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Monitoring of biofilm in cooling water system by measuring lactic acid consumption rate

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TITLE RUNNING HEAD: Monitoring of Biofilm

Abstract

Biofilm formation in cooling water systems causes many problems such as increase of the frictional resistance in tubes and decrease in the thermal exchange of the heat exchanger. Monitoring of biofilm formation in the system is necessary to avoid biofilm related problems and optimize biocide addition. However, detection of biofilm formation in the whole system is difficult. To solve this problem, a novel and simple method for monitoring biofilm was developed in this study. Biofilm consists of living microorganisms, and nonliving organic and inorganic substances. Microorganisms in the biofilm consume substrate in the circulating cooling water. Therefore substrate consumption rate reflects biofilm activity in the system. Experiments were conducted step wisely; that is beaker-scale, bench-scale, and operating plant experiments. The beaker-scale experiment was conducted to select suitable

1

substrate. Among the eight substrates examined, lactic acid was the most bio-consumable one by the biofilm formed on carbon steel coupon. The lactic acid consumption rate (R_{lac}) was estimated at 273 mg/m^2 h. The R_{lac} value was compared before and after the hydrogen peroxide wash of the bench-scale plant. The R_{lac} before the wash was 36.3 mg/m^2 h. On the other hand, the R_{lac} after the wash was negligible. An experimental study was next conducted in the operating cooling water plant. A rapid decrease of lactic acid concentration in the circulating water of a relatively unclean system was shown. On the other hand, R_{lac} in a clean system was low. This simple method provided us information on biologically active biofilm formation in the system.

Keywords: Biofilm, Monitoring, Cooling water, Fouling.

1. Introduction

Most of the bacteria in a cooling water system are likely to be in biofilm. Almost immediately after water enters a pipe, organic molecules grouped under the name conditioning factor adhere to the surface (1, 2). The conditioning factor neutralizes the surface charge and facilitates adhesion of bacteria. In the initial stage, pioneer bacteria attach themselves by electrostatic attraction and physical forces. The daughter cells produce polysaccharide matrix which facilitates maturation of the biofilm (3, 4). The presence of biofilm on a metal surface, as well as its metabolic activities, can cause microbiologically influenced corrosion (MIC) (5, 6). In addition to the MIC problem, biofilm increases heat transfer resistance of process heat exchanger, fluid friction resistance, and causes *Legionella*-related problems (7, 8). Biofilm formation on the surface of heat exchange tubes reduces the heat transfer rate because the thermal conductivity of biofilm is significantly less than that of metal.

Good monitoring systems of biofilm are a prerequisite for the optimization of the development of efficient strategies for controlling biofilm formation and maturation (9). The monitoring parameter is usually number of colony forming unit (CFU) per ml or ATP concentration in cooling water (10, 11). In

aqueous systems, bacterial cells are able to alternate between suspended and sessile forms, the latter generally known as biofilm. The concentration of suspended bacteria in circulating cooling water does not always reflect that of sessile forms. An increased biomass of biofilm did not affect microbial numbers in the water (12). Therefore, CFU or ATP value in cooling water will not be a parameter of biofilm formation.

In this study, a simple monitoring method of biofilm formation and maturation in a recirculating cooling system was investigated. A cooling tower is an ideal place for the growth of living organisms, because it provides air, heat, and light (5, 13). The major economic impact caused by biofilm in cooling water systems is because of energy losses due to increase of fluid frictional resistance and increase of heat transfer resistance (11). An accurate and simple method of biofilm monitoring may allow reduction of biocide use and save energy. The new method was based on monitoring biological activity of the biofilm in the system. The most direct biological activity of the biofilm is substrate consumption rate. This study aimed to incorporate the advantages of experimenting on both the laboratory and field scale. Use of biofilm under laboratory conditions allowed selection of the substrate for quantification of biofilm activity while biofilm developed within the bench-scale and the operating plant was used to confirm validity of the new method.

