Controlling bacterial growth and inactivation using thin film-based surface acoustic waves

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Abstract: Formation of bacterial films on structural surfaces often leads to severe contamination of medical devices, hospital equipment, implant materials, etc., and antimicrobial resistance of microorganisms has indeed become a global health issue. Therefore, the effective therapies for controlling the infectious and pathogenic bacteria are urgently needed. Being a promising active method for this purpose, surface acoustic waves (SAWs) have merits such as nanoscale earthquake-like vibration/agitation/radiation, acoustic streaming induced circulations, and localised acoustic heating effect in liquids. However, only few studies have been explored on controlling bacterial growth and inactivation behaviour using SAWs. In this study, we proposed to utilise the piezoelectric thin film-based SAW devices on silicon substrate for controlling bacterial growth and inactivation with and without using ZnO micro/nanostructures. Effects of SAW powers on bacterial growth for two types of bacteria, i.e., E. coli and S. aureus, were evaluated. Varied concentrations of ZnO tetrapods were also
added into bacterial culture to study their effects and the combined antimicrobial effects along with SAW agitations. Our results showed that when the SAW power was below a threshold (e.g., about 2.55 W in this study), the bacterial growth was apparently enhanced, whereas the further increase of SAW power to a high power caused inactivation of bacteria. Combination of thin film SAWs with ZnO tetrapods led to significantly decreased growth or inactivation for both *E. coli* and *S. aureus*, revealing its effectiveness for antimicrobial treatment. Mechanisms and effects of SAW interactions with bacterial solutions and ZnO tetrapods have been systematically discussed.

**Keywords:** Surface acoustic waves, ZnO tetrapods, thin film device, bacterial growth, bacterial inactivation,

1. Introduction

Biofilms or bacterial films formed on structural surfaces become critical issues in hospital environments, especially for biomedical devices such as valves, catheters, and implants, and *E. coli* and *S. aureus* are two well-known bacteria associated with the infectious diseases [1]–[4]. Survival rate of these bacteria species is influenced by intrinsic factors such as genetic mechanisms and metabolism, or external factors such as environmental conditions including pH value, temperature and humidity [5]–[8]. For preventing bacterial film growth, various passive and active methods have been implemented. The common passive methods include prevention and/or inhibition of bacteria adhesion/biofilm formation on surfaces by using antibacterial/antifouling surfaces or multifunctional coatings [9]–[12]. However, the passive surfaces or coatings would not easily cause effective bacterial resistance, and often be easily damaged *in vivo* because the plasma proteins from the biological fluids are easily transported back onto the surfaces, thereby inducing bacteria colonization and reducing their antibacterial effects [9], [13], [14].

Active methods include those using ultraviolet (UV) light, laser irradiation, ultrasound, and addition of antibacterial agents such as antibiotics, antimicrobial peptides (AMPs), and silver ions (Ag⁺), or zinc oxide (ZnO) micro- or nano-structures, and they have also been commonly used to eliminate the adhered bacteria on different surfaces [9], [15]–[18]. However, there has been various issues such as effectiveness and efficiency of these methods, instability and toxicity aspects with those AMPs, or increased concerns with safety and toxicity when utilising...
nanoparticles [19]–[21]. Ultrasound has been studied as a method to stimulate and enhance the growth of biological substances and microbial cells [22]–[26]. Strong shear and mechanical stresses are generated by low frequency (in KHz range) and high energy powered ultrasound for cleaning or mixing processes [22], [27], [28]. Whereas the high frequency (in sub-MHz and MHz level) and low power density ultrasound is extensively employed in applications such as in diagnosis and imaging. The later ones were also used for applications which require a huge quantity of redox radicals, such as the oxidation of organic contaminants in aqueous environments that involve large amounts of oxidising radicals [22], [27], [28].

