Water Research 118 (2017) 291–292

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Comment

Reply to comment on "Combination of cupric ion with hydroxylamine and hydrogen peroxide for the control of bacterial biofilms on RO membranes by Hye-Jin Lee, Hyung-Eun Kim, Changha Lee [Water Research 110, 2017, 83–90]"

Hye-Jin Lee, Changha Lee*

School of Urban and Environmental Engineering, Ulsan National Institute of Science and Technology (UNIST), 50 UNIST-gil, Ulju-gun, Ulsan, 44919, Republic of Korea

ARTICLE INFO

Article history:
Received 5 April 2017
Accepted 8 April 2017
Available online 8 April 2017

We appreciate the opportunity to respond to the comments made by Chen et al. (2017) on our recent article (Lee et al., 2017). Some of their comments may have resulted from misunderstanding regarding our study. However, we believe that the bactericidal effects of EDTA and sulfite in combination with Cu(II) need to be clarified since EDTA and sulfite are commonly used as quenching agents for copper ions and oxidants (e.g., H_2O_2 and HOCl), respectively. The issues raised by Chen et al. have been addressed below by providing additional experimental data.

First of all, please note that i) quenching with EDTA and sulfite was only applied for inactivation experiments on planktonic cells (Fig. 1) (as described in the methods for biofilm experiments, coupons or membrane samples are withdrawn from the reaction solution at time intervals and rinsed with DI water) and ii) the experimental conditions for planktonic cells ($[Cu(II)]_0 = 5$ μM, $[H_2O_2]_0 = 0.1$ mM, $[HA]_0 = 0.1$ mM) are different from those for biofilm cells ($[Cu(II)]_0 = 0.1$ mM, $[H_2O_2]_0 = 1$ mM, $[HA]_0 = 1$ mM).

We would like to clarify the procedure for quenching reactions in samples that was used in our study (Lee et al., 2017). In Section 2.3, we have stated that "*One-milliliter of sample was taken at predetermined time intervals and immediately quenched with EDTA and sodium sulfite. The sample was diluted with PBS to the required population of microbes*". In detail, five microtubes were generally prepared for each sample (one microtube with 10 μL of 20 mM EDTA solution and 10 μL of 20 mM sodium sulfite solution, and the other four microtubes with 0.9 mL PBS containing ca. 0.312 phosphate ion). One-milliliter of sample taken from the reactor was put into the first microtube containing EDTA and sulfite (final concentrations in the tube: 5 μM Cu(II), 0.2 mM EDTA, and 0.2 mM sulfite), and after gentle mixing, 0.1 mL solution was immediately taken from the first microtube and was added into the second microtube containing 0.9 mL PBS (10 times dilution). In the same manner, the solution was diluted consecutively into third, fourth, and fifth microtubes (100, 1000, and 10,000 times dilution, respectively). In this procedure, aside from the roles of EDTA and sulfite, dilution with PBS will quench the reaction (as also suggested by Chen et al.).

Additional experiments were performed to (re)examine the inactivation of *P. aeruginosa* by Cu(II) in the presence of EDTA and (or) sulfite (Fig. 1); after sampling, the reaction was quenched by consecutive dilution with PBS. First, the Cu(II)/EDTA combination caused only 0.3 log inactivation of *P. aeruginosa* cells in 60 min,

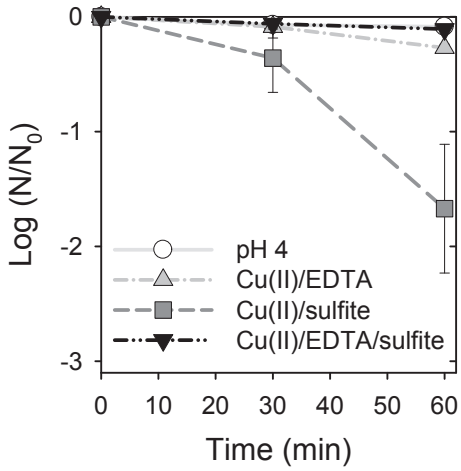

Fig. 1. Inactivation of planktonic *P. aeruginosa* cells by Cu(II) in combination with EDTA and (or) sulfite (Initial cell concentration: ~1 × 10^7 CFU/mL; $[Cu(II)]_0 = 0.1$ mM; $[EDTA]_0 = 2$ mM; [Sodium sulfite]$_0 = 2$ mM; $pH_i = 7$).

* Corresponding author.
E-mail address: clee@unist.ac.kr (C. Lee).

http://dx.doi.org/10.1016/j.watres.2017.04.018