

論文の内容の要旨

論文題目 Activated Sludge Bacterial Population Changes Caused by the Addition of Crude Extracts from Activated Sludge

(活性汚泥から得られた粗抽出液を添加することにより生じる活性汚泥中の微生物群集構造変化)

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Activated sludge processes are widely used in wastewater treatment. It is very important to study the factors affect the activated sludge bacterial population changes because bacterial population in activated sludge is known to affect the performance of wastewater treatment. There are some studies have also been conducted to understand the effects of different factors on activated sludge bacterial populations, but they mostly focused environmental factors which are given to bacterial ecosystems such as pH dissolved oxygen and temperature. The factors inside the activated sludge are not well paid attention. Recently, bacteriophages are also attracting interest of researchers as they are suspected to affect bacterial population.

In this thesis, the author focus on the activated sludge bacterial population changes affected by the interaction chemicals. In chapter 1, the background as well as the objective of the study was described. In addition, the thesis structure was also presented.

In chapter 2, a review on published report related to bacterial interaction mediated by chemicals has been presented. The bacterial interactions, like allelopathy and quorum sensing has been widely studied in plant sciences and single bacterial respectively, but their effects on the bacterial population changes yet very limited. However, the activated sludge bacterial population changes are very important to the wastewater treatment performance. It is necessary to investigate the activated sludge population changes affected by the interaction chemicals.

In chapter 3, the material and methods are described. The activated sludge used for incubation and chemical extraction was obtained from the lab sequencing batch reactor (SBR) and two full scale wastewater treatment plants. The activated sludge was incubated on the 96-well microplate for detection of the bacterial population changes caused by the addition of the crude extracts. Activated sludge bacterial population changes were analyze by polymerase chain reaction (PCR) in combination with restriction

fragment length polymorphism (RFLP) or by reverse-transcription-(RT-) PCR/RFLP and pyrosequencing methods, both targeting a partial 16S rRNA gene.

In chapter 4, the author studied the bacterial population changes affected by extracts of activated sludge by trial and error. Totally, 12 separate experiments were applied. The activated sludge used for incubation and chemical extraction was different by the operation day of lab SBR1. Based on the experiments of this chapter, the author found relative low F/M ratio and longer incubation time may enhance the detection of the bacterial population changes caused by the crude extracts, but the different strength of the extracts was also concerned.

In chapter 5, Firstly, the effects of F/M ratio and incubation time were preliminary investigated on detection of bacterial population changes caused by the addition of the crude extracts. The results showed the bacterial populations could be significantly affected by the extract even with small biomass increase and short time as short as 24 hours. Then the sensitivity of bacterial population of activated sludge from lab SBR1 and WWTP was compared and the results showed the WWTP activated sludge was much more sensitive than the lab SBR. Further, the activated sludge bacterial population changes affected by the crude extract with time up to 24 hours were investigated by monitoring the samples every two hours by RT-PCR/RFLP analyses. The results showed the bacterial population changes gradually with the time and the intensity fractions of some main bands like 425 bp, 316 bp, 206 bp, 158 bp and 91 bp significantly changed almost in the first 6 hours and then kept stable. The bacterial population changes affected by the different concentration of the crude extracts were also investigated simultaneously, and the results showed the bacterial population changed significantly when 0.3 time extract was added, and the main bands affected was 425 bp and 316 bp, that was identical with the affected bands in 24 hours experiment.

In addition, the activated sludge bacterial population changes affected by the crude extracts obtained from the different activated sludge were investigated. The results showed the band patterns affected by these different extracts were different, however, the extract E137 was most significant than others.

In chapter 6, For further elucidate the species of bacteria affected by the extract with time. The samples (chapter 5.3.3) were analyzed again by pyrosequencing method. Compared with the control, on class lever, *Gammaproteobacteria*, *Alphaproteobacteria* and *Sphingobacteria* were affected mostly. On the order lever, the bacteria of *Pseudomonadales*, *Sphingobacteriales*, *Flavobacteriales* and *Aeromonadales* were affected mostly. On the family lever, *Pseudomonadaceae*, *Moraxellaceae*, *Flavobacteriaceae*, *Aeromonadaceae* and One unclassified bacteria family belong to the order of *Sphingobacteriales* were affected mostly. On the OTUs lever, mostly affected OTU were OTU2850 and OTU110.

At the same time, species of bacteria affected by the extract of different concentration were (Chapter 5.3.4) analyzed again by pyrosequencing method. Compared with all the samples, the significant changes when 0.3 times extract were added. Especially, On class lever, *Gammaproteobacteria* and *Sphingobacteriia* were affected mostly. On the order lever, the bacteria of *Pseudomonadales*, *Flavobacteriales* and *Sphingobacteriales* were affected mostly. On the family lever, *Pseudomonadaceae*, *Moraxellaceae*, *Flavobacteriaceae* and one unclassified bacteria belong to the order of *Sphingobacteriales* were affected mostly. On the OTUs lever, mostly affected OTU were OTU2850 and OTU1299.

In the end, the comparison of RFLP bands patterns and the pyrosequencing results were applied, the results showed some OTUs positively were identical with the RFLP pattern, but some are not identical with the RFLP bands patterns.

In chapter 7, Conclusion and some recommendations for future study were suggested. In this present study, although the bacterial population changes could be detected by the extracts from the different activated sludge, however, bacterial population changes affected by the extract obtained from the day of 137 operation day was most significant compared with the other operation day. The study of the dynamics of the extracts with the operation day will be very interesting. In addition, the further fraction and separation should be done to finally identify the interaction chemicals.