

## How to Promote Formation of Biofilm

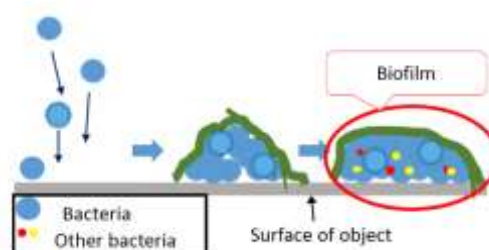
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### Abstract

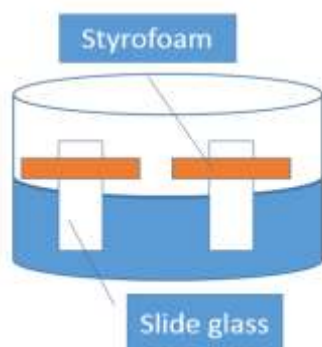
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### 1. Introduction

Biofilm is an aggregate of bacteria which has the ability to purify water. We conducted experiments to find a method to mass produce biofilm by changing the environment of the bacteria.



### 2. Method

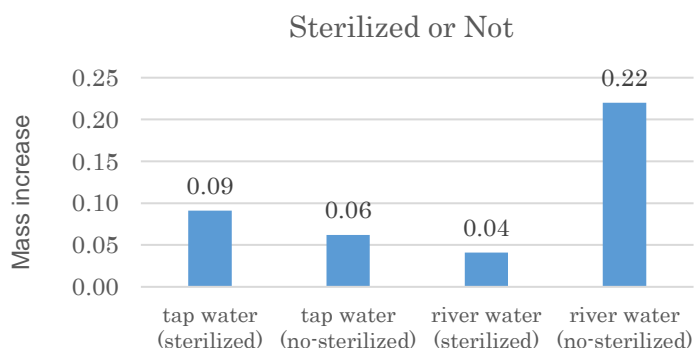


First, we got water from the river near our school and put them in the tanks. Six liters of water were in each tank. Second, we floated slide glasses in the water by using Styrofoam. Third, we left it for a week. Fourth, we took the glasses out of the tanks. Then, we measured how much mass had changed between before and after the experiment. We defined this change as the mass of the biofilm.

### 3. Result and discussion

#### Experiment 1

We used four tanks containing sterilized tap water, non-sterilized tap water, sterilized river water, or non-sterilized river water. We wrapped these tanks and left them in sunny place. The result is illustrated in Graph 1. When we compared sterilized tap water with non-sterilized tap water, we found little difference in biofilm mass. This implies tap water is originally disinfected and our sterilized treatment hardly effected it. When we compared sterilized river water with non-sterilized river water, we found a significant difference in



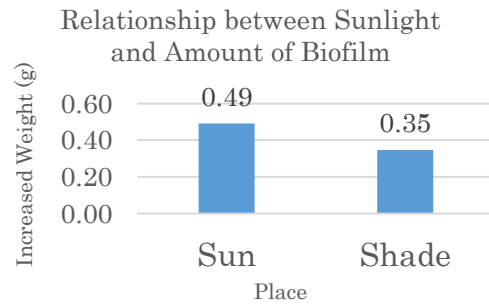
Graph 1

biofilm mass. This shows the number of bacteria in the water has great influence on the biofilm mass. From the above, we concluded bacteria in the water mainly makes biofilm.

### Experiment2-1

We put two tanks by the window and covered one of them with a cardboard case. Graph 2 shows standard deviation. We found the place in the sun has more influence on increasing the amount of biofilm than in the shade.

