

# Characteristics of Alcian-blue Dye Adsorption of Natural Biofilm Matrix

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**Abstract.** In this study, natural biofilm matrices formed on stones have been used for the adsorption of Alcian blue dye. Alcian blue is a member of polyvalent basic dyes that largely used from laboratory until industrial dyeing purposes. The adsorption of the dye onto the biofilm matrix has been carried out at different experimental conditions such as adsorption isotherm and kinetic of adsorption. The electric charge properties of biofilm matrix and its changes related to the adsorption of Alcian blue have been also investigated. Moreover, the results of Alcian blue adsorption to the biofilm were compared to those onto the acidic and neutral resin. The kinetics of adsorption result showed that the adsorption of the Alcian blue dye reached to a maximum adsorption amount within 60 minutes. The adsorption amount of Alcian blue to biofilm increased monotonously, and the maximum adsorption amount was greater compared to the resins. On the contrary, Alcian blue did not attach to the neutral resin having no electric charge. It seems that Alcian blue attached to the acidic resins due to electrostatic attractive force, and the same seems to be the case for adsorption of Alcian blue to biofilm. The adsorption of Alcian blue to the biofilm and acidic resins fitted to Langmuir type indicates that the binding of Alcian blue to the biofilm and acidic resins occurred in a monolayer like form. The maximum adsorption amount of Alcian blue on the biofilm (0.24 mmol/dry-g) was greater than those of acidic resin (0.025 mmol/dry-g). This indicates that the biofilm has many more sites for Alcian blue attachment than acidic resins. According to the result of this study, the biofilm matrix can be a good adsorbent for dye such as Alcian blue or other dyes that causing hazards in nature.

## 1. Introduction

The dyes are lost in industries effluents such as textile, leather, paper and plastics every year during the dyeing and finishing operations [1]. This problem becomes serious concern due to their effects on human and ecosystems [2,3]. The dangerous of dyes for ecosystems come from the high visibility, recalcitrance and undesirability, and thus, the removal of the dyes becomes a critical important effort to maintain clean environment. One of the dyes that largely use is alcian blue. This dye is a member of polyvalent basic dyes that largely used from laboratory until industrial dyeing purposes. When the dyes release to the environment such as aquatic ecosystems, the dyes may be accumulated in the interface surface including organism life on the surface.

Organism that found almost on the every interface in aquatic ecosystems is microbes formed biofilm. Biofilm consists of various microorganisms imbedded in a matrix composed of microbially produced extracellular polymer attaching to a surface [4,5]. Biofilm is formed when bacteria and other microorganisms attach to a surface and then replicate with producing extracellular polymer substrates [6,7].



The previous studies have shown that biofilm has high sorption capacities for many substances [8,9, 10,11]. The main driving force of the sorption process is an electrostatic interaction between the biofilm and the substances. Hence, the dyes such as alcian blue should be attracted and accumulated onto natural biofilm matrix. However, the study concerning this process has been rarely conducted. In the present study, the characteristics of alcian blue adsorption to natural biofilm matrix are investigated. According to the result of this study, the biofilm matrix can be a good adsorbent for dye such as alcian blue that causing hazards in aquatic ecosystems.

## 2. Materials and Method

### 2.1. Study area and sample preparation

Biofilm that used in this study was collected from Lake Biwa, Japan. This lake is located in the central part of Japanese Archipelago, having a surface area of 670 km<sup>2</sup> and total storage volume of 2.75×10<sup>10</sup>m<sup>3</sup>. The sample used in this study was collected from the shore of the southern basin (Akanoiwan) of Lake Biwa.

Samples for the characterization of the biofilm polymers were collected in November 2012 (winter) as the previous study founded that the biofilm thickness reaches the maximum amount in these seasons [6]. Stones were taken from the depth of 30 – 50 cm and brought back to the laboratory in a plastic container filled with nearby lake 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 biofilm pellets were prepared by centrifuging (8,000×g at 4°C for 10 min) the biofilm suspensions. The pellets of biofilm used as a sample in adsorption experiment were washed six times with 10 mM phosphate-buffered saline (PBS) (NaCl, 0.526 g; Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 0.358 g; distilled water, 1000 mL) of pH 7 and stored at -40°C until ion adsorptions analyses were conducted.

### 2.2. Maintaining biofilm wet/dry weight

The pellet of 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.

