## Dissertation Abstract

# Dynamics of Bacterial Population in Sludge and in Treated Water from Activated Sludge Processes

（活性汚泥およびその処理水中の細菌群集のダイナミクス）

by
Egodaha Gedara Wasana GUNAWARDANA
エゴダハ ゲダラ ワセナ グナワルダナ

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The present study focused to grasp bacterial population in treated water of the activated sludge processes. The activated sludge processes are widely used as a method of biological wastewater treatment. In these processes, treated water and activated sludge are usually separated by gravimetric settling, but the separation is not perfect and small amount of bacteria are left in treated water.

Bacteria in treated water affect the quality of treated water in three points of view. First is from the view of controlling pathogenic bacteria. Because this aspect is strongly related to human health, tremendous studies have been and are being done. Secondly, bacteria in treated water might affect ecosystems in receiving water bodies. And thirdly, these bacteria in treated water can interfere with advanced treatment processes for its reuse. Especially, considering the effects of these bacteria on receiving water bodies and water reuse, knowledge on whole bacterial population in treated water is essential. The amount of suspended solids (SS) and heterotrophic counts are indicators for the amount of whole bacterial population in treated water. Yet, these methods do not give the content of the bacterial population in treated water. Here is the need to understand the whole bacterial population in treated water.

Bacterial population in treated water is thought to reflect that in activated sludge. It is because treated water most probably contains small flocs of activated sludge. But there may also be differences. It is because some bacteria may prefer to live freely outside flocs and others may prefer to live in flocs. Therefore, it is expected that there are similarities and differences in bacterial populations in treated water and in activated sludge. Thus, the first objective of the study is to clarify the relationships of bacterial populations in treated water and in activated sludge.

In order to analyze bacterial populations in treated water and in activated sludge, the author employed one of molecular methods for bacterial population analysis in combination with a new method to prepare PCR compatible DNA extract from samples. The molecular method employed was the polymerase chain

reaction (PCR) followed by terminal restriction fragment length polymorphisms (T-RFLP) method. On the other hand, the new method to prepare PCR compatible DNA extract from samples is based on the destruction of cells by sonication followed, if necessary, by dilution to reduce inhibitory effects of substances in samples. Development of the new method is the second objective of this study. By the developed method, treated water samples are sonicated, then directly applied to PCR/T-RFLP analysis.

While analyzing the bacterial populations in treated water, the author found that filtrate of treated water through 0.2μm membrane filter contained small amount of DNA fragments, which could be amplified by PCR even without extraction of DNA. These DNA fragments (referred to as free DNA hereafter) might affect analysis of bacterial population in treated water from analytical point of view, but they may give any reflection of the dynamics of bacterial population. Thus, the third objective of the study was to investigate the behavior of free DNA in treated water.

With the above three objectives as the goal, this thesis contains seven Chapters. Chapter 1 is the introduction, and Chapter 2 is literature review. In Chapter 3, development of the methodology to prepare DNA template from treated water and activated sludge samples by the sonication based method was investigated. In Chapter 4, the developed method was applied to analyze the bacterial communities in treated water and activated sludge from laboratory scale activated sludge reactors. In Chapter 5, the bacterial communities in treated water and activated sludge in two full-scale wastewater treatment plants (WWTPs) were analyzed. In Chapter 6, composition of free DNA and its dynamics was studied. The source of the samples was filtrate of treated water analyzed in Chapters 4 and 5.

In Chapter 3, the experimental design and outcomes on the methodology development on the preparation of PCR-compatible DNA extracts from activated sludge and treated water were discussed. Treated water and activated sludge samples from full scale WWTP were used as the samples. They were sonified, and were diluted to different template concentrations. Then, using the 27f/519r primer set targeted at partial 16SrRNA gene, PCR was performed with different numbers of thermal cycles. The best results were obtained with the template DNA concentrations around 1-10pg/μL with 30 thermal cycles. The reproducibility was examined by comparing the composition of the PCR products by T-RFLP for triplicate analyses of both activated sludge and treated water samples; outcomes showed that the reproducibility of the developed method is satisfactory. The method of PCR template preparation developed here is easy and rapid to implement without the needs for chemicals and takes only a couple of minutes per sample.

