

Letter to the Editor

Inactivation and Regrowth of Antibiotic-resistant Bacteria by PAA Disinfection in the Secondary Effluent of a Municipal Wastewater Treatment Plant*HUANG Jing Jing^{1,3}, XI Jing Ying¹, HU Hong Ying^{1,2,#}, TANG Fang¹, and PANG Yu Chen¹

Inactivation and microbial regrowth of penicillin-, ampicillin-, cefalexin-, tetracycline-, chloramphenicol-, and rifampicin-resistant bacteria were studied to explore risks associated with selection and regrowth of antibiotic-resistant bacteria after PAA disinfection. The results showed that after exposure to 20 mg/L PAA for 10 min, inactivation of ampicillin-resistant bacteria reached 2.3-log, which was significantly higher than that of total heterotrophic bacteria with a decrease of 2.0-log. In contrast, inactivation of tetracycline-resistant bacteria was significantly less efficient, reaching only 1.1-log. Chloramphenicol- and tetracycline-resistant bacteria, as well as total heterotrophic bacteria regrew more than 10 fold compared to those in the untreated wastewater sample with 22 h stilling culture after exposure to 2 or 5 mg/L PAA as for 10 min. Selection and potential regrowth of tetracycline- and chloramphenicol-resistant bacteria are potential risks when utilizing PAA disinfection, which may induce the spread of specific antibiotic-resistant bacteria in reclaimed water.

Peracetic acid (PAA) is a strong oxidant which can react with a series of organic compounds such as amines, aromatics, and humic substances. Due to its strong oxidizing ability, PAA efficiently inactivates bacteria, viruses, and fungi^[1-2], while producing little mutagenic or toxic by-products^[3], thus making disinfection by PAA as an alternative method for wastewater treatment^[4-5].

The inactivation efficiency of PAA disinfected to individual species varies widely in wastewater effluents. As the most common indicator of fecal pollution, total coliforms, fecal coliforms, and *E.coli* are empirically used to indicate the inactivation efficiency of bacteria in wastewater effluents^[6]. Former studies showed that PAA disinfection can effectively eliminate total coliforms, fecal coliforms, and *E.coli*^[6]. However, coliforms can not represent of

all total heterotrophic bacteria. Previous study showed that the inactivation of total heterotrophic bacteria by PAA is not as efficient as that to total coliforms, fecal coliforms or *E.coli*^[4].

Potential regrowth of bacteria after PAA disinfection is another major disadvantage of PAA disinfection. As an organic chemical disinfectant, PAA oxidizes non-bioavailable carbon into a bioavailable carbon source; furthermore PAA exists in equilibrium with acetic acid and may yield additional acetic acid upon decomposition, which is also a commonly used carbon source. It has been demonstrated that these by-products can be utilized by bacteria for regrowth after neutralizing the PAA disinfection^[4,7]. Hence, the efficiency of PAA disinfection should be re-appraised to account for more than one indicator microorganism.

Antibiotic-resistant bacteria are considered as a potential microbial risk of novel contamination, and widely spread in wastewater effluents^[8-10]. Most antibiotic-resistant bacteria in wastewater effluents are pathogenic or opportunistic bacteria shed from humans and animals^[8]. Investigations of antibiotic-resistant bacteria showed that more than 50% of bacteria in wastewater effluent are antibiotic-resistant^[9]. However, there is little data on the inactivation efficiency of antibiotic-resistant bacteria, their regrowth after the PAA disinfection in reclaimed water or the selection of antibiotic-resistant bacteria may depend on the PAA effect.

The aim of this study was to determine inactivation of antibiotic-resistant bacteria in secondary effluents by PAA disinfection and the potential for microbial regrowth of antibiotic-resistant bacteria after disinfection in a simulated tank and/or pipeline containing standing reclaimed water. Penicillin-, ampicillin-, cefalexin-, tetracycline-, chloramphenicol-, and rifampicin-resistant bacteria were studied as typical antibiotic-resistant bacteria in the secondary effluent and represent antibiotics

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typically used to fight infections^[9]. Total heterotrophic bacteria were used to compare potential microbial regrowth of antibiotic-resistant bacteria.