2. Materials and Methods

2.1 Selection of substrate for the monitoring of the biofilm

To select a substrate which is readily consumed by the biofilm, model biofilm was formed on a carbon steel surface. Carbon steel coupons (30 mm × 75 mm) with a thickness of 0.35 mm were cut from a sheet stock. The specified composition of the carbon steel coupons was, in wt%, 99.71 Fe, 0.03 C, 0.19 Mn, 0.017 S, 0.013 P, and 0.01 Si. The surface was wet polished with an 800-grid polishing paper. The polished coupons were cleaned ultrasonically in acetone for 15 min, air-dried, and placed in

a water channel (Fig. 1A). The water channel contained 1.0 L medium containing (in mg/L) glucose 200, polypepton 320, $NaHCO_3$ 15, and KH_2PO_4 15. Activated sludge (10 ml) from a municipal wastewater treatment plant was used as the initial microbial inocula. Water was circulated by a tubing pump (Masterflex EZ-07520; Cole-Parmer Instrument Co., IL) at the rate of 800 ml/min. One tenth of the medium was exchanged with an identical volume of tenfold condensed synthetic waste water daily. After one week's incubation in the water channel, synthetic waste water was exchanged with tap water, and run for an additional day. The biofilm formed on the carbon steel was carefully transferred into a glass vessel (Fig. 1B) and used for selection of the substrate. Substrates used in this experiment were glucose, lactose, glycine, tryptophan, acetic acid, lactic acid NH_4^+, and SO_4^{2-}. Eight substrates were dissolved in 225 ml of distilled water respectively to make a final concentration of 20 mg/l, except the two ionic substrates (NH_4^+, and SO_4^{2-}). Total organic carbon (TOC) concentration was analyzed using a TOC analyzer (Shimadzu TOC-5000A, Shimadzu Co., Ltd., Kyoto, Japan). Tryptophan, acetic acid, and lactic acid concentration were analyzed by high performance liquid chromatography (LC-10AT, Shimadzu). Ammonium and sulfate ions were measured by ion-chromatography (LC-10AD; Shimazu). Experiments were conducted at 25˚C.

2.2. Lactic acid consumption in the bench-scale plant

A diagram of the bench-scale model cooling system is depicted in Fig. 1C. The plant consists of a water reservoir and a cooling tower. Operating conditions are summarized in Table 1. The surface areas of the plant are plastic (polyethylene and polyvinyl chloride): 29,840 cm^2, stainless: 500 cm^2, copper 155 cm^2, and glass 126 cm^2. The total surface area was about 3.06 m^2. The plant had been operated for half a year without washing. Tap water without any additives was circulated in the bench plant. The circulation rate was 11.5 times an hour. Therefore, it could be inferred that the incubation conditions in the bench-scale plant were nutritionally very poor (oligotrophic) and flow velocity was high compared with those of water channel used in the beaker-scale experiment. Blow is a physical

4

draining of water to avoid accumulation of salt in the circulating water. The blow rate was about 2 L/h. Water loss by evaporation was estimated at 7-8 L/h, which was replaced by adding fresh tap water. The experiments to monitor the consumption of substrate in the plant were conducted before and after the wash. Washing of the plant was carried out by adding 20 L of 35 %-H_2O_2 solution and rinsing the plant with tap water two times. A solution of the sodium lactate was added to the water reservoir to make a final concentration of 10 mg/L. The water sample was filtered through a membrane filter (0.22-µm pore size; Millipore), then subjected to HPLC analysis. The temperature of the circulating water was kept at 30 °C by a temperature controller equipped in the water reservoir. Before starting the experiment, 100 ml circulating water was sampled to examine the substrate consumption rate in it.

2.3. Lactic acid consumption in the operating plant

To demonstrate the validity of the newly developed method of biofilm monitoring, experiments were conducted in the operating plants. Plants used were named plant A, B, and C. Each plant consisted mainly of a cooling tower equipped on the roof and a heat-exchanger in the building. The main specifications of each plant are shown in Table 2. The total surface area in contact with circulating water was not exactly known. Plant A was relatively clean and new. On the other hand, plants B and C were relatively old and consequently foul. However, quantitative evaluation of cleanness of each plant was difficult. A solution of sodium lactate was added to the cooling tower to make a final concentration of 10 mg/L. Before the addition of sodium lactate to the plant, circulating water was sampled to monitor the R_{lac}. To estimate the physical loss of the water, sodium molybdic acid, which is biologically stable, was added to the plant to make a final concentration of 10 mg/L. The main physical loss of the substrate was due to blowing. Sodium lactate and molybdic acid dissolved in water was poured into the cooling tower within 5 minutes. The water sample was filtered through a membrane filter (0.22-µm pore size; Millipore), transported to the laboratory in a cooler-box, and concentrations of

molybdic acid and lactic acid analyzed. ATP concentration was analyzed by ATP analyzer (Lumitester C100N, Kikkoman Co., Chiba, Japan).