More recently, surface acoustic waves (SAWs) have been developed to realise biosensing and actuation functions which are based on acoustic wave propagation and vibrations on the structural surfaces such as LiNbO₃, silicon, polymer, metal or glass [29]–[32]. The SAWs have been widely utilised in various applications such as wireless communications, acoustofluidics, sensing, and particle/biological manipulations [31], [33]–[35]. When compared to those conventional ultrasound transducers, SAW devices exhibit various unique advantages which include minimised cell destructive, localised heating effect, miniaturized dimensions, and low effect of cavitation, etc. [36]. In fact, the less destructive and localised self-heating effects from SAWs are exceptionally critical for bacterial studies. As compared to those conventional ultrasound transducers, SAW energy is mainly confined within the surface or sub-surface of the material where the heat dissipation mainly occurs at one or a few wavelength depth from the surface [36]–[38]. Moreover, since the conventional ultrasound transducers tend to be operated at lower frequencies than the SAW devices, it was reported that higher frequency is unlikely to generate the significant cavitation effects on cell membranes thus not triggering cell death disrupting cell membranes [36], [39]–[42]. Thin film-based SAWs have recently been widely investigated, with their advantages for realising bendable/flexible lab-on-a-chip (LOC) platforms and implementing highly integrated functions with microelectronics or other microfluidic/sensing approaches [32], [43]. In addition to this, the thin film-based SAW devices can be used directly onto structural surfaces such as glass, polymer, silicon, or metal to produce acoustic wave propagation and surface vibrations [35], [44]. With all these excellent attributes, thin film-based SAWs should be suitably integrated for bacterial studies, in terms of bacterial growth and bacterial inactivation.

There are just few previous studies using SAWs for bacteria inactivation. For example, Kopel et. al (2011) reported that low-energy SAWs create an elliptical movement and microstreaming
in the medium, thus eradicating biofilm-residing bacteria when applied with an antibiotic simultaneously [3]. Loike et al. (2013) reported that SAWs can eradicate bacteria in a planktonic state and within biofilms [1]. Kazan et al. (2006) reported that low energy SAWs are effective towards the prevention of microbial biofilm formation [45]. It was also mentioned that the SAWs were repulsive to bacteria and the interfere/prevent the docking and adhesion of bacteria to solid surfaces, which were in the early stages of microbial biofilm growth [45]. Chew et al. reported that the acoustic radiation force induced on the bacteria was the dominant mechanism for bacterial inactivation and they observed a 75% reduction in bacterial load in 10 min treatment with the highest SAW excitation power of 348 mW [46]. Hong et. al reported that biofilm formation was strongly affected by the flows in the thin layers of the bacterial suspensions which were facilitated by surface waves [47]. Moreover, they discussed the various wave patterns which can either promote biofilms formation or prevent attachment of bacteria or biofilm formation [47].

Although there are a lot of studies using SAWs for combating and eliminating the bacteria biofilm formation in literature, there have been few studies for the key mechanisms behind the interactions of SAWs, bacteria, with or without semiconducting material microparticles such as ZnO. It is unclear how the SAWs affect the bacteria life cycle under these conditions, i.e., at which conditions for enhancing or eliminating bacteria growth and control with SAW agitations. In this paper, the bacterial growth (using *E. coli* and *S. aureus* as two examples) and antibacterial performance using thin film SAW ZnO/Si devices were studied with and without using ZnO tetrapods. First, we investigated the effects of various applied SAW powers (ranged from 0.36 to 14.0 W) on bacteria growth and evaluated the rate of growth or inactivation in a polydimethylsiloxane (PDMS) chamber. Subsequently, we applied varied concentrations of ZnO tetrapods (2 to 4 mg/mL) in the bacteria solution within the chamber as presented in Figure 1. The mechanisms of bacteria growth/inhibition and antibacterial performance using SAWs with ZnO tetrapods structures have been systematically studied.
Figure 1: An illustration of PDMS chamber on ZnO/Si SAW device for bacterial studies with thin film SAWs

2. Experimental details

2.1 Preparation and characterisation of SAW device

ZnO films with ~4.5 µm thickness were deposited onto 4-inch (100)-orientated silicon substrates using a direct current (DC) reactive magnetron sputtering (NS3750, Nordiko) process in an ultra-high vacuum system. The base pressure of the chamber was $1 \times 10^{-4}$ Pa before deposition. A zinc target with a purity of 99.999% was used for deposition of the ZnO films with a 20 cm distance from the substrate. During the deposition process, the surface of the substrate was pre-cleaned by means of a short bombardment (5 min) with Ar ions using a DC power of 300 W. The deposition conditions were as follows: DC sputtering power of 400 W; Ar/O$_2$ mixing flow ratio of 10/15 SCCM (standard cubic centimetre per minute); deposition rate of ~750 nm/h with a deposition pressure of 1 Pa.

Interdigital transducers (IDTs) were patterned and fabricated on the ZnO thin films using a conventional photolithography and lift-off process. A bilayer of Cr/Au with thicknesses of 20 nm/100 nm was prepared as the electrode in this study. Each IDT has 50 pairs of fingers, with a wavelength of 300 µm. The resonant characteristics (frequency and S-parameters) of the ZnO/Si SAW devices were measured using an RF vector network analyser (Keysight, FieldFox N9913A). An RF signal at the resonant frequency of the ZnO/Si SAW device was generated using a signal generator (Aim TTI, TG5011A) and then amplified using a power amplifier.
(Amplifier Research, Model 75A250). The RF power applied to the IDTs of the SAW devices was measured using a RF power meter (Racal Instruments 9104).

