UV Irradiation and Its "Sleeping Beauty Effect" on Bacteria

By Joan B. Rose, PhD

Until the late 1970's, chlorine was virtually the only disinfectant used to treat drinking water, thanks to a number of desirable attributes including its effectiveness against most known pathogens, residual protection, operational reliability and cost. New challenges in the past several decades, however, including the identification of chlorine-resistant parasites *Giardia* and *Cryptosporidium*, disinfection byproducts, and greater security considerations at treatment facilities, have helped to expand the available technologies for water disinfection.

One of the relatively recent options for water disinfection is ultraviolet irradiation or "UV treatment." New research[1] from the Chinese Academy of Sciences' Institute of Urban Environment is providing further information on how UV alone is not always effective at completely destroying certain waterborne bacteria, leaving them in a dormant state reminiscent of the princess in the fairy tale, "Sleeping Beauty."

The "Sleeping Beauty Effect"

Although UV irradiation is used primarily in conjunction with chlorine in US and Canadian municipal drinking water treatment (as UV delivers no residual or lasting effect in the distribution system piping), it is the sole disinfection method for some small-scale facilities and point-of-use treatment in some places. UV treatment is also becoming more widely used in China,[2] according to an article describing the recent research. What's more, UV treatment is "being proposed as an effective alternative to chlorination on a larger scale," according to the article.

Environmental engineer Xin Yu describes a series of experiments in which UV induced *E. coli* and *P. aeruginosa* bacteria into a dormant state in which they were viable but no longer culturable, but could later "wake up" and resume proliferating. Discovery of this sneaky trick provides important insight for water treatment operators enabling them to avoid the effect by combining UV with chlorination, according to Yu, because chlorine destroys the bacterial cell membrane.

Laboratory Results

[1] Zhang, S., Ye, C., Lin, H., Lv, L. and Yu, X (2015). UV Disinfection Induces a VBNC State in Escherichia coli and Pseudomonas aeruginosa. Environmental Science & Technology, 49 (3), 1721-1728.

[2] Author's note: Proposals to use UV exclusively for disinfection are likely not limited to China.

Xin Yu and his research team cultivated liquid cultures of the two species of bacteria, then exposed them to a range of UV radiation (0-300 millijoules/cubic centimeter). They then examined the effects of radiation on the bacteria in two ways. First, using "plate counting," the team counted live cultures remaining in a petri dish after irradiation, and noted only between 0.0001 – 1 percent of bacteria were still viable. The surprise came with the next test, however, involving a sophisticated method to examine the bacterial DNA.[3] That's when Yu discovered most of the bacteria *could still synthesize proteins*, a distinct sign that they were not dead, but merely dormant…the microbial equivalent of the princess in the fairy tale of Sleeping Beauty.

To gauge their ability to "awaken" from dormancy the researchers then provided a warm, nutrient-rich environment for the radiated bacteria. They learned that *E. coli* recovery was greater than that of *P. aeruginosa*. At 0.1 "Colony Forming Units" per milliliter, *E. coli* came back to life for all levels of UV radiation, whereas for *P. aeruginosa*, only those bacteria exposed to relatively low levels of radiation came back.

Refining the Toolbox

Yu concluded that treatment facilities should apply low doses of chlorine following UV irradiation to guard against dormant bacteria awakening and "sharing virulence genes with their neighbors." The significance of this advice should not be underestimated as gene exchange among waterborne pathogens is a contributing factor in promoting antimicrobial resistance, now a global threat.

As our disinfection toolbox grows, so must our knowledge of how to use, combine and monitor the technologies available to achieve safe drinking water globally. There is evidence that all disinfectants may induce some degree of "Sleeping Beauty" dormancy in human pathogenic bacteria, possibly as a survival strategy.[4] More research is needed to characterize the effect. For now, Yu and his team are to be congratulated for the significant information they have made available for water treatment professionals who battle waterborne pathogens daily.

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[3] Reverse transcription with polymerase chain reaction.

[4] See, for example, Oliver, J.D. 2009. "Recent findings on the viable but nonculturable state in pathogenic bacteria." *FEMS Microbiol Rev* 34, 415-425.