The results showed that the inactivation of bacteria resistant to 6 kinds of antibiotics by PAA disinfection was distinguishing (Figure 1). Following PAA treatment, penicillin-, ampicillin-, and cefalexin-resistant bacteria were inactivated at higher levels than heterotrophic plate count bacteria (HPC). The inactivation of chloramphenicol- and rifampicin-resistant bacteria was comparable to the inactivation of HPC. Remarkably, inactivation of ampicillin-resistant bacteria reached 2.3-log, which was significantly higher than HPC with a decrease of 2.0-log (One-way ANOVA, $P < 0.05$) after exposed to 20 mg/L of PAA for 10 min. The inactivation of tetracycline-resistant bacteria, however, was significantly less efficient (One-way ANOVA, $P < 0.05$). After exposure to PAA at 20 mg/L for 10 min, the level of tetracycline-resistant bacteria decreased by 1.1-log, while HPC decreased by 2.0-log. Hence, tetracycline-resistant bacteria showed a significant tolerance to PAA compared to HPC and other species of antibiotic-resistant bacteria.

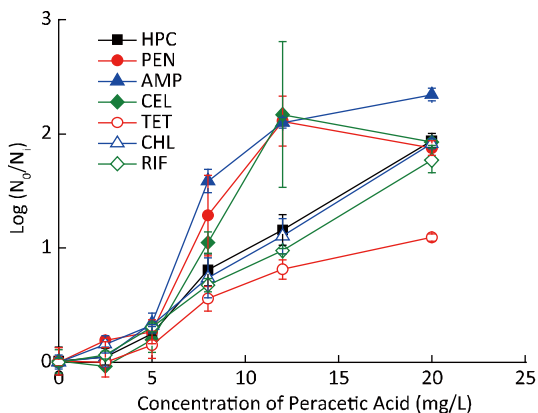


Figure 1. Inactivation curves of total heterotrophic bacteria and antibiotic-resistant bacteria by PAA disinfection in the secondary effluent. The contact time was 10 min. HPC (■), PEN (●), AMP (▲), CEF (◆), TET (○), CHL (△), and RIF (◇) represent total heterotrophic bacteria, penicillin-, ampicillin-, cefalexin-, tetracycline-, chloramphenicol-, and rifampicin-resistant bacteria, respectively. Error bars indicate the standard deviation of values obtained from a single sample analyzed in triplicate.

As reported by previous studies, the inactivation ratios of bacteria in secondary wastewater effluents by PAA disinfection at a fixed dosage are widely distributed. An investigation of PAA disinfection efficiency in a large wastewater treatment plant in Italy determined that the reduction of HPC in the secondary effluent was only 51.8% (0.32-log) after PAA disinfection at the dosage of 1.5-2.0 mg/L for 20 min^[6]. Caretti et al.^[5] indicated that the inactivation of HPC reached 2.11-log after disinfecting with 8 mg/L at PAA for 10 min in a pilot plant; results from Mezzanotte et al.^[11] showed that disinfection efficiency for HPC was much lower than total coliforms and fecal coliforms. The inactivation ratio of HPC in this study (bench disinfection) approached to the result of real wastewater treatment plant but was lower than those of bench disinfection or pilot studies. The results also inferred that inactivation efficiency of tetracycline-resistant bacteria could be lower than total coliforms and fecal coliforms.

The acetic acid introduced and produced during PAA disinfection contributes to microbial regrowth by providing a carbon source for the total heterotrophic bacteria. Results from Antonelli et al.^[7] showed that total heterotrophic bacteria regrew 24 h after disinfection with 2 mg/L of PAA for 12, 18, and 36 min to values 10-fold higher than the number of bacteria immediately after disinfection. Our experiment exhibited post-disinfection regrowth of HPC and antibiotic-resistant bacteria, after residual PAA was neutralized (Figures 2 & 3). The number of total heterotrophic bacteria after 22 h disinfecting by PAA at the dosage of 2 or 5 mg/L disinfection for 10 min was 1 order of magnitude more than the number of HPC before PAA disinfection. The results showed that there was a significant regrowth of HPC in the secondary effluent after 22 h post PAA disinfection at 8 mg/L for 10 min (Tukey's test, $P < 0.05$).