### 2.3. Electrophoretic Mobility

Electrophoretic mobility of biofilm polymer was investigated based on Kurniawan *et al.*, 2012 [6]. The pellet of biofilm polymer was washed three times as follows. The pellet was 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 pellet (ca. 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 biofilm polymer. The electrophoretic mobility (EPM) of the biofilm was measured on a ZETASIZER Nano-Z (Malvern Instruments, Ltd., Worcestershire, England) in 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. The pHs of PBS before and after the addition of biofilm and after measurement of EPM were recorded.

### 2.4. Kinetics of Adsorption

One wet-g of biofilm pellet was resuspended in 100 mL of 100 mM PBS of pH 7. The suspension was mixed vigorously with a vortex for 5 min, and then sonicated for 10 min, followed by the vortex for 30 s. Then, 6.0 mL of 10 mM alcian blue was added to the suspension. The temperature of the suspension was maintained at 25°C using a water thermostat, and mixed well using magnetic stirrer. The aliquots of the suspension were taken after 1 min – 60 min, then centrifuge (8,000 × g at 4°C for 3 min) to separate the supernatant and the pellet. The adsorbed amount of alcian blue by biofilm polymer

calculated from the difference of absorbance at 614 nm wavelength between controls (only PBS and alcian blue) and supernatants. The experiment was also conducted using one dry-g of acidic resin (SuperQ-650M; Toyopearl) and one dry-g of neutral resin (HW-65; Toyopearl) as an adsorbent.

### 2.5. Adsorption Isotherm

Several sets of biofilm suspensions were prepared (1 set was 0.1 wet-g of biofilm pellet resuspended in 5 mL of 100 mM PBS of pH 7). The suspension was mixed vigorously with a vortex for 5 min, and then sonicated for 10 min, followed by the vortex for 30 s. Then, a 10 mM of alcian blue solution, prepared with suspended alcian blue 8GX in 40 mL of 100 mM PBS of pH 7, was added to the biofilm suspension with various volumes (100 – 2000  $\mu$ L). The temperature of the suspension was maintained at 25°C using a water thermostat. After 5 minutes, the biofilm pellet and the supernatant was separated using centrifugation ( $8,000 \times g$  at 25°C for 3 min). The absorbance of alcian blue in the supernatant (abs-S) was measured calorimetrically by measuring the absorbance at 614 nm (the maximum absorption wavelength of alcian blue).

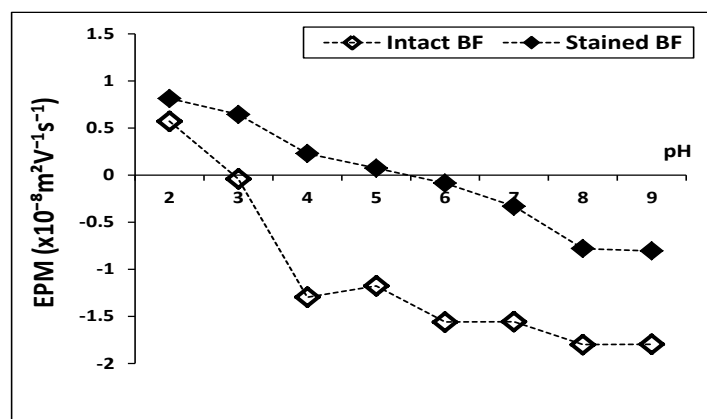
In the preliminary experiment, it was investigated that the supernatant (yellow supernatant) of the biofilm suspension also can adsorb alcian blue. Therefore, this supernatant was used as a control in the alcian blue adsorption experiment by biofilm polymer. To obtain the yellow supernatant, parts of the sets of biofilm suspension were centrifuged ( $8,000 \times g$  at 4°C for 3 min). The further steps were same with the experiment that used biofilm pellet as a sample. The result was the absorbance of alcian blue in the supernatant of control (abs-C). The adsorbed amount of alcian blue by biofilm polymer was calculated from the difference between abs-C and abs-S that equal with the adsorbed amount of alcian blue by biofilm polymer. The adsorption experiments (kinetics of adsorption and adsorption isotherm) were also conducted using one dry-g of acidic resin and neutral resin. The resins used were TOYOPEARL CM-650M and HW-65, respectively.