For evaluation of the induced surface heating effects upon SAW agitations, an infrared (IR) camera (FLIR, T620bx) was used to monitor the temperature changes on the SAW device at different RF powers. A K-type thermocouple was also used on the SAW device’s surface to compare and verify with the results obtained from the IR camera. The temperature measurement was taken in front of the IDT area, where the bacteria solution was located during the testing. In addition, the temperature measurements at each of the RF powers were taken in triplicates during the SAW agitations, and it is worth noting that both the IR camera and the thermocouple showed nearly identical temperature readings during SAW agitations.

### 2.2 Processing and characterisation of ZnO tetrapods

Preparation methods of the ZnO tetrapods was reported previously in literature [48]–[50]. Briefly, they were fabricated via flame transport synthesis (FTS) technique which was a single-step process to convert Zn metallic particles directly into ZnO tetrapods. In a conventional FTS process, a mixture of Zn tetrapods (from Goodfellow, Huntington, UK) with an average diameter of 5 mm and poly (vinyl butyral) (PVB) powder (Mowital B 60H from Kuraray Europe GmbH, Hattersheim, Germany) at a weight ratio of 1:2 was first placed into a ceramic crucible and then heated to 900 °C in a furnace [48], [50]. The PVB polymer in this instance was acted as a sacrificial layer that was decomposed at a high temperature during the heating process in the furnace. During the heating process, the Zn microparticles was converted into Zn atomic vapour, which was participated in nucleation and growth processes of tetra-ZnO micro- and nanostructures via a solid-vapour-solid growth mechanism with the presence of native oxygen molecules [48], [49]. The growth of fluffy tetra-ZnO micro- and nanostructures networks with higher aspect ratios was achieved when the sacrificial polymer was replaced with a process that involved heating Zn powder under a nitrogen atmosphere [48]–[50].

The ZnO tetrapods were analysed based on their crystal structures using X-ray diffraction (XRD, D5000, Siemens, Cu-Kα radiation, 40 kV, 30 mA), utilising a Cu-Kα radiation source (λ = 0.15406 nm) operated at 2 kW. The ZnO microstructure solution was then prepared via dissolving the tetra-ZnO structures in deionised (DI) water with a concentration ranging from 2 to 4 mg/mL. The ZnO solution was ultrasonically mixed for 1 hour to achieve a homogeneous
mixture. Morphology of these ZnO tetrapods was observed using a scanning electron microscope (SEM, Tescan Mira 3).

2.3 Bacterial culturing of E. coli and S. aureus

*Escherichia coli* K12 (*E. Coli*, DSMZ 3925) and *Staphylococcus aureus* (*S. aureus*, DSM 2569) were grown in a Luria Bertani (LB) broth overnight in a static incubator (37 °C) for 16 hours. From the culture, 1 mL of the bacteria solution was placed into a fresh 50 mL LB broth in orbital shaking incubator at 37 °C for about 3 hours. Once this was done, the optical density (OD$_{600}$) value of the bacteria solution was measured. To obtain the standard growth curves for both bacteria cultures, the inoculum for both cultures were standardised to an OD$_{600}$ value of 0.01 and were placed in the Tecan plate reader. In addition, the LB broth was used as the control group. The OD$_{600}$ measurements were taken hourly over a period of 24 hours.

2.4 SAW agitation and bacterial growth tests

The PDMS chamber was fabricated by using the PDMS elastomers, Sylgard® 184 from Dow Corning®, with vinyl groups (part A) and hydrosilane groups (part B) in a ratio of 10:1. Once the mixture was completed, it was placed in a petri dish where de-gassing was conducted inside a gas chamber. After the degassing process, the PDMS mixture was placed in an oven at 60 °C for 4 hours before it was cut into multiple 3 x 3 x 3 cm$^3$ cubes.

