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Homogenous Sono-Fenton reaction can trigger long term bactericidal effect against *Acinetobacter baumannii* due to residual stress induced by reactive oxygen species

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A B S T R A C T

Gastrointestinal diseases caused by microbial contamination of water has been one of the enduring public health challenges of the mankind, wherefore demands continual innovation in the contemporary water treatment techniques. Here, a systematic study was carried out to elucidate the bactericidal mechanism of ultrasound assisted Fenton reaction (Sono-Fenton: SF) against multidrug resistant *Acinetobacter baumannii*. Inactivation of $\approx 5 \times 10^6$ CFU/mL of *A. baumannii* was achieved within 90 min of SF under weak acidic conditions using 20 mg L^{-1} of H_2O_2 and 2 mg L^{-1} of Fe^{2+}. Having more than 99 % inactivation efficiency, no reactivation of the bacteria was observed for 96 h. After SF, a combination of ultrasound and H_2O_2 was found to be more efficient although complete inactivation was not achieved within the same time frame. Experimental evidence suggests the generation of multiple reactive oxygen species (ROS) such as H_2O_2, $^{\bullet}OH$ and $O_2^{\bullet-}$. The synergistic effect of ROS and ultrasound may have resulted in the membrane damage of the bacteria as evident from the electron microscopy analysis. Interestingly, our data also suggests that the residual stress associated with SF could have long term bactericidal effect and is influenced by incubation temperature. Comparative transcriptomic analysis showed that NirD/YgiW/YdeI family stress tolerance protein, KGG domain containing proteins, and PspC domain containing proteins, all known to control the stress induced responses in bacteria was significantly upregulated after SF treatment. Additionally, genes regulating transcription (GntR) and translation (rpsQ) have been downregulated which may induce a nutrient and metabolite limiting conditions in the bacterial cell. The bacterial inactivation ability of SF process was further validated with real water samples collected from natural systems and therefore, advocate the possible real-world applications.

1. Introduction

The interplay between water, public health and society can best be understood from a perspective that contemplates the long-term dynamics and demonstrates proposition from an integrated, interdisciplinary perspective [1]. Therefore, from the conceptualization of early civilization, availability of potable water has been the "*sine qua non*" for the survival and welfare of the human society. The certitude of this fact is conceded by the United Nations (UN) in its sustainable development goals (SDG6: Clean water and sanitation) [2]. However, in spite of stupendous and dedicated efforts of governments and humanitarian organizations, globally 2 billion people lack access to safe drinking water and water borne diseases cause 3900 child deaths each year [3]. In India alone, water borne infections have resulted in the loss of 73 million days of work putting negative impact on the regional economy [4]. As per the report of the World Health Organization (WHO), drinking water

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