## 3. Result and Discussion

### 3.1. Electric charge properties of biofilm polymers

The EPM value of biofilm was measured (Fig. 1). The result was used to analysis the surface charge distribution of biofilm polymer and estimates the functional groups in biofilm polymer. The EPM of biofilm ranged from negative to positive values as a function of pH. It seems that both negative and positive charges exist on biofilm polymer. In the lower pHs the EPM of biofilm showed greater positive value. This indicates the decreasing of negative charge; the ionization of functional group such as carboxylic acids seems to be depressed at lower pH [6].

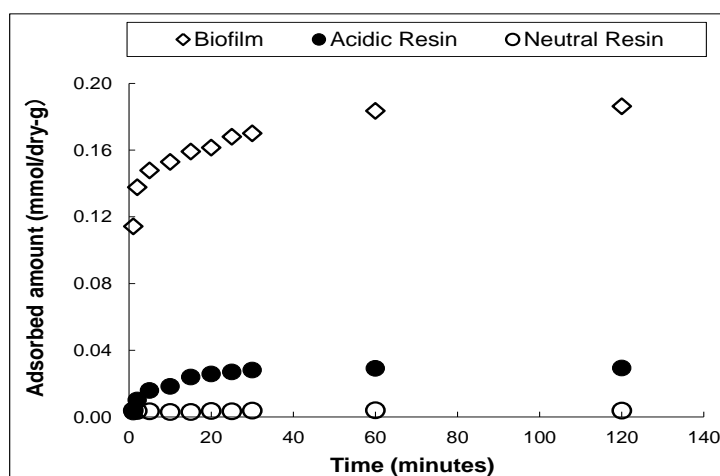
The EPM changed greatly around pH 4. This indicates there are some functional groups that have maximum proton adsorption ability around pH 4. This fact strengthens the estimation that carboxylic groups exist within BF polymer. To analyze the influent of alcian blue attachment on the electric charge characteristics of biofilm polymers, the EPM dependence on pH of the intact and alcian blue stained biofilm were compared (Fig. 1). EPM of alcian blue stained biofilm showed greater positive value than intact biofilm. It was indicating that biofilm polymers adsorb alcian blue which carries positive charge, and thus, makes biofilm polymers to have more positively charged sites. Alcian blue that designed to be adsorbed by electrostatic forces could attach to some surfaces having oppositely charges sites. This reveals that one of the main factors that promote adsorption of the dye to BF polymer is electrostatic attractive force.



**Figure 1.** Electrophoretic mobility (EPM) of intact (♦) and alcian blue stained (◊) biofilms. EPM values were measured in from pH 2 until pH 9.

### 3.2. Time course of adsorption

The time courses of alcian blue adsorption to biofilm, acidic resins and neutral resin were investigated (Fig. 2). The adsorption of alcian blue to various resins occurred quite quickly, i.e., reached to a maximum adsorption amount within a few minutes. This result and the smaller adsorption amount of alcian blue on a neutral resin, indicate that the driving force of alcian blue adsorption is an electrostatic attractive force between the positively charged alcian blue and the negatively charged resin surface.



**Figure 2.** Time courses of adsorption of alcian blue to biofilm (◊), acidic resin (●) and neutral resin (○).

The adsorption of alcian blue to biofilm was also a quick process (Fig. 2), indicating that the adsorption of the dye to biofilm is a process as in the case of ion exchange resins. The adsorption amount of alcian blue to biofilm increased monotonously, and the maximum adsorption amount was greater compared to the resins. Thus, it can be deduced that the sites in biofilm for alcian blue adsorption have similar electrostatic properties and their number per dry gram is far greater than that of ion exchange resins.

### 3.3. Adsorption isotherm

Alcian blue (positively charged) attached to the biofilm and acidic resins (negatively charged on their surfaces) (Fig. 3). The adsorbed amount of alcian blue leveled off at higher concentration of the dye. On the contrary, alcian blue did not attach to the neutral resin having no electric charge (Fig. 3). These