To evaluate the microbial growth under SAW agitations, OD measurements and cultivation in LB agar plates were performed for bacteria quantification. For the OD$_{600}$ measurements, a bench-top spectrophotometer (WPA biowave, CO8000 Cell Density Meter) was used. An aliquot of 3 mL of fresh *E. coli/ S. aureus* inocula (OD$_{600}$ 0.5, $10^7/10^8$ CFU/mL) was placed inside the PDMS chamber, and they were divided into two groups: SAW test groups which were exposed to nine RF powers (0.36, 0.95, 1.4, 1.9, 2.55, 3.21, 4.01, 6.64, and 7.8 W) and control, which was not exposed to any SAW agitation. Both the PDMS chamber for control and test groups were conducted at room temperature. Different powers were evaluated individually for 30 minutes, and the inocula in the PDMS chamber was replaced in each subsequent test to maintain the initial OD$_{600}$ at 0.5. At the end of 30 minutes, an aliquot (10 µL) of the bacteria solution was taken from PDMS chamber for serial dilution in a 96 well plate, and 5 µL solution were drop casted onto LB agar plates from both the test and control groups to carry out bacterial quantification. After this process, the LB agar plates were incubated at
37 °C for overnight. To obtain the CFU/mL (colony forming unit/mL) value, the following equation was utilised:

\[
\frac{\text{CFU}}{\text{ml}} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{volume of culture plated in ml}}
\]  

(1)

2.5 ZnO tetrapods combined with SAW

After SAW tests, *E. coli* and *S. aureus* cells were evaluated for antibacterial assays using ZnO tetrapods combined with SAWs. Following the similar conditions set for SAW tests previously mentioned, microbial inocula at OD600 0.5 (control and test groups) was placed inside the PDMS chamber, in which three concentrations of ZnO tetrapods (2, 3, and 4 mg/mL) were added to the culture, with five RF powers applied for the SAW testing groups. After 30 minutes for each RF power tested, OD measurements and microbial quantification through serial dilutions and cultivation in LB agar plates were carried out. The plates were incubated at 37°C, for 16 hours and quantified in CFU/mL.

3. Results and Discussions

3.1. Characterisation of ZnO tetrapods and SAW device

SEM images of the ZnO tetrapod structures are shown in Figures 2(a) and 2(b) at low and corresponding high magnifications respectively. The average diameter of the ZnO arms is in the range of few 100 nm to few micrometres. Each tetrapod consists of four arms converging from the centre with angles of ~105° to 110°. This structure combines the features of one-dimensional ZnO micro and nanorods, which results in a highly porous linked network with numerous interconnecting (bridging) junctions [48]. The tetrapodal shape of these nanostructures is very effective in the construction of flexible, structurally intact, and mechanically stable 3D networks with very high porosities (up to 98%) and large surface-to-volume ratios, hence prompting the interaction between ZnO tetrapods with bacteria, along with SAWs.

The obtained XRD diffractograms of ZnO tetrapods and ZnO thin film are shown in Figure 2(c), and all the observed peaks are in accordance with those of wurtzite-type ZnO (JCPDS 36-1451). The sputtered ZnO film has a strong texture along (0002) orientation, which is good for Rayleigh acoustic wave generation and propagation. The obtained dominant reflections of ZnO tetrapods, e.g., the three theta peaks at 31.9°, 33.9° and 57.9° are attributed to those of (10-10), (0002) and (10-11) planes of the wurtzite ZnO [48].
Figure 2: (a) and (b) SEM images of ZnO tetrapods used; (c) XRD diffractogram of ZnO tetrapods, with an inset of the XRD diffractogram for ZnO thin film; (d) Frequency response (S$_{11}$) of ZnO/Si SAW device with a wavelength of 300 µm.

**Figure 2(d)** shows the obtained reflection spectra (S$_{11}$) of the ZnO/Si SAW device with a wavelength of 300 µm. The resonant frequency of the Rayleigh waves was found at ~15.81 MHz from the experimental result. The multiple peaks centred around 31.64 MHz were assigned to the Sezawa wave mode. The corresponding velocities were calculated to be ~4743 and ~9492 mm/s, for both Rayleigh and Sezawa wave modes, respectively.

### 3.2. Effects of SAW on *E. coli* and *S. aureus*

The bacteria growth curve of both *E. coli* and *S. aureus* under the standard growth conditions (i.e., placed in a 37 °C incubator) are illustrated in **Figure 3(a)**. For the growth curve of *E. coli*, the cells enter the first lag phase in the first two to three hours. When the cells were adapted to
their new environment, they begin to divide exponentially and enter into the exponential growth phase which was denoted as the period of up to 24 hours. As for the growth curve of \textit{S. aureus}, the cells enter the first stage in the first 5-6 hours, and begin to divide exponentially and enter into the exponential growth phase after 12 hours. Once the nutrients in the medium are depleted, the bacterial culture enters a stationary phase which is denoted between 12 to 22 hours. Cultures in the stationary phase collect catabolic products in the environment over time, which then result in a slight decrease in the number of viable cells known as the death phase, denoted from 22 hours [7].