3. Results and Discussion

3.1. Selection of substrate for a monitoring of the biofilm

Biofilm was rapidly formed on the surface of the carbon steel coupon exposed in the water channel (Fig. 1A) inoculated with activated sludge from a municipal wastewater treatment plant. The condition in the water channel was eutrophic (nutritious) and the flow speed was comparatively low. After one week's exposure, steel coupon was rinsed with fresh tap water for a day and transferred to a glass vessel (Fig. 1B) to estimate the substrate consumption rate (R_{sub})(Fig. 2). The respective R_{sub} were calculated based on the data obtained during 6-hour incubation period and expressed as (mg-substrate/m^2 h) in Table 3. Among the six organic compounds (glucose, lactose, glycine, tryptophan, acetic acid, and lactic acid) consumption rates of glucose and lactic acid were higher than those of the other four compounds. Since glucose was used as a carbon source in synthetic wastewater, bacteria which utilize glucose as a carbon source might grow dominantly in the biofilm. Consumption rates of the two ions, NH_4^+ and SO_4^{2-}, were small compared with those of organic compounds. This suggested that nitrification and sulfate reduction activities in the biofilm might be low. Lactic acid was not used as a substrate during biofilm formation. However, consumption of lactic acid was the highest among the substrates used in this experiment. Therefore, lactic acid was selected as a tracer substrate to estimate the biological activity of the biofilm.

3.2. Lactic acid consumption in the bench-scale plant

Since the biofilm formed in the water channel used in the bench-scale experiment was thought to be different from the biofilm formed in the operating plant, a bench-scale plant which had been operated

6

for half a year under conditions similar to those of an operating plant, was used to further experiments conduct. The decrease of lactic acid concentration in the circulating water before the wash was proportional to time (Fig. 3). After 6 hours, the value reached half of the initial value. Possible reasons for loss of lactic acid in the system were biological consumption by the biofilm on the material surface and bioflock in the circulating water, and loss due to blow. Biological loss of lactic acid due to bioflock in the circulating water before the wash was estimated as 0.093 mg/L h. The values for blowing and total system were 0.098 and 0.746 mg/L h, respectively. Therefore, lactic acid consumption rate by the biofilm before the wash was estimated at 0.555 mg/L h. Since the total surface area of the system and total volume of the water circulating were estimated at about 3.06 m^2 and 200 L, respectively, the lactic acid consumption rate by the biofilm could be calculated as 36.3 mg/m^2 h. The same value for the biofilm formed on the carbon steel coupon was 274 mg/m^2 h (Table 3). Biological activity of the biofilm formed in the water channel was higher than that of bench-scale plant. Biofilm grown at low flow velocities exhibits low density and high effective diffusivity but can not resist higher shear stress, whereas biofilms grown at higher flow velocities are denser and can resist higher shear stress but have lower effective diffusivity (10, 14). Diffusion of the lactic acid through the biofilm formed in the bench-scale plant might be a rate limiting step.

A similar experiment was conducted after the H_2O_2 wash solution. Before the wash, water in the reservoir was transparent. On the other hand, after the wash the water in the reservoir became turbid due to presence of biofilm detached from the system. The water was discharged and rinsed with tap water twice. Then, lactic acid solution was added to the bench-scale plant to evaluate consumption rate. Decrease of lactic acid in the system was 0.082 mg/L h, which was almost the same as loss due to blow (0.098 mg/L h). The R_{lac} in the circulating water was negligible, suggesting that the biological activities of both biofilm and bioflock were at trace level after the wash. Based on this bench-scale experiment, we were convinced that the lactic acid consumption rate might reflect activity of biofilm formed in the system.