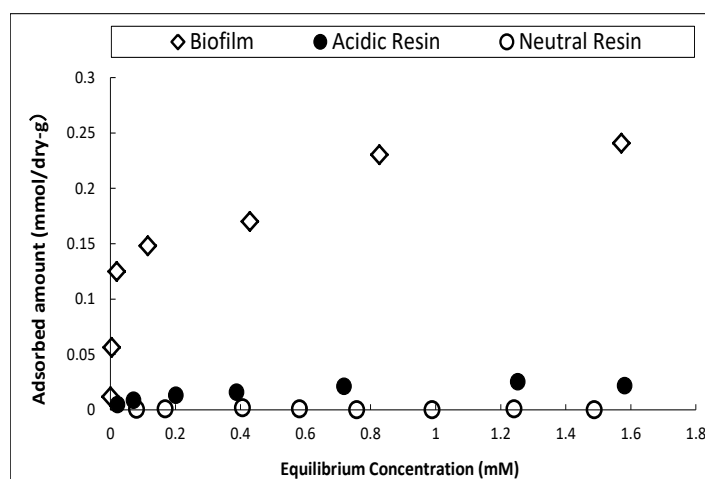
results indicate that alcian blue attached to the acidic resins due to electrostatic attractive force, and the same seems to be the case for adsorption of alcian blue to biofilm [11,12,13].

The maximum adsorption amount and the adsorption equilibrium constant for alcian blue adsorption were calculated using a variant of the Langmuir isotherm equation [13,14,15], as described below.

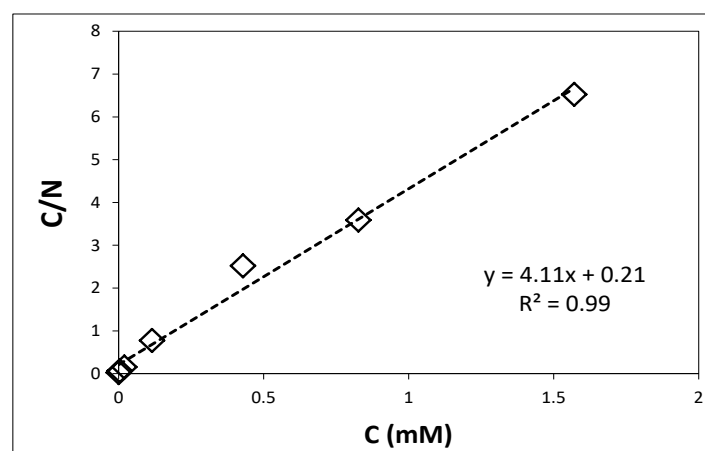
$$\frac{C}{N} = \frac{1}{(N_{\text{max}})b} + \frac{C}{N_{\text{max}}}$$

The plot of  $C/N$  against  $C$  yields a straight line with a slope of  $1/N_{\text{max}}$  and a y-axis intercept of  $1/(N_{\text{max}})b$ , and thus, the values of  $N_{\text{max}}$  (the maximum amount of adsorbed ion; mmol dry-g<sup>-1</sup>) and  $b$  can be calculated [13].

The adsorption of alcian blue to the biofilm and acidic resins fitted to Langmuir type adsorption (Fig. 4). Thus, it seems that the binding of alcian blue to the biofilm and acidic resins occurred in a monolayer like form. The maximum adsorption amount of alcian blue on the biofilm (ca. 0.24 mmol/dry-g) was greater than that of acidic resin (0.025 mmol/dry-g). This indicates that the biofilm has many more sites for alcian blue attachment than acidic resins.



**Figure 3.** Adsorption isotherm of alcian blue to biofilm ( $\diamond$ ), acidic resin ( $\bullet$ ) and neutral resin ( $\circ$ ).



**Figure 4.** The plot of  $C/N$  against  $C$  of alcian blue adsorption to biofilm based on the variant of Langmuir isotherm model.

#### 4. Conclusion

The present study revealed the characteristics of alcian blue adsorption to the biofilm polymers. The adsorption amount of alcian blue to biofilm increased monotonously, and the maximum adsorption amount was greater compared to the resins. On the contrary, alcian blue did not attach to the neutral resin having no electric charge. It seems that alcian blue attached to the acidic resins due to electrostatic attractive force, and the same seems to be the case for adsorption of Alcian blue to biofilm. The adsorption of Alcian blue to the biofilm and acidic resins fitted to Langmuir type indicates that the binding of Alcian blue to the biofilm and acidic resins occurred in a monolayer like form. The maximum adsorption amount of Alcian blue on the biofilm (0.24 mmol/dry-g) was greater than those of acidic resin (0.025 mmol/dry-g). This indicates that the biofilm has many more sites for Alcian blue attachment than acidic resins. According to the result of this study, the biofilm matrix can be a good adsorbent for dye such as Alcian blue that causing hazards in nature.

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