**Figure 3**: OD measurements of (a) standard curve of \textit{E. coli} with and without SAW; CFU/mL readings for (b) \textit{E. coli}; (c) \textit{S. aureus}; (d) Temperature readings at different RF powers

**Figure 3(b)** compares the growth results for the control group and test group of \textit{E. coli} in the PDMS chamber when it was subjected to various SAW RF powers (from 0.23 W up to 14.1 W). As the RF power is increased, the CFU/mL readings are increased until at a certain RF power where the CFU/mL readings become decreased at approximately 3.21 W. With the further increase of SAW power of above 6.64 W, there are no colonies of bacteria detected. The dramatic decrease in CFU readings for SAWs at higher powers can be explained by the
possibility of SAW disrupting the cell walls of E. coli upon SAW agitations and streaming, causing the strong inactivation effects. Acousto-thermal effect causes temperature changes, which might be detrimental for the bacteria growth. It should also be addressed that sonochemical reactions can result to the production of highly reactive radicals and molecular products when SAWs induce nanoscale acoustic cavitation in liquids[51], thus the bacterial cells being stressed and growth being prevented. Figure S1(a) in the supporting information compares the OD values for the control and test groups of E. coli in the PDMS chamber when subjected to various RF powers. It was observed that as the RF powers were increased, the OD values were increased minimally with a few fluctuations before it started decreasing at higher RF powers. This implies that with increasing RF powers, the bacterial growth was induced with SAW via the increment of OD values, before it started to decrease at higher RF powers, revealing the bacterial inactivation effect. As compared to the control group, the OD values were observed to be of similar readings with minor fluctuations.

Figure 3(c) compares the bacterial growth results for the control group and test group of S. aureus when subjected to various RF powers (0.23 W to 4.01 W). Bacterial quantifications were observed to be similar to that of E. coli, with increased cells number until the threshold of 4.01 W, where the decreased cells number, or no cells number was observed at higher RF powers. Figure S1(b) in the supporting information compares the OD values for the control and test groups of S. aureus in the PDMS chamber when subjected to various RF powers (0.36 to 4.01 W). It was observed that as the RF powers were increased, the OD values were also observed to be increasing. When compared to the control group, the OD values were observed to increase minimally. Similarly to E. coli, with increasing RF powers, S. aureus bacterial growth was induced with SAW via the increment of OD values. Figures S2(a) to S2(d) in the supporting information present the confocal microscopy images of both the control and test groups at two different RF powers (i.e., 0.23 and 7.8 W).

As mentioned above, another effect induced at applying SAW powers is the strong acousto-thermal effect, which can increase the temperature of the liquid. Along with the mechanical vibrations from SAWs, enhanced temperature will have a significant effect on the bacteria’s life cycle. The temperature readings against time up to 30 minutes at various applied SAW powers from 0.24 W to 6.76 W are presented in Figure 3(d). Clearly the temperature is increased with the duration of SAW power, and maximum temperature has reached to 76.1 °C at the power of 6.76 W. It is worth mentioning that as the temperature readings for both 0.61
and 1.01 W were too dense, these two RF powers were not presented in Figure 3(d).
Comparing the CFU/mL readings obtained from Figure 3(c), it can be implied that when the
RF powers applied induced preferable temperature (for example, near 37 °C), it would promote
the bacterial growth, and this was annotated with the increase in the CFU/mL values. However,
when much higher RF powers is applied, significant acousto-thermal effects occur (along with
strong agitation and streaming effects), and temperature increase would become a detrimental
effect for bacterial growth, hence eliminating them instead at higher RF powers due to the high
temperatures induced. Moreover, both *E. coli* and *S. aureus* are mesophilic strains, with the
37 °C as optimum temperature at, but can grow at temperature ranging between 10 °C to 40 °C
and 15 °C to 45 °C for *E. coli* and *S. aureus*, respectively [52], [53]. However, extended
exposures above 42 °C are not recommended for *S. aureus* [53]. As for *E. coli*, it was reported
that although it can grow at temperatures up to 49 °C and was also observed to be alive at 53 °C
[52], it was demonstrated that *E. coli* is sensitive to heat treatment from 60 °C to 70 °C which
resulted in inactivation [54]. Hence, this further imply that the acousto-thermal effect from
SAW have an effect on the bacteria.

