Appendix C

論文の内容の要旨

Dissertation Abstract

論文題目
Dissertation Title

Study on Relationship between Dewaterability and Bacterial Population in Activated Sludge

（活性汚泥の脱水性と細菌群集構造の関係に関する研究）

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In this study, relationship between dewaterability and bacterial population in activated sludge was of interest. With the developed dewaterability testing method, water content of dewatered sludge (WCDS) testing method, and combination of two molecular biology methods-T-RFLP and pyrosequencing, dynamics of dewaterability and microbial population were monitored and their correlations were analyzed by multiple regression analysis. The detailed compositions of this thesis are introduced as follows:

Chapter 1 and 2 introduced the background of this study and relevant research results. Additionally, the significance of this study was mentioned as well.

Dewatering is a physical unit process to reduce the volume of sludge by removing water from wet sludge. Many factors, such as extracellular polymer substances (EPS) concentration, particle size distribution, specific surface area, density, particle charge, bound water content, pH, and organic concentration, have been reported to affect dewaterability of sludge.

In the case of dewaterability of excess activated sludge, bacterial population in the sludge would be one of possible factors that affect dewaterability. Yet, so far, very limited studies have been done to clarify the effects of bacterial population on dewaterability. And those limited studies often focused on the morphological characteristics of bacteria. However, bacterial characteristics other than morphology might also affect dewaterability. Thus, it is worth to study the relationships between whole bacterial population in activated sludge and its dewaterability.

Chapter 3 demonstrated the development of WCDS testing method and the details of selected molecular biological methods.

Dewaterability testing methods, such as capillary suction time (CST), specific filtration resistance (SRF) are popularly applied by other researchers on dewaterability comparison and improvement aspects. However, CST and SRF come with quite high deviation (up to 10% of CST and 24% of SRF) which may not reflect the dynamics of dewaterability during operation. Therefore, there had not been a

convenient method to determine dewaterability of excess sludge with small amount from laboratory scale activated sludge reactors.

In present study, firstly, 4×25 ml mixed liquor was centrifuged at 2000g (5 min) for thickening at the same time, supernatant was decanted, and then around 300mg-wet thickened sludge pellets each were loaded on piece membrane filters (for 4 samples) for dewatering. The filters were placed in a Swinnex filter holder (25mm, Millipore) individually, and the filter holder was set in a bucket for 50 mL conical tubes (No. 053-5010, Kubota). Then, further the loaded sludge pellets were again centrifuged at 2000g for 5 min to exert dewatering process. At last, the dewatered sludge cake (about 100 mg) was moved onto a piece of prepared aluminum foil (for which dry weight (A) had been predetermined), and the dewatered sludge weight including aluminum foil (B) was measured. In measuring the dewatered sludge weight, the foil was folded to cover the sludge to avoid drying during weight measurement. The sample was unwrapped, dried at 105°C for 60 min, cooled to room temperature in a desiccators for 30 min, and the weight after drying was measured. Water content of dewatered sludge (WCDS) was calculated to assess dewaterability by (B-C)/(B-A). Measurement of weight was done with an analytical balance XS105 (Mettler Toledo, USA) with a resolution of 0.01 mg. Analyzes were done in quadruplicate.

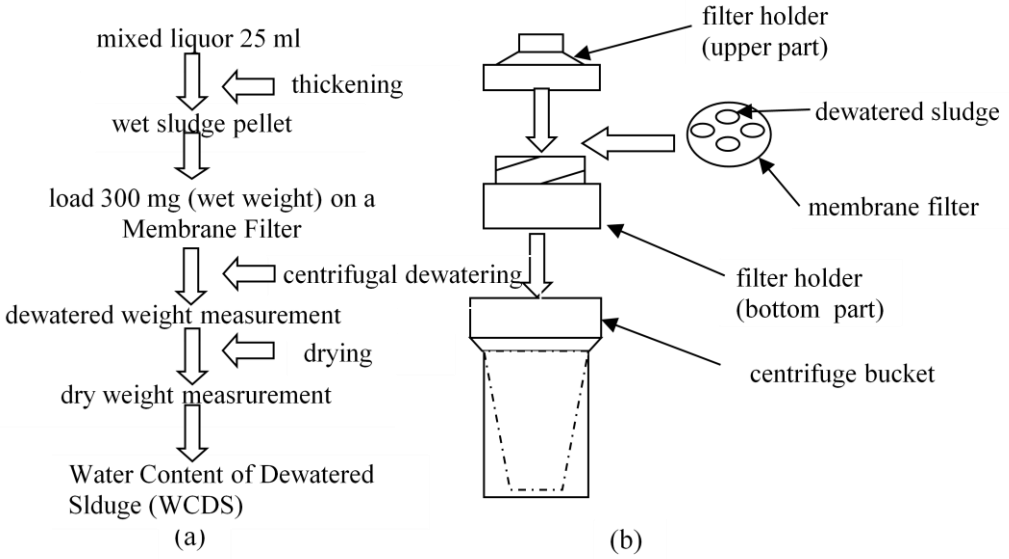

Figure 1 Flowchart of dewaterability testing. (a) Whole procedure, and (b) Schematic of dewatering step.