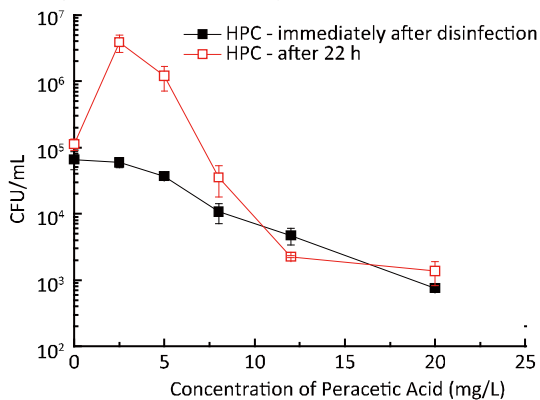


Figure 2. Regrowth of total heterotrophic bacteria in the secondary effluent post PAA disinfection. The dark time was 22 h. The contact time of PAA disinfection was 10 min. Error bars indicate the standard deviation of values obtained from a single sample analyzed in triplicate.

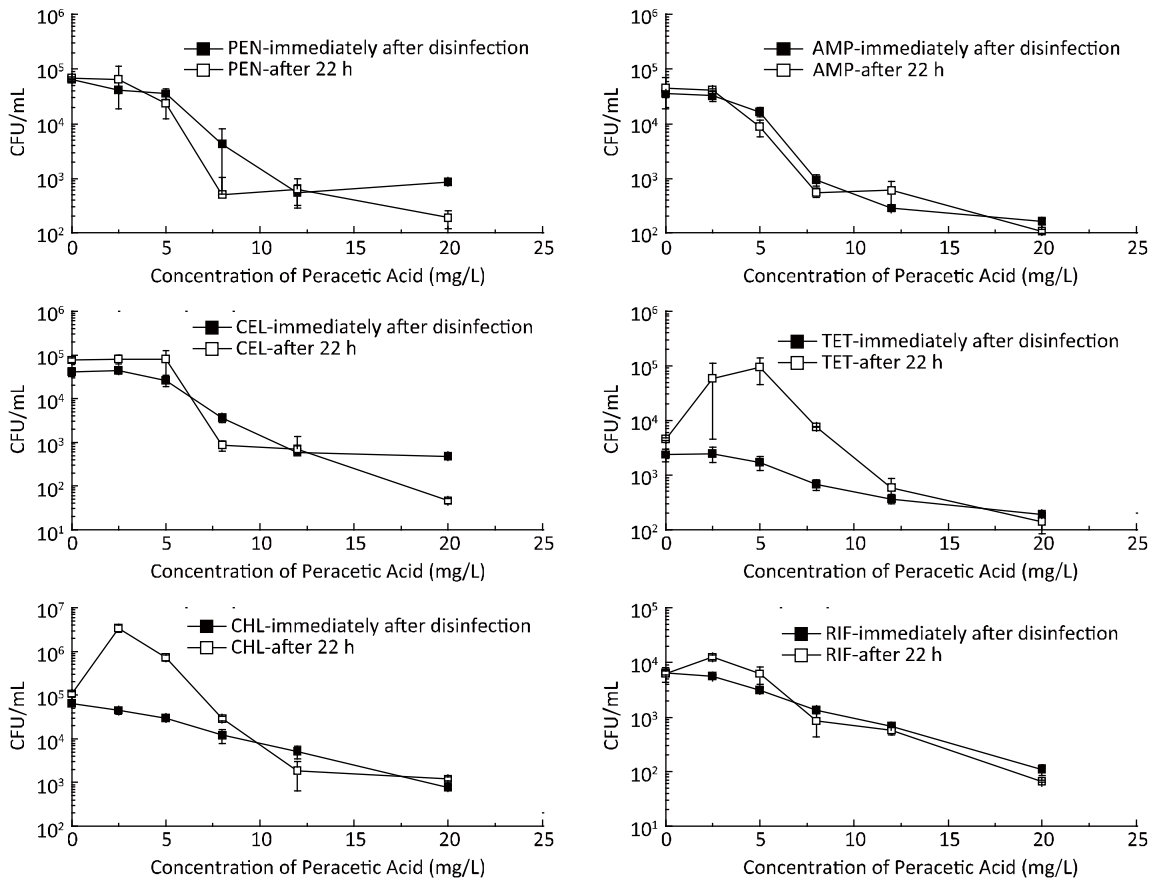


Figure 3. Regrowth of antibiotic-resistant bacteria in the secondary effluent post PAA disinfection. The dark time was 22 h. The contact time of PAA disinfection was 10 min. Error bars indicate the standard deviation of values obtained from a single sample analyzed in triplicate.

Chloramphenicol-resistant bacteria regrew after 22 h incubation when the dosage of PAA was below 8 mg/L (Tukey's test, $P < 0.05$); no growth was observed when dosage of PAA exceeded 12 mg/L. The number of colonies of chloramphenicol-resistant bacteria after 22 h incubation the dosage of with 2 mg/L of PAA exceeded those in the secondary effluent unexposed to PAA, by 50 fold. At the dosage of 5 mg/L PAA, an increase of approximately 10 fold number was observed in the colonies of chloramphenicol-resistant bacteria relative to untreated samples; after 22 h, the regrowth number of tetracycline-resistant bacteria with 10 min exposure was nearly 40 fold relative to the number of colonies of untreated secondary effluent. Unlike chloramphenicol-resistant bacteria, regrowth of tetracycline-resistant bacteria was observed when the dosage of PAA exceeded 8 mg/L. Previous research in our laboratory has shown no apparent regrowth of total heterotrophic bacteria, tetracycline- and chloramphenicol-resistant bacteria

in the secondary effluents after 22 h incubation when no PAA is applied demonstrating that acetic acid from PAA is an essential carbon source for regrowth in the secondary effluent^[10].