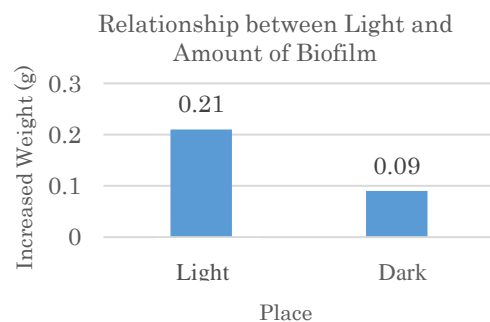
We thought of two reasons. First, photosynthetic bacteria reacted to the sunlight. Second, the water temperature rose because of the sunlight, so bacteria tended to proliferate in the environment.



Graph 2

### Experiment2-2

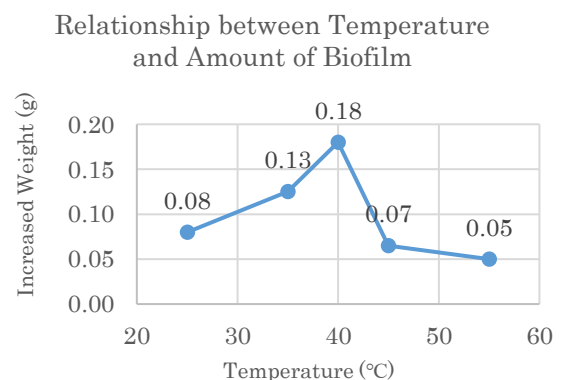
In a darkroom, we put two tanks which were illuminated by fluorescent light and covered one of them with blackout curtains. We tried not to change the water temperature. The water temperature in sun was 16°C, and in shade it was 16.5°C. Graph 3 shows that existence of light did not have an influence on the formation of biofilm.



Graph 3

### Experiment2-3

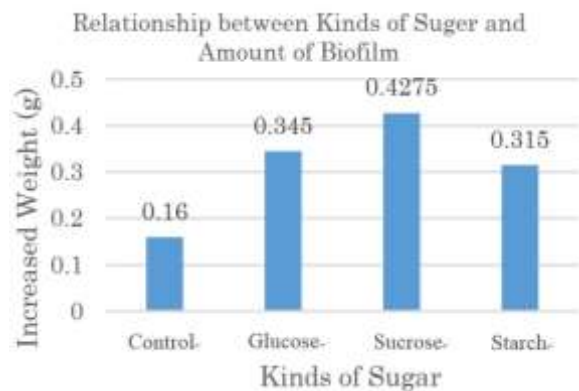
We used a yogurt maker to maintain the temperature (Graph 4). The best condition of water temperature for biofilm production was 20~40°C.



Graph 4

### Experiment3-1

We changed the type of sugar. Because the main substance of biofilm is sugar and bacteria use sugar, we thought sugar would affect the amount of biofilm. We used glucose, sucrose and starch. Glucose is a monosaccharide, sucrose is a disaccharide and starch is a polysaccharide. The result of our experiment is illustrated in Graph 5. It shows that the amount of biofilm is greatly influenced by sugar.

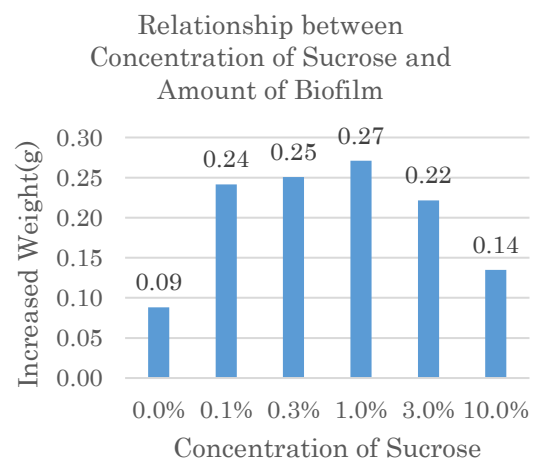


Graph5

When we used the water which contained sucrose, we were able to get the most amount of biofilm. We expected that a monosaccharide has the optimum influenced on the amount of biofilm because bacteria can use it easily. However, the result was different from our expectation.