7

3.3. Lactic acid consumption in the operating plant

The R_{lac} in the operating plant was analyzed (Fig. 4). The average circulation time of cooling water in the plant estimated according to the water storage and circulating rate (Table 2) were 2.8, 3.4, and 2.4 min for plant A, B, and C, respectively. The substrate and molybdic acid added to the cooling tower were expected to mix rapidly in the circulating water. However, the concentration of the molybdic acid was scattered during the initial several hours after the addition. The R_{lac} of each plant was calculated from the data in the Fig. 1. Biofilm activity of substrate consumption was calculated by subtracting loss by blowing and consumption in the circulating water from the total loss in the plant (Table 4). In plant A, the relatively clean one, lactic acid added to the plant was not completely consumed during the test period. After the experiment, plant A was opened to observe inside. Pipes used in the heat exchanger of plant A were made of copper. No distinguishable fouling was observed through fiberscope on the surface of the pipe. Substrate consumption rate of the film in plant A was smaller than those of plant B and C. On the other hand, R_{lac} in the circulating water of plant A was the highest among the three plants. It is interesting to know that the R_{lac} in the circulating water was inversely proportionate to that of biofilm. Plant B showed the highest biofilm activity and the lowest activity of the circulating water. It was interesting that the increase in microbial activity in biofilm had no long term effects on microbial activity in water. Biofilm formed on the surface might catch bioflock in the circulating water and reduce the biological activity of the water. This finding suggested that the biological activity of the circulating water evaluated by the ATP concentration may not reflect the biofilm formation in the system. And there is no direct linkage between microbial numbers in biofilm and in water.

Change of the ATP concentration in the circulating water after the addition of the lactic acid was analyzed in the laboratory (Fig. 5). Before the addition of the lactic acid, ATP concentration in the circulating water of plants B and C was around 1,000 pmol/L. The value did not change after the

8

addition of lactic acid. On the other hand, the initial concentration of ATP in plant A was about 2,000 pmol/L and the value rapidly increased after the addition of lactic acid. This observation indicated that the microbes in plant B and C were resting and could not be converted into vegetative state immediately after the addition of substrate. On the other hand, microbes in plant A started metabolizing substrate and produced ATP, indicating that the microbes in plant A were mostly vegetative.

4. Conclusions

Biological activity of the biofilm formed inside in the cooling water plant was investigated. Substrate consumption rate was used as a index to estimate the biological activity. Among the eight substrates used in this study, lactic acid showed the highest consumption rate (R_{lac}) in the beaker-scale experiment. Following the beaker-scale experiment, bench and operating plant experiments were conducted. Within these experiments, we have reached the following conclusions:

1. Biofilm formed in the bench-scale plant showed lactic acid consumption activity before the H_2O_2-wash.

2. After the H_2O_2-wash, R_{lac} of the bench-scale plant was of trace level, indicating the R_{lac} value reflects biological activity of the biofilm formed in the plant.

3. The R_{lac} of the relatively clean operating plant was low, and high in the fouled plants.

4. The R_{lac} value of the biofilm was in inverse proportion to the R_{lac} value of circulating water.

5. ATP concentration of the circulating water did not reflect the biological activity of the biofilm.

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Figure legends

Fig. 1. (A) Water channel used for the formation of biofilm on the carbon steel coupons. Steel coupons (30 mm × 75 mm × t=0.35 mm) were placed in the channel circulating oxygen saturated medium, (B) Glass vessel for analyzing biofilm activity of substrate consumption. The biofilm formed on the steel coupon in the water channel was placed in the vessel filled with substrate solution, (C) Flow sheet of bench-scale cooling water plant.

Fig. 2. Substrate consumption by the biofilm formed on the steel coupon (A) Glucose, lactose, and glycine concentration were analyzed by the TOC analyzer, (B) Tryptophane, acetic acid, and lactic acid concentration were analyzed by the HPLC, (C) Ion concentration of NH_4^+ and SO_4^{2-} were analyzed by the ion-chromatography.

Fig. 3. Lactic acid consumption in the bench-scale plant. Lactic acid concentration changes in the plant (circle) and in the circulating water (triangle) were analyzed. Experiments were conducted before (solid-symbols) and after (open-symbols) the H_2O_2-wash.

Fig. 4. Lactic acid consumption in the operating plant. Three operating plants, plant A (A), plant B (B), and plant C (C) were analyzed. Lactic acid concentration changes in the plant (circle) and in the circulating water (triangle) were analyzed. The concentration change of molybdic acid in the plant (square) was also analyzed.

Fig. 5. ATP concentration changes in the circulating water. Circulating water was sampled from plant A (circle), plant B (triangle), and plant C (square). Arrow indicates the time of lactic acid addition.