrient (nitrate) can be adsorbed into the biofilm matrix. Under the same conditions, the amount of adsorption of cationic nutrients into the biofilm matrix was more significant than that of anionic nutrients. The adsorption of nutrient ions by the biofilm is a passive uptake process that occurs quickly. The Nmax for ammonium and nitrate to the biofilm was 0.2 mmol/wet-g and 0.1 mmol/wet-g, respectively, while the b values for ammonium and nitrate were 0.04 l/mmol and 0.08 l/mmol, respectively. According to the results of this study, the passive uptake of nutrient ions into the biofilm matrix is an essential ecological factor providing nutrient ions for aquatic microbial life.

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