Mechanism consideration of photocatalytic and photoelectrocatalytic inactivation of *E. coli* inactivation

Guiying Li [1,*], Hongwei Sun[1,4], Taicheng An[1], Po-kueng Wong[2], Huijun Zhao[3]
[1]*The State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China*;
[2]*School of Life Sciences, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, China*
[3]*Centre for Clean Environment and Energy, Griffith University, Queensland 4222, Australia*
[4]*University of Chinese Academy of Sciences, Beijing 100049, China*
**Corresponding author: Tel.: +86-20-85291572; E-mail address: ligy1999@gig.ac.cn*

Introduction

Various biohazards such as viruses, bacteria, and fungi have currently received considerable attention because of their abundance in water. Furthermore, the generation of toxic byproducts like trihalomethanes during conventional disinfection process is another public concern. Since Matsunaga et al. first reported their pioneering work on photocatalytic (PC) inactivation of microorganisms using UV-irradiated TiO_2 powder, extensive studies have been dedicated to this research area. Although it is widely accepted that reactive species (RSs) generated in PC system are responsible for bacterial inactivation, the lethal killing mechanisms are still under intensive debate and the relevant evidences were not fully provided. Further, catalyst is often used in aqueous suspended system in most studies on water disinfection, which limits the technological development of photocatalytic disinfection owing to relatively low photocatalytic efficiency and post-treatment need of catalyst from aqueous slurries.

Hence, in this work, an immobilized catalyst onto a conducting substrate was prepared and employed as photoanode to photoeletrocataltically inactivate *E. coli.* Further, PC and photoelectrocatalysis (PEC) inactivation mechanisms of *E. coli* were investigated in detail.

Materials and Methods

All PEC, PC and electrochemistry (EC) inactivation experiments were conducted in a three-electrode photoelectrochemical reactor. The photo-anode was a piece of Ti foil with highly ordered TiO_2 nanotubes array as reported in our previous work. In PEC and EC experiments, a platinum foil and a saturated Ag/AgCl were served as counter and reference electrodes with 0.2 M $NaNO_3$ as electrolyte under a constant anode bias potential of +1.0 V versus Ag/AgCl. The illumination source consisted of 3 LED with maximum emission at 365 nm and light intensity was 10 mW cm^{-2} for PEC and PC treatment. Fifty milliliters of the prepared *E. coli* K-12 (1.10×10^7cfu/mL) suspension was inactivated in the reactor. Samples were taken at regular intervals for further analysis including the inactivation contribution of RSs, the intracellular RSs levels and cell envelope permeability changes during treatment.

Results and Discussion

Results showed that no inactivation effect existed for PC or EC within 60 min treatment, while PEC inactivated all cells within 45 min. the highest inactivation efficiency in PEC was due to the suppression of the recombination and prolonged lifetime of both h^+ and e^-. Scavenger experiments demonstrated that h^+ played an important role in PEC bactericide *via* direct attack of *E. coli* cells or production of other RSs such as $^{\bullet}OH$ and H_2O_2. $^{\bullet}OH$, as expected, was not the dominant bactericidal species because of its insufficient half-life and half length.

Furthermore, the PC and PCE inactivation mechanism was investigated detail. Firstly, the fluorescent monitor of intracellular esterase activity and RSs levels during PEC showed that FDA fluorescent intensity increased dramatically in the initial 10 min of inactivation, and decreased gradually thereafter, while DCFH-DA fluorescence kept rising up until 30 min of process. Secondly, both superoxide dismutase and catalase levels were shown to be enhanced during the initial 10 min of PEC inactivation as oxidative stress induced by the uprising oxidative strength inner- and outer-membrane. Decrease of enzyme activity was observed for both enzymes after 10 min of PEC treatment, indicating that the oxidative strength at high level exceeded the antioxidative protection system of the bacteria, companioned by accelerated loss of cell culturability. Finally, during PEC inactivation process, the bacterial viability was determined by Live/Dead fluorescent staining method and results showed that all the untreated cells were stained green (0 min), and red fluorescent cells increased as PEC treatment time prolonged. All bacterial cells were stained red at 60 min, suggesting that the membrane integrity of all the cells was lost.

Significance

The bacterial cells were inactivated mainly due to the attack from photo-generated holes assisted by elevated intracellular RSs level. The detail mechanism results confirmed that at the initial stage of inactivation, the oxidative stress of bacterial cells such as increased superoxide dismutase and catalase activity was induced by RSs stimulation, which protects the cells from RSs attack. Bacteria resistance finally failed with the inactivation of protective enzymes, followed by loss of membrane integrity.

Acknowledgements

Financial supports form NFSC (No. 21077104 and No. U1201234) and Science and Technology Project of Guangdong Province, China (2012A032300010).