博士論文(要約)

Effects of Chlorination on Bacterial Regrowth Kinetics and Community Structure in Tertiary Treated Reclaimed Water
(三次処理再生水における細菌再増殖の動力学と群集構造に対する塩素消毒の影響)

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Reclaimed water is gaining importance as a stable, alternate source of freshwater to address ever increasing water demand as well as to mitigate the effects of changing rainfall patterns and climate change. Deterioration in microbial water quality during storage and distribution is a major concern for reclaimed water industry. Specifically, problems related to microbial regrowth - which can lead to or involve regrowth of opportunistic pathogens, changes in aesthetic quality of water, biofouling of cooling towers and corrosion of the infrastructure - have plagued the reclaimed water industry. In order to prevent microbial growth, reclaimed water is disinfected before being supplied. Chlorination is one of the most widely used disinfectants and chlorine doses in the range of 5-20 mg Cl_2/L are used in reclaimed water. However, at the distal ends of the distribution system, decay of residual chlorine and concomitant bacterial regrowth are often reported. Hence, a better understanding of bacterial groups that survive chlorine treatment and can regrow in the distribution system is essential. Additionally, insights into the role of chlorine dose and dosing patterns in delaying regrowth may help in developing more efficient control via disinfection.

The main objective of this study is to understand the effects of chlorination on bacterial regrowth kinetics and community structure in tertiary treated reclaimed water. Specific objectives were: (1) To examine the role of tertiary treatment processes in shaping reclaimed water bacterial community, (2) To study the effect of chlorine dose and dosing pattern on bacterial regrowth by chlorinating reclaimed water under laboratory conditions, (3) To develop mathematical model to explain the regrowth phenomenon and (4) To identify bacterial groups involved in regrowth. This study focuses on non-potable, tertiary-treated municipal wastewater applied for urban uses; more precisely, treated wastewater with low ammonia content and subjected to ozonation. Further, indigenous bacteria present in reclaimed water systems can exist in two forms - planktonic or sessile (biofilm), however, only planktonic forms are dealt with.

Water samples for experiments were collected from two water reclamation plants from Tokyo Metropolitan area (referred to as Plant A and Plant B) which have similar tertiary treatment configuration - incoming secondary effluent of municipal WWTP (Waste Water Treatment Plant) is treated with biofiltration + ozonation + chlorination at Plant A and with sand filtration + ozonation + chlorination at Plant B. To study the role of tertiary treatment in shaping reclaimed water bacterial community, water samples were collected at each step in the treatment train of Plant A (thrice) and Plant B (once). Additionally, two points in the distribution system (DS) of Plant A, namely PoC (Point-of-Compliance) and PoU (Point-of-Use) were sampled to examine the water quality changes during distribution. Residual free chlorine, bacterial cell counts (Total cell counts (TCC), intact cell count (ICC) using flow cytometry and Heterotrophic plate count (HPC)) were measured for each sample along with 16S rRNA gene based bacterial community profiling. DNA extracted from triplicates were pooled and amplified using nested PCR and sequenced by Illumina MiSeq. The reads obtained were subjected

to QIIME analysis.

For chlorination experiments, unchlorinated reclaimed water was collected after ozonation from Plant A and Plant B on four sampling occasions (referred to as A1, A2, A3 and B1). The water was chlorinated (in triplicates of 2 L each) such that initial chlorine doses were 1.5, 3, 4.5 and 6 mg/L along with unchlorinated set as control and incubated at 20 °C under dark for 20 days (Experiments A1, A2 and B1). Residual free chlorine, bacterial cell counts (TCC, ICC and HPC) were measured at regular intervals along with samples for bacterial community profiling. For Experiment A3, a single dose of 6 mg/L was compared with multiple smaller dosing scenarios having identical cumulative chlorine dose (1.5 mg/L added 4 times; (3+1.5+1.5) mg/L and (3+3) mg/L) in order to understand how chlorine dosing pattern alters regrowth. Incubation conditions and parameters measured were similar to the previous experiments.

A major change (1 to 2-$\log_{10}$ order) in bacterial counts is brought about by chlorination process in Plant A whereas by ozonation in Plant B. Chlorine decay along the DS of Plant A (values below 0.05 mg/L of free chlorine) allowed bacterial regrowth to be detected at PoC and PoU (2 out of 3 sampling occasions). *Proteobacteria* was the most abundant phyla in all samples sequenced. As the raw water moves along the treatment chain, the diversity of bacteria reduces, especially due to ozonation and chlorination (phylum Proteobacteria constitutes >80% of relative abundance in ozonated and chlorinated samples). However, along the DS in the event of regrowth, bacterial diversity increases. Tracking abundant OTUs demonstrated that OTUs were either present in raw water and could survive treatment process to regrow in DS or introduced in the storage tank of Plant A.

