論文の内容の要旨

論文題目 Behavior of bacteriophages in the course of wastewater treatment and attempts to identify their hosts based on the analysis of 16S rRNA in supernatant

（下水処理の過程におけるバクテリオファージの挙動と上清中の 16S rRNA の分析による宿主同定の試み）

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In this study, daily behaviors of bacteriophage in treated water and behavior of bacteriophages in the course of wastewater treatment were clarified. In addition, attempts to identify their hosts based on the analysis of 16S rRNA in supernatant were also conducted. Activated sludge process is the most widely employed method to treat wastewater from households and industrial activities. This process depends deeply on the activity of microorganisms including bacteria. Lots of studies have been done on the factors which impact the system performance, such as PH, temperature, substrate contents, bacterial community structures. Researchers in marine study found that virus mediated disturbance of microorganism community and controls the size of specific host population. Large amount of bacteriophages have been found in the wastewater treatment process, however, few efforts have been done on the effect of bacteriophage behaviors of wastewater treatment performance.

Preliminary experiments were conducted to evaluate the approaches to analyze bacteriophages before real monitoring experiments. The Pre-experiments concluded as follows: for PFGE analysis (1) effect of fluorescence detectors on the quality of PFGE profiles, (2) limitation amount of bacteriophage DNA for PFGE analysis, (3) recover rate of bacteriophages during the process concentration using ultracentrifuge and ultrafilter, (4) effect of diluents on bacteriophage DNA concentration; selection of restriction enzyme for

bacteriophage digestion for polymerase-RFLP; effect of pretreatment at 95°C for 3 min on DNase activity and damage of bacteriophages capsid for analysis of 16S rRNA gene in bacteriophage DNA. Filtrate (0.2μm membrane filter) of supernatant of activated sludge mixture from laboratory-scale sequencing batch reactor after centrifuge was used for the studies in this section. Sensitive of Gel Doc XR and FLA-3000 were compared for generate PFGE profiles using pure λDNA and phage DNA concentrated from real wastewater treatment plant (WWTP) to reduce original sampling volume. The minimum DNA amount with which clear bands can be recognized on gel images were 0.3μg (phage DNA prepared from WWTP samples) and 0.1μg (pure λDNA) using Gel Doc XR and 0.05μg (both pure λDNA and phage DNA from WWTP) for FLA-3000. In addition, recover rate of phage DNA in the process of concentration using ultracentrifuge and ultrafilter was evaluate for calculation necessary volume of supernatant for PFGE analysis. The recovery rate of ultracentrifuge was 18-37% and of ultrafilter was 22-72%. Thus, 45 mL supernatant is enough for PFGE analysis. Sample supernatant was recommended for recover phage pellet from ultracentrifuge tube for 1×TE and sterilized distilled water could cause capsid. Comparing with *Hha*I, *Kpn*I, *BamH*I, *Sal*I, *Hind*III showed good activity of digesting DNA products of amplification phage DNA using polymerase phi29. Proteinase K digestion was necessary to prepare phage DNA for 16SrRNA amplification.

Method of investigation dissolved 16SrRNA released from bacterial host lysed by acteriophages in supernatant using RT-PCR amplification was developed. The possibility, reproducibility, and condition for samples storage were determined. Finally, the amplification was confirmed using pure incubated mixture of E.coli (IFO3301) and biacteriophage T4. Supernatant of activated sludge mixture was used as 16SrRNA template after centrifuge at 2,000g for 5 min and filtrate through 0.2μm membrane filtrate. Universal primer pair of 27f/519f was used as primer. 25 thermal cycles of RT-PCR successfully amplified dissolved 16SrRNA in supernatant using TaKaRa PrimeScript® One Step RT-PCR Kit Ver. 2 (Takara, Japan) and noisy DNA around 100bp was avoided. RFLP profile generated by Agient 2100 Bioanalyzer (Agient Technologies, Palo Alto, CA) with DNA 1,000 Assay Kit for DNA digestion fragments of *Hha*I showed good reproducibility of this method. The possibility of this method was also confirmed by 25 cycles of RT-PCR amplification of 16SrRNA released from E.coli (IFO3301) lysed by bacteriophage T4. RFLP profiles of both *Alu*I and *Hha*I

digestion products show satisfactory reproducibility. -20°C was the recommended condition for samples storage.

Finally, a laboratory-scale sequencing batch reactor was operated 50 days. Daily dynamics of bacteriophages and 16SrRNA in treated water and dynamics of bacteriophages and 16SrRNA in supernatant in the course of wastewater treatment was monitored. To induce new phage species emerge during operation period, stresses, such as increase temperature, substrate over feeding, and mytomicin C addition, were given to the operated reactor. Totally, 12 cycle of sequencing batch reactor was monitored dynamics of phage in the course of wastewater treatment process and supernatant was collected every 20 min. effluent from whole effluent was also collected. PicoGreen, PFGE, polymerase-RFLP methods were applied for phage concentration and community structure studies. Developed RT-PCR method was used for 16SrRNA investigation.

During monitoring period, phage DNA concentration in treated water fluctuated. Phages were more active in previous period (from begin to the 33rd day) than in the period from the 34th day to the end. Community structure changed with time during operation. Long term stably band, short term and one day emerged band was observed. More phage species emerged in period of the 15th to 32nd day. On the 28th day, phage DNA concentrations significantly increased compared with new band (around 100kb) emerge on PFGE profile and DOC removal inefficient. Bulking sludge addition did not cause phage community change. The behavior of dsDNA phages in activated sludge in the course of synthetic wastewater treatment was found to be very diverse and complicated. Increase of phage DNA concentrations was observed not only during aerobic phase, but also during anaerobic and settling phases. The increase rate was diverse: sometimes significant, and sometimes gradual. In addition, decrease and no change of dsDNA virus were also observed in the course of wastewater treatment process. Obviously large amount of phages were released during the first 6 min of anaerobic phase. Comparing with temperature increasing and mytomicin C addition, overfeeding substrate worked well on inducing phages burst. Sometime same phage species were induced (Cycle 7, 8, 16, 17, 30), sometimes new phage specie was induced (6 min of Cycle 6 and on the 6th day of supernatant). Responded to phage activity, 16SrRNA community also showed active dynamics during anaerobic phase according to RFLP profile of DNA products of digested with *Hha*I.