論文 / 著書情報
Article / Book Information

題目(和文)	環境水中における塩素消毒後の大腸菌の再増殖に関する評価とモデリング
Title(English)	Assessment and modeling of the regrowth of Escherichia coli in environmental water after chlorine disinfection
著者(和文)	WANG Manna
Author(English)	Manna Wang
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種別(和文)	論文要旨
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（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	土木・環境工学　系 土木工学　コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of (Philosophy)
学生氏名： Student's Name	WANG MANNA	指導教員（主）： Academic Supervisor(main)	吉村千洋
		指導教員（副）： Academic Supervisor(sub)	

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Domestic wastewater is a crucial source of waterborne pathogens and a point source of disseminating antibiotic resistance through discharge or reuse. Therefore, thorough disinfection for eliminating the microbial risk is essential to public health and the environment. However, bacterial regrowth after wastewater disinfection has been frequently reported, making the disinfection effect temporary. Thus, the assessment of bacterial regrowth after disinfection is needed (as stated in **Chapter 1**). Literature review (**Chapter 2**) identified two research gaps: (1) the irrelevance of the tested conditions in the laboratory to the natural environment; (2) the adoption of the conventional culture-dependent method for bacterial quantification. Therefore, this study aims to assess and model the regrowth of *Escherichia coli* (*E. coli*) in environmental water after chlorine disinfection. Specific objectives are (1) to develop and verify a culture-independent method for quantifying viable *E. coli* in environmental water, (2) to assess the regrowth of *E. coli* in environmental water after chlorination, and (3) to model the regrowth pattern of *E.* coli after chlorination.

Disinfection (e.g., chlorination and UV irradiation) could act as environmental stress for bacteria and induce them to enter the viable but non-culturable (VBNC) state. VBNC bacteria can reactivate in favorable conditions and regain culturability, which is one of the main mechanisms of bacterial regrowth. The commonly adopted plate count method can only count the culturable fraction of the total viable cells, leading to underestimating the potential regrowth. Quantification of viable bacteria is thus necessary. A novel fluorescence spectroscopy-based method using Live/Dead Kit (SYTO 9 and propidium iodide) was developed and verified for quantifying viable bacteria (including VBNC and culturable cells) (**Chapter 3**). The fluorescence emission peak area of SYTO 9 in the range of 500–510 nm at the excitation wavelength of 470 nm correlates linearly with the viable cell counts ($R^2 > 0.99$, $p < 0.0001$) with only slight variations in the complex water matrix. This approach could quantify viable *E. coli* in 0.85% saline solution, river water and treated wastewater, with a detection limit of 3.67×10^4 to 2.70×10^7 cells/mL. In addition, the method is simple and fast (less than 30 minutes per sample), showing a broad range of potential applications such as laboratory-scale disinfection experiments, antimicrobial susceptibility tests and integration into a real-time monitoring system.

In previous studies, regrowth evaluation after wastewater disinfection did not reflect the realistic

water conditions, which can be critically influential in bacterial regrowth. Therefore, **Chapter 4** and **Chapter 5** of this study focused on assessing and modeling the regrowth of *E. coli* in various types of water after chlorination with various initial chlorine doses. The multiplex detection method (i.e., the combination of the fluorescence-based viability test and plate count) for bacterial quantification was applied to understand the regrowth mechanism. As a result, chlorination treatment (initial free chlorine of 0.2, 0.5 and 1.0 mg/L) induced *E. coli* to enter the VBNC state, and a higher initial chlorine concentration could speed up this transition. The regrowth mechanism after chlorine disinfection was demonstrated to be the reactivation of *E. coli* from the VBNC state in the tested conditions. The regrowth tests showed that removing environmental stress (i.e., oxidative stress from chlorine) was adequate to reactivate the VBNC subpopulation of *E. coli*.

The second-order regrowth model (survival ratio (%) as a function of regrowth time (day)) well fitted the experimental data (R^2: 0.73-1.00 with a median of 0.99). It suggested that the post-chlorination regrowth followed a typical regrowth kinetic, including a lag phase, an exponential increase phase and a stabilization phase within the 3-day observation. The model application revealed that increasing the applied chlorine dose (0.07-3.08 mg Cl_2 min/L) could effectively limit the maximum real regrowth rate (% day $^{-1}$) and the maximum survival ratio (%), and the regrowth was also dependent on the initial chlorine concentration and receiving water body. Though the specific influential components in water could not be identified, the environment-relevant water (i.e., Tama River water and Nomi River water) resulted in a higher degree and rate of regrowth. In addition, this study proposed a framework of the integrated chlorination-regrowth model for estimating the culturable *E. coli* during regrowth. Even though the integrated model was not good enough to make an accurate estimation of bacterial regrowth due to the data limitation, the demonstration of the model framework revealed that it could be a promising tool in evaluating the regrowth under a defined disinfection and regrowth condition.

Overall, this study developed a novel fluorescence-based method for quantifying viable *E. coli* in environmental water and presented a new approach with multiplex detection to understand the regrowth mechanism and assess post-chlorination regrowth in environmental water (**Chapter 6**). In this way, this study contributes to the scientific knowledge of post-chlorination regrowth in environmental water. The presented approach to assess and model the regrowth also contributes to wastewater management by highlighting the importance of regrowth monitoring and providing a scientific basis for better design of the disinfection process.