In Chapters 4 and 5, the method developed in Chapter 3 was applied to investigate the bacterial population in treated water and in activated sludge. In Chapter 4, samples were obtained from laboratory scale reactors, while in Chapter 5, they were obtained from full scale treatment plants. In Chapter 4, samples were obtained two laboratory scale reactors operated with synthetic wastewater as the feed. For one of the reactors, samples were daily collected for 11 days, while for another; samples were collected weekly for 4 weeks. The bacterial populations in treated water and in activated sludge were successfully profiled by the template DNA, which were prepared by the developed method. The results showed that the bacterial community structures in treated water had significant differences from those in activated sludge. Some of peaks in the T-RFLP profiles were found more intense in treated water while other peaks were found more intense in activated sludge. The principal component analysis (PCA) on T-RFLP data showed that there was a similar trend of bacterial population changes in activated sludge and in treated water samples.

In Chapter 5, bacterial population in treated water and in activated sludge samples collected from full scale WWTPs were analyzed. Monthly samples were collected for a year from February 2010 to January 2011 to observe seasonal fluctuations, while daily samples for 5 successive days were collected in February, May, August, and November 2010 to observe daily fluctuations. The suspended solids (SS) in

treated water had an average of 6mg/L and 7mg/L in plant A and B respectively, and total DNA concentrations in sonicated treated water was in the order of 100μg/L. The outcomes clearly suggested that the bacterial populations in treated water had clear differences from those in activated sludge, as same as observed in laboratory reactors. Yet, the outcomes of PCA analysis on the T-RFLP data for monthly collected samples clearly showed a similar trend of bacterial populations changes in activated sludge and in treated water from both WWTPs. Year-round trend was confirmed by the PCA analysis, as the plots had a tendency to draw year-round circles. An additional experiment in Chapter 5 suggested that abundance of some of bacterial species was increased in treated water after the sludge had been stored for a couple of hours. The outcomes of experiments in Chapters 4 and 5 can be interpreted as follows. Peaks or bacterial species corresponding to those peaks, found more intense in treated water are associated with pin flocs or freely living in the bulk liquid. Those found more intense in activated sludge are associated with more stable flocs.

In Chapter 6, the behavior of free DNA in treated water of the activated sludge processes was investigated with basically the same samples as used in Chapters 4 and 5. As additional samples, influent wastewater to the full scale WWTPs were collected on the sampling occasions of October and December 2010. The average concentrations of free DNA in treated water were around 10pg/μL, while those of activated sludge mixed liquor and treated water samples were around 10.000pg/μL and 100pg/μL respectively. The concentration of free DNA was only about one tenth of that of total DNA in treated water. And major T-RFLP peaks in free DNA were not observed in treated water. Thus, it was concluded that free DNA does not affect the analysis of bacterial population in treated water by the method developed here.

The T-RFLP patterns of free DNA were very different from those of activated sludge. Yet, by PCA analysis, a similar trend in bacterial population change in activated sludge and free DNA were found. This outcome suggests that one of the sources of free DNA is bacteria in activated sludge. On the other hand, there were peaks that found in free DNA and in fluent samples but not in activated sludge samples. This suggests that another source of the free DNA is bacteria in influent. Yet, many of the peaks that were found intense in free DNA were weak or not detected in influent nor in activated sludge.

In conclusion, the outcomes showed that, it is enabled to use the developed methodology to analyses bacterial populations in treated water and in activated sludge. The bacterial populations found in treated water had significant differences from those in activated sludge from both laboratory reactors and WWTPs. However, similar seasonal trend of the changes of bacterial populations in treated water and in activated sludge was found. The free DNA concentration in treated water was only about one tenth of that of total DNA in treated water and it was found that the free DNA was not affected the analysis of bacterial population in treated water by the method developed in this study. The outcomes of this study are important in both practical point of view and scientific points of wives. From the practical point of view, conducting further studies to see the possibility of suppressing leak of dominant bacterial species to treated water are very important. From the scientific point of view; the outcomes of free DNA in treated water are important as it may reflect the microbial ecology in activated sludge.