# Visible-light-driven microbial inactivation by $TiO_2$ composite: advanced biological evidence of 3-D tomography and cellular mechanisms

Shang-Ming Huang[1], Chih-Huang Weng[2], Jing-Hua Tzeng[1], Li-Ting Yen[1], Che-Jui Chang[1], Yao-Tung Lin[1,*]

[1]Department of Soil and Environmental Sciences, National Chung Hsing University, Taichung, Taiwan
[2]Department of Civil and Ecological Engineering, I-Shou University, Kaohsiung 84008, Taiwan
*Author to whom correspondence: Prof. Yao-Tung Lin; e-mail: yaotung@nchu.edu.tw

## Abstract

Pathogenic microorganisms are a major concern for water safety, as their presence may lead to the spread of waterborne diseases. Chemical biocides are widely used in the environment to remediate water pathogen contamination, while these chemicals are only effective for short-term antimicrobial control and may themselves be toxic. The use of visible-light-responsive photocatalyst is a promising alternative for water disinfection. In general, the profiles of photocatalytic inactivation can be characterized into three regions, including initial shoulder, log-linear, and tail regions. However, defining the cellular mechanisms that lead to microbial inactivation has been the preoccupation of many researchers. There is still much debate over which process or set of processes lead to the death of an organism exposed to photocatalytic action. Most studies have indicated that the destruction of the cell wall/membrane is a key process for inactivation mediated via photocatalysis-triggered ROS (reactive oxygen species). Nevertheless, such inactivation process differs among microorganism species. Not only are the individual properties of these cellular constituents important, but also the manner in which they are structurally arranged confers additional resistance to inactivation. Nowadays, the imaging technique has greatly advanced our understanding of cell structure and function. However, no single imaging method proves to be the perfect solution. A multimodal methodology for cellular imaging is desired for understanding the photocatalytic inactivation of microbes.

In this study, biological atomic force microscopy (AFM) and full-field transmission hard X-ray microscopy (TXM) were carried out for underlying the visible-light-driven inactivation mechanisms between varying types of microbial strain. AFM can not only provide the images but also analyze the cellular height, roughness, and adhesion at the same time. TXM provides the three-dimensional (3D) nanoscale structures, which has the potential to examine thicker cells and tissues as well as to benefit from increasing spatial resolution. We have found that the bacterial inactivation was associated with the appearance of crack-like structures on the bacterial membrane. When the photocatalytic nanoparticles interacted with intact cells, the membrane appears slightly disordered. After inactivation, the crack-like damages occurred along with visible nanoparticles on the bacterial surface. During the photocatalytic inactivation, the adhesion force increased at the beginning of the inactivation but decreased after that. When the cell adhesion over 1.5 nN, the cells suffered relatively serious damages leading to loss the cultivability in agar culture. Higher adhesion force may manifest itself as a decrease in the lipid bilayer mobility, an increase in the viscosity of lipid bilayer, and the cytoplasm released from the bacterial cell during inactivation. The 3D images from TXM showed that the bacterial structure has crashed after 2 h (shoulder region) by applying photocatalysts under visible-light irradiation. Although most of the cell still showed ball-shape at $0^o$ angle, the cell structure has damaged at the same time in a different direction. Clearly, TXM can provide 3D images of both clustered and single-cell structure under an atmosphere environment. In this study, we have demonstrated that AFM and TXM are potential to characterize the cellular structures of microbes during visible-light-driven inactivation, which helps to understand the inactivation mechanisms between different types of microbial strain.

**Keywords: visible-light, photocatalytic inactivation, mechanism, AFM, TXM**

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