In contrast, there was no significant regrowth of penicillin-, ampicillin-, cefalexin- and rifampicin-resistant bacteria 22 h after PAA disinfection (Tukey's test, $P < 0.05$). At the dosage of 12 mg/L PAA, no apparent regrowth of total heterotrophic bacteria, tetracycline- and chloramphenicol-resistant bacteria was observed. This trend is continued with higher concentrations showing that microbial regrowth is inhibited by PAA with high dosages.

As a method for wastewater treatment, PAA disinfection has been put into practice in some wastewater treatment plants. The disadvantages and risks of wastewater disinfection by PAA, however, which include low inactivation efficiency for some specific bacteria and high potential regrowth of microorganism, still limit its widespread application. Our experimental results showed that

penicillin-, ampicillin-, and cefalexin-resistant bacteria were inactivated more efficiently than total heterotrophic bacteria. However, tetracycline-resistant bacteria could survive when exposed to 12 mg/L of PAA and the inactivation efficiency was much lower than total heterotrophic bacteria. Low concentrations of PAA during disinfection could be selected for tetracycline-resistant bacteria in secondary effluents. Both tetracycline- and chloramphenicol-resistant bacteria regrew 22 h post PAA disinfection, while the number of colonies of penicillin-, ampicillin-, cefalexin- and rifampicin-resistant bacteria remained constant. Concerns regarding regrowth of bacteria in a tank and/or pipeline after PAA disinfection should be directed not only towards total heterotrophic bacteria, but also to specific antibiotic-resistant bacteria. Regrowth of total heterotrophic bacteria, tetracycline-, and chloramphenicol-resistant bacteria can be controlled when the dose of PAA disinfection reached 12 mg/L. Following PAA disinfection, tetracycline- and chloramphenicol-resistant bacteria could become dominant species in secondary effluents. The spread of tetracycline- and chloramphenicol-resistant bacteria from effluents could increase microbial risks in natural waters.

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