3.3. Enhancing SAWs effect with added ZnO tetrapods

Bacterial quantifications under different ZnO tetrapods concentrations at five RF powers are
presented in Figures 4(a) to 4(e). The CFU/mL readings for both control and test groups do
not differ significantly at an RF power of 0.36 W and 0.95 W for *E. coli*. The main reason for
this observation could be due to the insufficient SAW agitation to promote the interaction
between the ZnO tetrapods and bacteria. In addition to this, temperature increment induced by
the SAWs at such power range might also be insufficient to promote any growth or killing to
occur.
Figure 4: CFU/mL readings at different ZnO concentrations with an RF power of (a) 0.36 W, (b) 0.95 W, (c) 1.4 W, (d) 1.9 W, (e) 4.01 W, Comparison to S. aureus at (f) 1.4 W, (g) 1.9 W, (h) Acoustic streaming of bacteria solution in terms of velocity (mm/s) versus power (W) upon SAW agitations when subjected to various RF powers.

As the ZnO concentration increases as depicted in Figures 4(c) and 4(d), the CFU/mL readings were observed to decrease in the SAW test groups, which implies that SAW induced agitation and streaming effects enhanced the interactions of ZnO tetrapods with bacteria and promoted the inactivation mechanism. Upon the application of a higher RF powers of 4.01 W as shown in Figure 4(e), the SAW test group do not show any CFU values which indicates that SAW dramatically enhanced the ZnO inactivation effect on bacteria growth, along with the acousto-thermal effect induced by SAW. As a general trend, at lower ZnO concentrations, higher CFU/mL readings were observed before they were started to decline as the RF powers and ZnO concentrations were increased. When there was no presence of ZnO tetrapods, the CFU/mL values were observed to increase with increasing RF powers. However, the CFU/mL values began to decrease with increasing ZnO concentration and RF powers. This further implies that there is a synergetic effect between ZnO concentration and RF powers on the antibacterial property for E. coli, along with the accompanied acousto-thermal effect induced by SAWs. As compared to those of S. aureus as presented in Figures 4(f) and 4(g), a similar trend was obtained as those of the E. coli whereby as the RF powers were increased with increasing ZnO concentration, the CFU/mL readings were observed to be decreasing.

3.4. Discussions on mechanisms for bacteria inactivation

SAW effects on E. coli and S. aureus

The quantification results of E. coli shows an increased number of CFU/mL with increasing RF powers. The increase in the CFU readings for the SAW test groups could be explained with the increase in liquid convection via three mechanisms. One of the mechanisms is known as the acoustic streaming (or internal flow) where the momentum from the propagating sound waves is being transferred to the liquid, resulting the liquid to flow in the direction of the propagating sound waves and providing more nutrients and other supplies for bacteria growth [22], [23], [55], [56]. The other mechanism is the SAW agitation or radiation effects for bacteria. SAWs can cause bacteria vibration, which can serve as an energy source to promote growth by
allowing bacteria to multiply faster alongside with obtaining more nutrients from the streaming of the LB broths upon SAW agitations [57].

Localised acousto-heating effect induced by the application of RF powers is another key factor for the life modulation of bacteria. At very low RF powers, the acousto-thermal effects are insignificant, thus there are not apparent bacteria growth enhancement. However, with increase of SAW power, the acousto-thermal effects are increased significantly, which could encourage the bacterial growth. However, at very high RF powers, the induced acousto-thermal effects would be so significant that SAWs become inhibit or cause the death of bacteria cells.

Figure 4(h) shows the experimentally obtained velocity readings against various RF powers as the bacteria solution in the PDMS chamber was subjected to SAW agitations. With an increase in the RF power applied on the ZnO/Si SAW device, the velocity of the acoustic streaming of bacteria solution is increased. In this case, acoustic streaming can be analysed quantitatively via the following equations [34], [58]:

\[ \frac{\partial p}{\partial t} + \nabla \cdot (p \mathbf{v}) = 0, \quad p \frac{\partial \mathbf{v}}{\partial t} + p (\mathbf{v} \cdot \nabla) \mathbf{v} = -\nabla p + \mu \nabla^2 \mathbf{v} + \left( \mu_b + \frac{\mu}{3} \right) \nabla \times \nabla \times \mathbf{v}, \quad (2) \]

