3D Internal Ultra-Nanostructures of Cellular Responses of Microorganisms-Materials Interaction during the Disinfection Process

Than Thi Nhu Anh[1], Chih-Huang Weng[2], Jing-Hua Tzeng[1,3], Li-Ting Yen[1,4], Yao-Tung Lin[1,5,*].

[1] Department of Soil and Environmental Science, National Chung Hsing University, Taichung 40227, Taiwan
[2] Department of Civil and Ecological Engineering, I-Shou University, Kaohsiung 84008, Taiwan
[3] Department of Civil and Environmental Engineering, University of Delaware, Newark, DE, USA
[4] Department of Plant, Soil and Climate, Utah state University, Salt Lake, Utah, USA
[5] Innovation and Development Centre of Sustainable Agriculture, National Chung Hsing University, Taichung 40227, Taiwan

[*]**Author to whom correspondence should be addressed:** Prof. Yao-Tung Lin (E-mail: yaotung@nchu.edu.tw)

Abstract

S. aureus (Gram-positive), *K. pneumoniae* (Gram-negative), and *E. coli* (Gram-negative) are emergent human pathogens arised from the consumption of foods contaminated by foodborne pathogens. Although chemical disinfection is the most widely used method nowadays, it has been raised concerns over the producing of toxic disinfection by-products. Many researchers have lay out the future plans by using photocatalysis disinfection, however the changes of cellular structure in response to the disinfection remains unclear. In this study, the performance of chitosan (CTS) derived from acetylation of the shrimp shell and calcined oyster shell (COS) powder were fabricated and used to investigate the disinfection efficacy of *S. aureus, K. pneumoniae*, and *E. coli*. The disinfection study is evaluated by vary initial bacteria concentrations (10^5, 10^6, and 10^7 CFU/mL) and amounts of dosage effect, i.e., CTS or COS (0.01 ~ 1.00 g/L). Results show that microorganisms were successfully inactivated below the detection limit within 12 h in all tests, and the disinfection rate is way much higher than other photocatalyst methods, such as TiO_2. The disinfection rate is nearly the same even simulated with the different kinetics, indicating that the time required to complete an inactivation is not as significant as the changes in initial bacterial concentration. Findings revealed that the use of COS in the disinfection process could generate oxidative active oxygen species (ROS), and then the ROS attached to the surface of the microbial membrane. Intuitively, the positively charged CTS could interfere with the negatively charged surface of microbial and alters the cell permeability. Due to the interactions of cationic NH_3^+ groups on the CTS surface with negatively charged cell membranes, the membrane permeability consequently increased and membrane lysis. Obviously, the disruption of the cell structure of *S. aureus*, *K. pneumoniae*, and *E. coli* by CTS and COS lead to leakage of cytoplasm, thereby increasing the solution relative conductivity. Results of SEM, TEM, FM, and AFM observations showed that all the microbial strains were found to lose their cell structure integrity after being disinfection treated with CTS or COS. As to gain insight into the disinfection mechanism by using materials CTS and COS, microbial 3D topographies for in-situ cellular discharge analysis during the disinfection was analysed by using synchrotron-based TXM (01B1). TXM permits further investigations into the diversity of 3D internal ultra-nanostructures of the two bacteria species and enables exploring the evolutionary trends of 3D cellular structure. When the reaction time was 0 h, the bacteria cells were spindle-shaped and intact. After the reaction time proceed for 2 h, the cell walls of Gram-negative (*K. pneumoniae*) had obvious cell sag and damage, that is a solid evidence of a severe attack by ROS and influenced by NH_3^+. Nevertheless, the ultrastructure of Gram-positive (*S. aureus*) was nearly unaffected by the interactions with CTS and COS after 6 h, but the cell had deformed on the morphology by TXM 3D images. Such an approach, as exemplified, is very helpful to identify the structural morphology of a cell with its physiological characteristics during a disinfection process.

Keywords* – Transmission X-ray Microscopy, Disinfection Mechanisms, *Chitosan, Calcined Oyster Shell

Introduction

The disinfection is a highly potential method to treat the microbial contaminated substances. Of particular, the use of CTS and COS is a cost-effective way in disinfection purpose [1-3]. With the help of electronic morphology (EM) methods, it is able to understanding more on the performance of antimicrobial materials. So far, the deformation of bacteria under the pressure of antibacterial substances remains unclear even the shape of the cell and the SEM 2D images have been explored extensively. Currently the specific preferential damage of the microorganisms reported in the relevant articles has not been reached a conclusive point. However, the exploring of how the microorganism is damaged under a photocatalytic process is a vital knowledge as to gain insight into the disinfection mechanism.

Experiments

Specimens on flat grids are placed in a vacuum on a cryo-tilt stage adapted from electron tomography with a tilt range of ±80° [4]. Alternatively, the specimens mounted in thin-walled capillaries can undergo a full 360° rotations and data collection involves recording projection images every 1–2° through a 180° rotation [5].

