Biofilm Formation Derived from Ambient Air and the Characteristics of Apparatus

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Abstract. Biofilm is a kind of thin film on solidified matters, being derived from bacteria. Generally, planktonic bacteria float in aqueous environments, soil or air, most of which can be regarded as oligotrophic environments. Since they have to survive by instinct, they seek for nutrients that would exist on materials surfaces as organic matters. Therefore, bacteria attach materials surfaces reversibly. The attachment and detachment repeat for a while and finally, they attach on them irreversibly and the number of bacteria on them increases. At a threshold number, bacteria produce polymeric matters at the same time by quorum sensing mechanism and the biofilm produces on material surfaces. The biofilm produced in that way generally contains water (more than 80%), EPS (Exopolymeric Substance) and bacteria themselves. And they might bring about many industrial problems, fouling, corrosion etc. Therefore, it is very important for us to control and prevent the biofilm formation properly. However, it is generally very hard to produce biofilm experimentally and constantly in ambient atmosphere on labo scale. The authors invented an apparatus where biofilm could form on specimen’s surfaces from house germs in the ambient air. In this experiment, we investigated the basic characteristics of the apparatus, reproducibility, the change of biofilm with experimental time, the quality change of water for biofilm formation and their significance for biofilm research.

1. Introduction

Biofilm[1-4] can be defined as the slimy matter formed on materials surface by bacterial activity. Strictly speaking, it is not any film, but just a thin matter on a solid-like matter. Actually, it is a mushroom-like or tower-like slimy matter composed of water, EPS (Exopolymeric substances), bacteria etc. Usually, many substances in water are incorporated into biofilm at the same time. Fig.1 shows the schematic illustration for biofilm formed on materials. It brings about many kinds of chronic diseases in our human bodies[5]. And it also leads to serious materials failures of structures from the mechanical or functional viewpoints, such as corrosion, cracks, fractures etc. of metallic or other...
However, the biofilm has not only negative sides, but also many positive sides for our society. Separation and recovery of metallic elements by biofilm reactors is one of such a promising possibility for sustainable circulating society in the near future. At any rate, all of them would be related to biofilm. Therefore, it would be very important to investigate the biofilm behavior, structures etc. on laboratory scale and also to utilize it for various purposes practically. To fulfill the versatile purpose, an appropriate experimental apparatus on laboratory scale was needed.

Recently, the authors invented a certain type of biofilm reactor to investigate biofilm formation behaviour on laboratory scale from both of fundamental and practical-applicable viewpoints. If it would work well for various purposes, lots of technical problems in practical industries could be solved and much more knowledge would be accumulated. However, various fundamental characteristics for the apparatus must be investigated and clarified to run it properly and also to apply the results to practical problems precisely and correctly. In this investigation, we produced and operated the biofilm formation reactor based on the cyclic water system from various viewpoints and discussed on the results.

2. Experimental

Fig. 2 shows the schematic illustration for the biofilm reactor used for this investigation. It enables biofilm to be formed by planktonic germs in ambient air mainly. The apparatus is basically composed of a column, water tank, pump and pipes connecting those units. All of them constituted a cyclic water system. The column was made of transparent acryl cylinder. The clean water was accumulated at the bottom tank. Then it was pumped up through the pipe to the upper column. The water was flown into the column and out to the pipe at the other end. In the column, some specimens (sheets of austenitic stainless steels, carbon steels and glasses, 10x20mm, the thickness ranges from 0.5mm to 2mm.) were placed with the supporter. The flow direction in the column was parallel to the specimen’s surface and specimens could occupy the upper and lower positions. The water flowing out of the column was introduced into the tank. However, a plate was placed just above the tank and the water was designed to run down on it before it was poured down into the tank. A fan was also set above the plate and the ambient air was designed to blow down on it from the fan. On the intermediate plate, air including planktonic germs was mixed into the water and the mixed water was circulated. The cycle was repeated during the test. The test term was 10 days at maximum.

The specimen after the exposure was taken out of the system properly and served for the following some observations and analyses. The specimen was observed by a low pressure SEM-EDX (Hitachi Miniscope, TM-1000) and also by an optical microscope (Keyence VW-9000). The former enabled us to analyse biofilms containing lots of water and organisms in biofilm. An the latter had a function to produce 3D images of samples where the observation stage moved up and down around a middle position slightly and automatically. The quality change of water in the system was checked by a multi pH meter periodically.

3. Results and Discussion

3.1 Biofilm formation and its reproducibility in the circulation system.

Firstly, we investigated if the biofilm formation could occur properly in the circulation system. Fig. 3 shows some results for the observation of an exposed specimen (carbon steel). Fig. 3-1 shows the conventional optical-microscopic photo for carbon steel exposed in a week. Bubble-like matters
were observed on the surface. By using the function for 3D analyses of the microscope, the color was assigned to the image according to the height of surface. The result was shown in Fig.3-2. The red color was given to the higher part, while blue to the lower one. The observation angle was changed virtually on the software and the result was shown in Fig.3-3. The final one shows the typical tower-like shape of biofilm.