where \( \mathbf{v} \) is the flow velocity, \( t \) is time, \( p \) is the pressure of the fluid, \( \mu_b \) and \( \mu \) are the bulk and shear viscosities of the fluid respectively. The left-hand side of Equation 2 represents the inertia force per unit volume of fluid where the first term represents the unsteady acceleration, and the second term represents convection acceleration [33]. On the right-hand side of Equation 2, the net forces per unit volume includes the pressure and viscosity gradients, where these equations can be used with the boundary conditions and linear relationship between pressure \( p \) and mass density \( \rho \) to predict the motion of the fluid [34], [58]:

\[ p = c_0^2 \rho \quad (3) \]

where \( c_0 \) refers to the speed of sound in the fluid. Moreover, there are two components of liquid flow, i.e., fluid acoustic motion and streaming motion. At lower RF powers, slow streaming of the bacterial solution is induced when the velocity of the acoustic component is considerably larger than the streaming component [34], [55]. This is known as linear streaming where the convection acceleration can be ignored [34], [55]. In addition to this, the effect of inertia on the streaming motion is insignificant as compared to viscous effects, and thus a slow streaming happens when the flow is laminar [34], [55]. However, at higher RF powers, fast streaming of the bacterial solution was induced when the streaming velocity is of the same order or greater than the acoustic component. Such streaming causes the bacteria to experience flowing,
allowing the continuously supplied nutrients from the LB broth, thus promoting bacteria
growth.

SAWs was utilised to loosen cell bunches created during the microbial cultivation phase [59].
This allows the improvement in nutrient utilization by the bacterial cells, resulting in increased
cell biomass and better target substance output [22], [59]. Due to this reason, SAWs enhance
membrane permeability, and this results in an increase in cell growth and multiplication by
speeding up the transport of chemicals [22], [60]. Moreover, SAWs can be utilized to modify
culture medium for the creation of an ideal environment for microbial growth and proliferation.
In other words, SAW has effects on cellular components, functioning, and genetics, which
further allow micro-organisms to multiply faster [22], [61].

Another possibility from SAW would be the cavitation bubble-driven strategy where
continuous motion of particles can be propelled by cavitation of bubbles during SAW agitations
[62], [63]. In this mechanism, the cavitation bubbles grow and collapse continuously which
result in the generation of periodic pulse thrust to drive the particles or cells to move in the
liquid [62], [63]. In this manner, it would suggest that upon SAW agitation at various RF powers,
SAW can allow the ZnO tetrapods to interact with bacteria in LB broth to facilitate either
growth or inactivation. It was also noted that cavitation bubbles can expand spherically and
collapse asymmetrically, which makes it push on the particle generated by the bubble
expansion which is greater than the pull on the particle generated by the bubble collapse [62],
[63]. However, as it is well-known, the higher frequency would be difficult to cause effective
generation of bubbles, and the higher the frequency, the smaller the bubbles. Therefore, such
cavitation effect should not be a key mechanism for the bacterial inactivation [64].

Effects of ZnO tetrapods

Upon the addition of ZnO tetrapods without applying SAWs (i.e., the control groups), the
CFU/mL readings were observed to decrease with increasing ZnO concentrations, these ZnO
tetrapods interacts with E. coli bacteria causing the reduction of bacteria [65], [66]. With the
introduction of SAW agitation at various RF powers, it induced acoustic streaming, enhancing
the strong interaction/reactions between the ZnO tetrapods and E. coli bacteria. Other than
acoustic streaming, it is presumed that the forces on particles exposed to acoustic waves are
those induced by direct irradiation of acoustic field and indirect irradiation from the scattering
of the acoustic field from other particles [34], [67]. The primary acoustic radiation pressure \( (F_{\text{ARF}}) \) is the force applied on a single particle in a fluid due to SAW whereas the secondary acoustic radiation pressure is about the force due to the acoustic interactions with other particles in the fluids [34], [67]. The surface integral of the time-averaged second-order pressure \( p_2 \) and momentum flux tensor \( p_0 v_1 v_1 \) at a fixed surface outside the oscillating sphere is used to calculate the acoustic radiation force [68]. Consequently, the general solution can be expressed as:

\[
F_{\text{ARF}} = -\int_{\partial \Omega} \langle p_2 \rangle n + p_0 \langle (n \cdot v_1)v_1 \rangle \ (4)
\]

where \( n \) is the unit normal vector of the particle surface directed into the fluid. In general, the bacterial solution and ZnO tetrapods are exposed to the net acoustic radiation force and the SAW acoustic streaming induced Stokes drag force \( F_{\text{drag}} \) where the dominant force is dependent on the size of the particles [34], [57], [69], [70]. Hence, particles that are larger than a given threshold size are typically dominated by the acoustic radiation force, and the size threshold is dependent on several factors such as the acoustic frequency, acoustic contrast factor, and kinematic viscosity [34], [57], [69], [70]. The Stokes drag force, \( F_{\text{drag}} \), is influenced by the fluid flow field, fluid viscosity, and particle size and shape. Consequently, \( F_{\text{drag}} \), is governed by the follow equation for a spherical particle of radius \( r \), with medium viscosity \( \mu \), and relatively velocity \( v \) [68]:

\[
F_{\text{drag}} = 6\pi \mu r v \ (5)
\]