Meanwhile, there are different methods available to analyze whole bacterial community in activated sludge, such as polymerase chain reaction/denaturing gradient gel electrophoresis (PCR/DGGE) and PCR/terminal-fragment length polymorphism (T-RFLP). Recently, pyrosequencing technology has been developed, the resolution of bacterial community analysis has also rapidly improved with this

technique. DGGE, with its drawbacks on difficult comparison with others implementation, was not adopted in this study. PCR/T-RFLP was reported as a semi-quantification method which had been applied in this study. Meanwhile, pyrosequencing offered the possible way on identification of the T-RFLP profilings and was utilized in this study as well.

Based on the analysis of relationship above, the responsible bacteria were further identified on monitored dewaterability dynamics of activated sludge with the pyrosequencing pipeline and QIIME basing software-OTUMAMI.

Chapter 4 discussed the results obtained by methods introduced in Chapter 3.

Totally, three reactors were monitored with dynamics of dewaterability of activated sludge. As the main results, monitored dewaterabiity of activated sludge in reactor III was shown in Figure 2.

Apparently, from day 50th to Day 120th, WCDS value increased at a relatively rapid speed. (Figure 2), while, from day 130th to the end, the fluctuation of WCDS value fluctuated within more narrowed range. With about initial 60 days of relatively lower value, WCDS increased to more than 90% which meant the general deterioration of dewaterability during the operation. Those changes may come from the shift of bacteria consortia and the competition among them also resulted in the fluctuation during the monitoring.

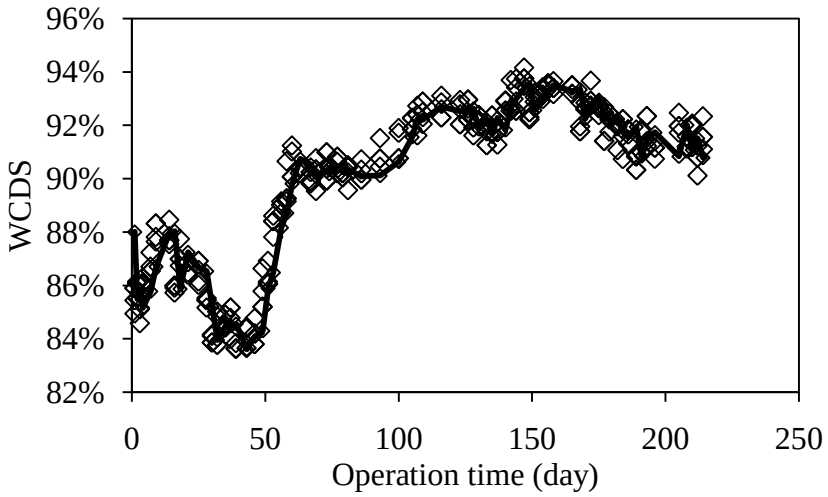

Figure 2.WCDS dynamics of activated sludge from operated reactor III (n=4) Average values are connected by solid line

Then, the microbial population was analyzes by the PCR/T-RFLP method and further identified with the corresponding T-RFs by virtual digestion of pyrosequencing.

For instance, relationship was revealed by the following equation which showed the corresponding T-RFs and their correlationship with dewaterability.

Reactor III:

$$WCDSest = -0.16005 * RH_{79}^{\cdot} + 0.34496 * RH_{93}^{***} + 0.22374 * RH_{106}^{*} - 0.56820 * RH_{111}^{***} + 0.35380 * RH_{421}^{***} + 0.13187 * RH_{466}^{***} - 0.05258 * RH_{482}^{*} + 0.83785^{***} \quad (3)$$

In particular, the estimated WCDS value well matched the observed WCDS value with R^2 of 0.84.

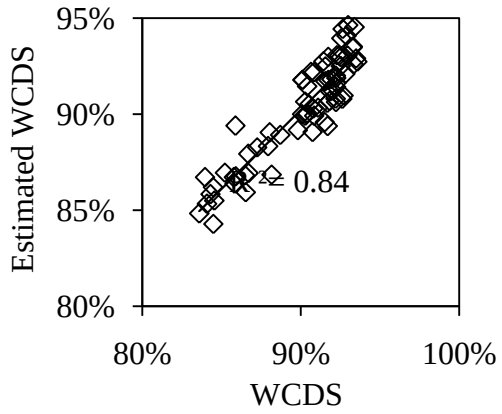

Figure 3 Correlation between estimated WCDS and measured WCDS of reactor III

Regarding corresponding bacteria, virtual digested corresponding T-RFs were selected and by classification with QIIME, the T-RFs were successfully classified though some could not be classified at genus level.

EPS was also analyzed with the correlation to dewaterability. No obvious correlation was found.Besides the 3 reactors, samples from 2 WWTPs were also collected to see the correlation between bacterial population and dewaterability.

Chapter 5 showed the results from 2 trial experiments to clarify the impacts of two factors –overloading and storage time. Overloading may to some extent improve dewaterability of activated sludge (4% in terms of WCDS value). Storage time certainly influences dewaterability of activated sludge because of the changed nature. However, different sludge showed different trend which on the other hand confirmed the behavior of dominant bacteria has different impact on dewaterability.

Chapter 6 summarized the present study and gave some suggestions on the future study.

In summary, this study demonstrated that the specific relationship between dewaterability and bacterial population was worth investigating. The approach established in this study to some extent obtained the preliminary results on the correlation toward some specific bacteria.