

Light Dependent Microbial Responses to Cu-doped TiO₂ Nanoparticles

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Abstract:

Shewanella oneidensis MR-1 is an environmental bacterium that can reduce metals and survive in both anaerobic and aerobic conditions. *Mycobacterium smegmatis* is a pathogenic bacterium, but a non-virulent species. Titanium dioxide (TiO₂)-based nanoparticles, commonly used as catalysts, have photocatalytic properties that allow the nanoparticles to absorb ultraviolet (UV) light. The results showed that neither the TiO₂ nor the copper (Cu)-doped TiO₂ nanoparticles reduced the viability of *S. oneidensis* MR-1 under dark or fluorescent light conditions because the bacterium has evolved the strong capability to reduce metal ions and oxidative stresses. *S. oneidensis* MR-1 is highly sensitive to ultraviolet (UV) light, but the presence of TiO₂ nanoparticles or Cu-doped TiO₂ nanoparticles dramatically increased the cell viability. *M. smegmatis* showed a dramatic decrease in viability at a higher doping amount of Cu-doped TiO₂ nanoparticles. The UV light absorbing properties and aggregation of the nanoparticles may contribute to our findings.

Introduction:

Titanium dioxide (TiO₂) is a semiconductor with photocatalytic properties. TiO₂ nanoparticles can produce free radicals and exert strong oxidizing capabilities [1]. Doping the TiO₂ with transition metals enhances the photocatalytic properties of the nanoparticles [2]. The process of doping the semiconductor reduces the band gap energy, thus performing photoactivity even in the visible range of the spectrum [2]. Metal doped TiO₂ nanoparticles in the anatase crystalline form is a strong bactericidal agent when exposed to near-UV light or visible light [3]. This is due to the fact that metal doped TiO₂ nanoparticles produce hydroxyl radicals which oxidize the complex proteins in the cell inhibiting the enzymatic function thus leading to cell death [3].

Methods:

S. oneidensis MR-1 was grown in a minimal MR-1 medium at 30°C at a shaking speed of 200 rpm. When the growth of *S. oneidensis* MR-1 approached the stationary phase (OD₆₀₀ ~ 1.0), cells were diluted with 5 mL minimal MR-1 medium in the absence of carbon source in a glass bottle to an optical density at 600 nm ~ 0.1 (~ 10⁸ CFU/ml). *M. smegmatis* was grown in a Sauton liquid medium at 37°C at a shaking speed of 200 rpm. When the growth of the *M. smegmatis* approached the stationary phase (OD₆₀₀ ~ 1.0), cells were diluted with 5 mL of the carbon-free medium in a glass bottle to an optical density at 600 nm ~ 0.1 (~ 10⁸ CFU/ml).

After determining the correct volume of bacteria to obtain the OD of 0.1, nanoparticle stock solution was added after sonication for one minute to prevent aggregation.

After the bacterial cells had been exposed to dark, or to fluorescent light or to UV light for a period of time, 100 μl of the sample was taken and then diluted in a series, before being spread onto the agar plates. The total number of viable cells was estimated based on a colony-forming unit (CFU) after one day's incubation at 30°C for *S. oneidensis* MR-1 and at 37°C for *M. smegmatis*.

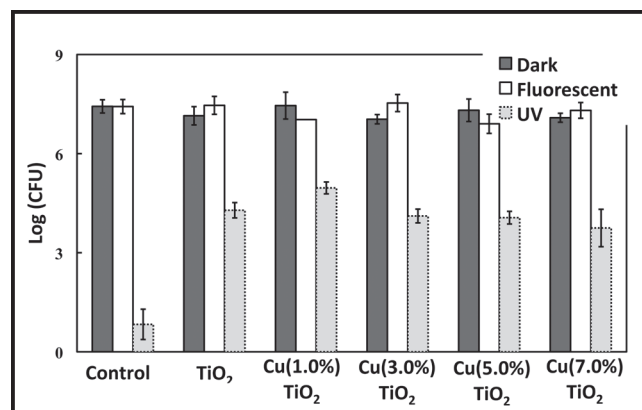


Figure 1: Responses of *S. oneidensis* MR-1 to Cu-doped TiO₂ NPs.

