Mechanisms of Enhanced Antibacterial Activity by Reduced Chitosan-intercalated Nontronite

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The proliferation of antimicrobial resistance is a growing global concern. Ubiquitously distributed, naturally occurring iron-containing clays can be utilized as an antibiotic alternative and kill antibiotic-resistant pathogens [1]. However, the coulombic repulsion between negatively-charged bacteria and clay surface makes this process inefficient. This repulsion can be overcome through charge reversal of clays from negative to positive via chitosan intercalation [2].

Charge-reversed, reduced chitosan-intercalated nontronite (rC-NAu-2) attracts negatively-charged pathogens and is demonstrated to be more effective and persistent in killing *E. coli* cells than unmodified reduced nontronite (rNAu-2). A closer spatial association between positively-charged rC-NAu-2 surface and negatively-charged bacteria increases the chances of cell membrane attack by extracellular ROS, influx of soluble Fe^{2+} and yield of intracellular ROS. All these factors contribute to the enhanced antibacterial activity of rC-NAu-2 relative to rNAu-2. In contrast to rNAu-2 treated *E. coli* cells, where membrane damage and intracellular ROS/Fe accumulation are restricted to the polar regions, the closer bacteria-clay association in rC-NAu-2 results in nonselective membrane damage and more uniform intracellular ROS/Fe distribution across whole bacterial cells. These results advance the antibacterial model by highlighting the importance of bacteria-clay interactions to the antibacterial activity of Fe-bearing clays.

[1] Wang et al. (2017), *Environ. Sci. Technol.* **51**, 7639-7647.
[2] Singh et al. (2017), *Geochim. Cosmochim. Acta* **210**, 25-41.