A PhD thesis conducted by David Spiteri on the ultrasonic action as a means of wastewater treatment, has revealed new molecular understanding of mechanisms occurring at the RNA level, indicating the use of ultrasound as a means of disinfection.

Currently one of the most used treatment methods is chlorine, which is cheap and highly effective in killing pathogens. However, this results in disinfection by-products and may affect the palatable taste of water. For this reason, alternative methods of disinfection have long been considered, such as ultrasound, which has recently received wide attention in water and wastewater treatment and environmental remediation areas, including the application for disinfection purposes.

Ultrasound creates cavitation phenomena, resulting in the formation of several free radicals, namely OH• and H•, due to the breakdown of the H_2O molecule. These radicals affect the cellular integrity of the bacteria, causing the inactivation of several processes, and thus it is important to unravel the mechanism of action of this technology in order to understand long-term effects on micro-organisms.

This study looked into the application and process of ultrasound technology as a means of disinfection by acoustic cavitation. Several genes used in the defence mechanism were observed, with the study showing for the first time, the possible role of the GAD system in ultrasound treatment and oxidative stress, as these have shown that they are essentially crucial in the protection from oxidative stress.

Another part of the study focused on identification of expressed genes, with the investigators observing that many are playing a role in stress protection. High throughput sequencing technology was used to identify such genes, which concluded that ultrasound is leaving an impact on the bacteria, by changing its mechanism making them better or worse in their defence mechanisms.

Finally, the study also looked at long term mutations by running Whole Genome Sequencing to assess the mutation effects of ultrasound treatment on the *E. coli* K-12 bacteria. Variations were observed at four different areas of the genome, which although may not be having a significant effect on the bacteria, indicate how the environmental stresses influence the bacteria.

In conclusion the current research identified several genes which were involved in several pathways, which play a role in metabolic and biological pathways. These pathways would allow the bacteria to become more efficient in their growth under different environmental conditions, which might allow them to grow faster, or use less resources in their metabolic pathways.

In the context of the wastewater recycling and reuse, the aim was to find a treatment capable of eliminating or substantially reducing all the pathogens to reduce pollution of the receiving waters and to provide public health protection. Ultrasound treatments can be a potential technology for this type of treatments.

The findings of this research project have also been the subject of a peer reviewed paper with more papers planned soon.

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