Conventionally in our laboratory, the biofilm has been observed only by a low-pressure scanning electron microscope. Even though it could confirm the biofilm clearly, the difference between biofilm and corrosion was sometimes hard to be fixed. On the other hand, a 3D analysis by the optical microscope could clarify the difference to some extent, shown in Fig.4. Fig.4-(1), the corrosion occurred more remarkably, being confirmed by the observation of naked eyes. The typical red rust was scattered on the surface in the latter observation. In such a case, concave parts were observed more, even though many tower-like biofilms were observed. On the other hand, tower-like biofilms were much more remarkable in the case when the corrosion was controlled well (Fig.4-(2)). In this case, the concave parts were relatively few. Therefore, it suggests that the ratio and extent of tower-like films to the concavo parts could fix which would be dominant, corrosion or biofilm.

Fig.5 shows the specimen alignment in the column to investigate the effect of flow on biofilm formation. In our previous paper, we confirmed that the biofilm was hard to form without the flow parallel to the specimen surface and shear force as a result. In this investigation, we surveyed how the biofilm formation differed depending on the place from the viewpoint of flow strength. The part (a) in the figure 5 has stronger flow than at the part (b). Fig.6 corresponded to the results that the surfaces were observed by the 3D analysis of optical microscope. Fig.6-(a) corresponds to the result at the part (a) in Fig.5, while Fig.6-(b) to Fig.5-(b). When the specimen was placed at part (a), the biofilm was more remarkable, being compared with that at part (B). On the other hand, the extent of convex-concavo pattern at part (B) was much more remarkable suggesting that the corrosion occurred dominantly.

Fig. 7 shows the result to investigate the
reproducibility, when the different apparatuses having the same systems and structures were used to produce biofilms. Being compared each other, both specimens showed a similar extent of biofilm formation. As shown in Fig.1 schematically, the attachment of bacteria to material surfaces, the original cause of biofilm formation, would be the interfacial phenomenon with a certain probability between materials and organisms. Therefore, the biofilm shape and pattern might change for each opportunity. However, Fig.7 shows almost the similar amounts and thickness of biofilms were produced every time, when the experimental condition was the same.

3.2 Change of water quality and element condensation in biofilm

Fig.8 shows the change of pH and electric conductivity during the exposure test. As shown in Fig.8, the pH increased with time gradually, while the temperature was kept almost constant. The conductivity also increased with time, even though the plots varied widely to some extent. The increase of conductivity and pH suggest that various complex ions were produced with some metallic ion.

Fig.9 shows the results for biofilm formation on a glass specimen. The substrate glass was a commercial fluorine doped tin oxide. In the SEM photos for the specimen’s surface, the dark part corresponds to the biofilm area. When the electron beam was irradiated in the region, various metallic elements were identified. Regarding indium, the surface concentration was kept constant and low. For the doped fluorine (that was not detected by the EDX analysis.) covered the under layer including indium etc. almost completely. Therefore, it suggests that the other elements reflected the concentration in biofilm on the FTO specimen. Silicon and calcium increased with the biofilm formation to form silicate, calcium carbonate, their complex ions, compounds etc. The condensation of the two elements in biofilms has been pointed out so far[11]. On the other hand, some metallic ions, iron and zinc were also concentrated in biofilm with time. At this point, it is not clear why these metallic elements were condensed in biofilm. We presume that those metallic ions would react with calcium compound or silicate to form complicated complex compounds. However, they might react with EPS (Exopolymeric substances), one of the main components of biofilm to form bioorganic compounds. The problem would be an important mission to be carried out from the viewpoint of biofilm utilization.
4. Conclusions
A unique reactor based on the circulation water system was made and the characteristics were investigated as a preliminary research to investigate biofilm formation and to utilize it for practical application. The following results were obtained.

#1: Biofilm was produced on the specimen surface exposed to the clean water in the system.
#2: Typical tower-like biofilms were observed by the optical-microscopic 3D analyses.
#3: The biofilm was reproduced under the same experimental condition, even though the different apparatus with the same structure and system was used.
#5: The biofilm formation needed a certain flow strength and shear forth. Without them, corrosion dominated the biofilm on steel specimens.
#6: The pH and conductivity of circulated water increased with the exposure time. It might be attributed to the complex compounds.
#7: Silicon and calcium were concentrated in biofilm also for this investigation, just like the previous studies showed.
#8: Iron and zinc were concentrated in biofilms, even though the reason is not clear at this point. It should be solved in the near future for the practical application of biofilm.

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