Results and Conclusions:

Figure 1 showed that neither the TiO₂ nor the Cu-doped TiO₂ nanoparticles reduced the viability of *S. oneidensis* MR-1 under dark or fluorescent light conditions because the bacterium has evolved the strong capability to reduce metal ions and oxidative stresses. *S. oneidensis* MR-1 is highly sensitive to ultraviolet (UV) light, but the presence of TiO₂ nanoparticles or Cu-doped TiO₂ nanoparticles dramatically increased the cell viability. The 1% Cu-doped TiO₂ nanoparticles had the greatest increase in cell survival by nearly 196-fold. The aggregation of the nanoparticles shielded the bacteria from the UV light. In addition, the characteristics of nanoparticles, such as absorbing the light within a specific wavelength and tuning the reflection light density, could lead to control their effectiveness for protection of *S. oneidensis* MR-1 against UV stresses.

Figure 2 showed that nanoparticles decreased the viability of the *M. smegmatis* bacteria under dark or fluorescent light conditions. The *M. smegmatis* could tolerate the stresses of UV light, but after the nanoparticles were added into the cell culture, the survival cell amount decreased. Hydroxyl radicals were released from the TiO₂ nanoparticles when exposed to UV light; the *M. smegmatis* cannot tolerate these radicals, which was associated with a decrease in *M. smegmatis* survival. Figures 3 and 4 show the interaction of *S. oneidensis* MR-1 and *M. smegmatis* with Cu-doped TiO₂ nanoparticles. We found that the surface structure of stressed cells became twisted and rougher with regular wrinkles, although without significantly morphological changes.

Acknowledgments:

I would like to thank the NNIN Research Experience for Undergraduates Program for the opportunity to participate in this program. I am also grateful to the National Science Foundation for funding the research project. I want to thank my Principal Investigator, Dr. Yinjie Tang, and my Mentor, Bing Wu, for all of their guidance throughout the research project. I would like to thank Yogesh Goyal, for working on the project with me. Thanks to Kate Nelson, Brent Riggs, and Kristy Wendt for providing training on all of the instruments I used for my research project. Finally I would like to thank God for giving me such a fantastic opportunity to participate in this amazing program, and my family for supporting me and encouraging me to follow my dreams.

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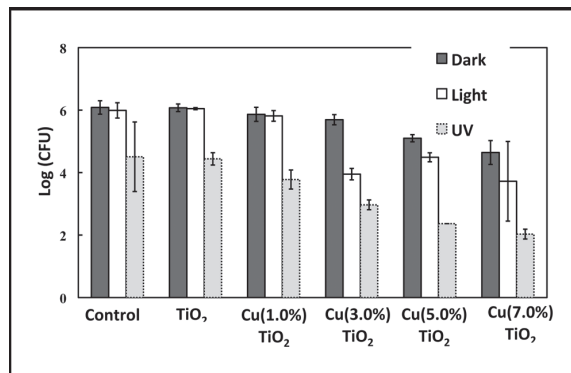


Figure 2: Responses of *M. smegmatis* bacterium to Cu-doped TiO₂ NPs.

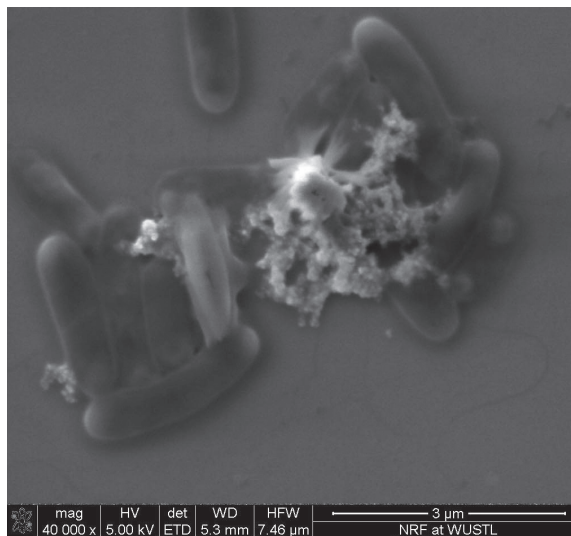


Figure 3: SEM image of *S. oneidensis* MR-1 with Cu (1.0%) TiO₂ after UV exposure.



Figure 4: SEM image of *M. smegmatis* with Cu (3.0%) TiO₂ after UV exposure.