In the laboratory chlorination experiments, regrowth was not observed until residual free chlorine had declined below detection limit (i.e., 0.03 mg/L) emphasizing the need to maintain chlorine residual in order to suppress regrowth. In Experiments A1 and A2, when 1.5 and 3 mg/L was dosed, ICC reached stationary state with maximum cell counts of ~10^6 cells/mL, whereas at higher doses, regrowth was slower with maximum cell counts being 1 to 2-$\log_{10}$ order lower than the previous cases. In Experiment B1, regrowth characteristics was different due to the lower bacterial counts in ozonated water as well as presence of higher ammonia nitrogen (0.3 mg/L), which lead to formation of combined chlorine, than Plant A samples. In Experiment A3, regrowth was observed under all chlorine doses and dosing pattern did not significantly alter regrowth. Yield number decreased at higher chlorine dose of 4.5 mg/L (Experiments A1 and A2) indicating differences in bacterial groups regrowing when treated with varying levels of chlorine, probably due to selection based on chlorine resistance.

Mathematical model was proposed to explain the differences in bacterial inactivation and regrowth observed under various chlorine doses in the identical experiments, A1 and A2. Modeling was split up into several sections- Parallel Second Order model was used to capture chlorine decay, which was in

turn used with Chick-Watson model to simulate inactivation of bacteria based on ICC; bacterial regrowth was modeled using Monod model (using TCC). Three groups of bacteria namely Group A (moderately chlorine resistant), Group B (highly chlorine resistant) and Group C (chlorine sensitive bacteria which are completely killed on addition of chlorine and does not regrow) were assumed to explain the inactivation plot. On simulation of inactivation model, it was found that both Group A and Group B survive chlorine doses of 1.5 and 3 mg/L, but only Group B survives 4.5 mg/L. Further assumptions were made that regrowth in case of 1.5 and 3 mg/L cases were predominantly due to Group A, to which higher observed yield value was assigned. The lower yield value obtained in case of 4.5 mg/L was assigned to Group B. Using TCC and Monod model, specific growth rate and Monod's half-saturation constant were predicted. Group A had similar predicted values for both experiments A1 and A2 while Group B did not.

Bacterial community profile revealed a decline in the number of major bacterial taxa that regrew with increase in chlorine dose. In Experiment A1, major OTUs belonging to unclassified *Oxalobacteraceae* (OTU-9), *Methylophilaceae* (OTU-10) were found in samples dosed with lower chlorine doses (1.5 and 3 mg/L), while OTUs belonging to *Acidovorax* (OTU-8), *Rheinheimera* (OTU-22), unclassified *Betaproteobacteria* (OTU-7) and *Pseudomonas* (OTU-23) were detected both below and in 4.5 mg/L treated samples. When dosed with 6 mg/L, *Pseudomonas* (OTU-23) emerged as single dominant species. Though community profile differed between Experiments A1 and A2, some major OTUs like *Acidovorax* (OTU-8), unclassified *Oxalobacteraceae* (OTU-9), *Methylophilaceae* (OTU-10) and *Rheinheimera* (OTU-22) were common. However when treated with 6 mg/L dose, another single dominant OTU belonging to the same genus, *Pseudomonas* (OTU-25) regrew in Experiment A2. A significant shift in dominant bacterial groups were observed at 4.5 mg/L of chlorine dose. Based on the experimental data, the bacterial community that survives chlorination and regrows in reclaimed water can be divided into two major groups thus supporting the assumptions of Group A and Group B made during modeling. In Experiment A3, bacterial community profile under different dosing patterns differed widely despite no significant differences observed in regrowth kinetics. The species composition and relative abundance of dominant OTUs constituting Group A and Group B were calculated from bacterial community profile for the Experiments A1 and A2 and were correlated with results obtained from modeling. Major OTUs found in DS of Plant A, specifically *Acidovorax* and unclassified *Oxalobacteraceae*, were also known to regrow in at least one of the laboratory experiments indicating that the laboratory experiments were able to capture regrowth phenomenon occurring at the field scale.

Thus, chlorine dose not only determines the inactivation and regrowth in terms of bacterial counts, but also by selecting bacterial communities that can regrow. Hence, appropriate chlorine dose to disinfect reclaimed water should be carefully chosen based on intended use and water age.