### Experiment3-2

We changed the concentration of sucrose. We used water whose concentration of was 0%, 0.1%, 0.3%, 1.0%, 3.0% and 10% sucrose. The result of the experiment illustrated in Graph5? We were able to get the most amount of biofilm when we used the water with a sucrose concentration of 1.0%. We concluded when we used the water with a sucrose concentration of 3.0% and 10%, bacteria have difficulty reproducing in high osmotic pressure. On the other hand, when we used water with a low sugar concentration, such as 0.1% and 0.3%, it did not have an effect on the amount of biofilm.



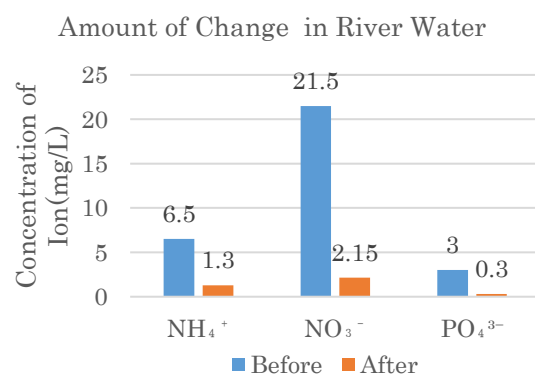
Graph 6

### Experiment4

We researched what substances are cleaned by biofilm, because we observed that water in tanks became gradually clear when we produced biofilm on slide glasses for a long time. We used three kinds of ions which are likely to be included in dirty water, to confirm that whether biofilm have an ability to clean up water or not.

They are  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ . We put

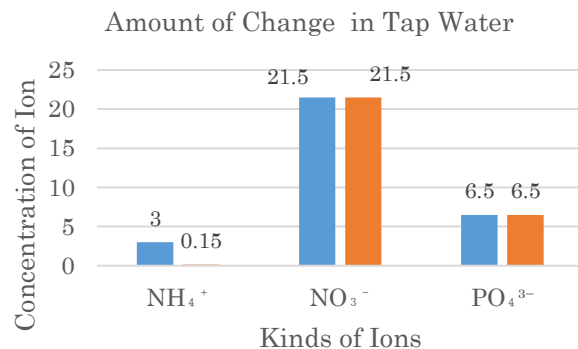
them into a tank and measured how much the concentration of ions increased or decreased



Graph 7

through the experiment with a water analysis kit.

When we used tap water, which had few bacteria, concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  did not change, however, the concentration of  $\text{PO}_4^{3-}$  had decreased a lot. (Graph 7) This shows that  $\text{PO}_4^{3-}$  decreased if we put it in water in the sun regardless of the existence of biofilm. On the other hand,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  decreased when we used river water. (Graph 8) This shows that biofilm affects  $\text{NH}_4^+$  and  $\text{NO}_3^-$ .



Graph 8

From the above, we concluded that biofilm have an ability to clean up  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in water.

#### 4. Conclusion

The optimum condition for producing biofilm is using river water, in the sun, at about 40°C, and using water with a sucrose concentration of 1.0%. Also, we proved that biofilm has ability to clean up water. We should find out how to use it in the future.

#### 5. References

- ・日本微生物生態学会 (2005) . 「バイオフィルム入門—環境の世紀の新しい微生物像」 . 日科技連
- ・兼松秀行・生貝初・黒田大介・平井信充 (2015) 「バイオフィルムとその工業利用」 . 米田出版
- ・栗原伸一・丸山敦史.(2017). 「統計学図鑑」 .オーム社
- ・上田拓治.(2009) . 「44の例題で学ぶ統計学検定と推定の解き方」 .オーム社
- ・Okubo Takuya. (1994) . Effects of Riverbed Biofilm on Changes in Water Quality in a Small Channel

<http://iss.ndl.go.jp/books/R100000002-I000000082150-00>

#### 6. Key words

Biofilm

Bacteria

Slime