Considering that the PDMS chamber is consisted of bacterial solution and ZnO tetrapods, the acoustic waves are presumed to attenuate the boundary interaction with the PDMS walls, leading to a boundary-driven streaming. When an acoustic wave propagates towards a solid boundary, the non-slip boundary will create a high-velocity gradient which is perpendicular to the solid surface, thus creating a steady boundary layer vorticity [34], [57], [69], [70]. This is well-known as the inner boundary streaming [34], [57], [69], [70]. The stronger the inner boundary streaming flow, the more counter-rotating streaming vortices are generated within the fluid [34], [57], [69], [70]. This is referred to as outer boundary streaming or Rayleigh streaming [34], [57], [69], [70]. In brief, SAW agitation allows the interaction of ZnO tetrapods with bacterial solution due to acoustic streaming and acoustic radiation force, as well as acousto-thermal effects.

**Integrated effects of SAW and ZnO micro and nano tetrapods**
The decrease in CFU readings was observed when the bacteria solution was subjected to ZnO tetrapods. ZnO is known to be a transition metal oxide and a semiconductor with a wide band gap of 3.37 eV [71]–[73]. When the radiation energy is greater than the bandgap of ZnO, electron-hole pairs are created where the electrons will be promoted to the conduction band (CB) [74], [75]. The hole which was generated in the valence band (VB) will possess strong oxidising character, and oxidising sites will be created that are capable of oxidising water molecules or hydroxide anions, thus generating strong oxidising species [74], [75]. This reaction can then result to the redox chain reaction with the creation of reactive oxygen species (ROS) formed via hydroxyl radical (OH⁻), hydroperoxide radical (HO₂⁻) and superoxide radical anion (O₂⁻) as pathways of bactericidal action, and this can be represented in the following equation [73]:

$$\text{ZnO (s) + H}^+ \text{ ions (gas) -> Zn}^+ \text{ ions (solid) + H}_2\text{O (gas)} \quad (6)$$

ZnO tetrapods can react with hydrogen ions (which can be from the DI water of the LB broth) to produce molecules of H₂O₂ and the generated H₂O₂ can penetrate the cell membrane and thus killing the bacteria. The generation of H₂O₂ is strongly dependant on the surface area of the ZnO tetrapods, which lead to more oxygen species. Hence, the generation of reactive oxygen species (ROS) could be one major factor that caused cell wall damage due to localised ZnO interactions, enhanced membrane permeability, internalization of nanoparticles due to the loss of proton motive force and uptake of toxic dissolved zinc ions [73], [74].

The other possibility could be that ZnO particles kill the bacterial cells via inducing oxidative stress where the increase in oxidative substances produced in the internal and external environment of cells can cause damage to intracellular biomolecules [74]. In other words, oxidative stress can be induced by the ROS generation produced from ZnO tetrapods, which then result to the inhibition of protein synthesis and DNA replication. This electronic excitation can in turn destabilise the charges present in the cytoplasmic membrane of the bacterial cell, causing rupture. Therefore, the ZnO can damage the cytoplasmic membrane by releasing Zn⁺ ions from the dissolution of ZnO in aqueous solution where the Zn⁺ ions are toxic to microbial cells and can damage important biological molecules such as cell membranes, proteins, and nucleic acids, leading to microbial death [73], [74].
4. Conclusions

Both active and passive methods were utilized to study the mechanisms and interactions of SAW with bacteria solution and ZnO tetrapods. This was achieved through the concept of incorporating varied concentrations of ZnO tetrapods with various RF powers. Our results revealed that with increasing ZnO concentrations and RF powers resulted in the inactivation of bacteria, which was characterized by a low CFU/mL reading. The decrease in CFU/mL reading is mainly due to the synergistic effects of acoustic streaming, acoustic radiation force from SAWs and the generation of ROS species and oxidative stress induced by ZnO tetrapods upon SAW agitations at various RF powers.

Data availability statements

No primary research results, software, or code have been included and no new data were generated or analysed as part of this review. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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