Results

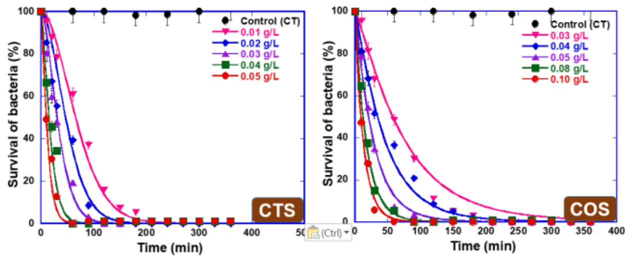

Figure 1. Bactericidal effects of CTS and COS dosage on *K. penumoniae* treated by disinfection process
[*K. penumoniae*] = 10^5 CFU/ml, [COS] = 0.03 - 0.10 (g/L), and [CTS] = 0.01- 0.05 (g/L)

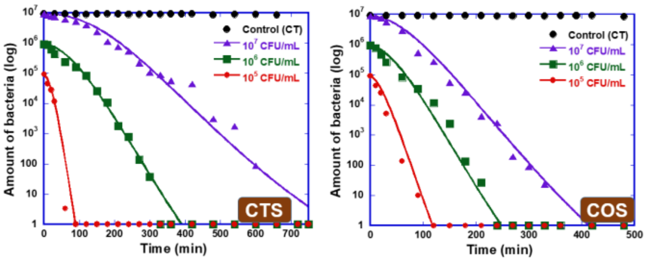

Figure 2. Bactericidal effects of initial microbial concentration on *K. pneumoniae* treated by CTS/COS. [CTS/COS] = 0.05 g/L, [*K. penumoniae*] = 10^5 – 10^7 CFU/mL.

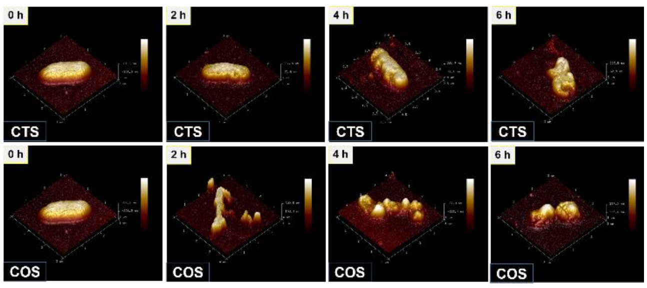

Figure 3. The AFM of *K.pneumoniae* treated with CTS and COS at (a) 0 min (b) 120 min (e) 240 min (f) 360 min.

[*K. penumoniae*] = 10^8 CFU/mL, [CTS] = 1 g/L, [COS] = 0.2 g/L, temperature = 25°C±1°C

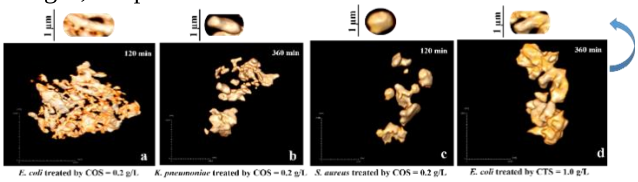

Figure 4. *The* 3D images of *E. coli*, *K. pneumoniae*, *S. aureus* after 2 h and 6 h disinfection process with vary amount of either CTS or COS dosages.

Discussion

The results of TXM (Figure 3 and 4) show the 3D images of *E. coli*, *K. pneumoniae*, *S. aureus* after disinfection treatment. The preliminary results show that synchrotron-based TXM (01B1) can be used to reveal the difference of cellular structure during the disinfection process with CTS and COS.

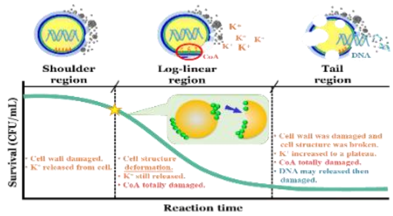

Figure 5. The disinfection mechanism using CTS and COS.

Acknowledgments

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References

1. Benhabiles, S., et al., *Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste.* Food Hydrocolloids, 2012. **29**(1): p. 48-56.
2. Devlieghere, F., A. Vermeulen, and J. Debevere, *Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables.* Food Microbiology, 2004. **21**(6): p. 703-714.
3. Seo, J.H., et al., *Calcined Oyster Shell Powder as an Expansive Additive in Cement Mortar.* Materials, 2019. **12**(8).
4. Heroux, A., et al., *Macromolecular crystallography beamline X25 at the NSLS.* Journal of Synchrotron Radiation, 2014. **21**: p. 627-632.
5. Larabell, C.A. and K.A. Nugent, *Imaging cellular architecture with X-rays.* Current Opinion in Structural Biology, 2010. **20**(5): p. 623-631.