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Understanding the Interaction of *Escherichia coli* with ZnO Tetrapods at Microwave Frequencies

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Abstract

This work deals with the effect of ZnO tetrapodal nano-micro structures on the permittivity of *Escherichia coli (E. coli)* using transmission method at microwave frequencies. Based on method, the permittivity of *E. coli* with and without tetrapodal ZnO particles was calculated. During these studies, it has been found that ZnO tetrapods have the ability to disturb the electrical properties of this bacterial strain. Comparative analyses through conventional microbiological agar plating analysis showed decreased colony formation of *E. coli* in the presence of ZnO tetrapods in a broth medium. These experimental results demonstrate the ability of ZnO tetrapodal structures to alter the electrical properties of *E. coli*.

Keywords—Transmission method, Zinc oxide, Agar plating method

1. Introduction

The microwave frequency spectrum of 300 MHz to 10 GHz received more attention in the literature due to its better absorption characteristics within biological material. The interaction of electromagnetic waves with biological material depends upon the tissue boundaries, body size and their dielectric properties [1]. The published studies on interaction between microwaves and bacteria showed that microwave produce major effect on bacteria, which vary from killing of bacteria to enhance the growth of bacteria [2]. For environmental engineers and physicists, bacteria are important for a number of reasons. Pathogenic bacteria are one of the major concern in food and water treatment facilities because of their rapid growth and pathogenic effects while non-pathogenic bacteria are one of the major concern in industries for making food products and medicines [3]. It has been expected that 37.7 million Indians are affected by water-borne diseases and 1.5 million children die due to diarrhoea alone [4]. Characterization of bacteria and development of its precise and rapid detection technique has been an important topic of research. Although, there are many detection methods such as optical, nucleic acid detection, molecular whole cell recognition, enzyme substrate method, electro chemical method etc for the identification and detection of bacteria [5]. But in order to minimize the cost and detection time researchers have developed many microwave techniques for detection of pathogenic bacteria [6-8]. These microwave techniques are inexpensive and more easy hand approach than other techniques. The ZnO tetrapods used in this research are synthesised at Kiel university, Germany, by flame-transport-synthesis (FTS) method and their application in the area of treating herpes simplex virus type-2 infections and fast ZnO based ultra-sensitive and selective hydrogen nanosensors are reported [9,10]. The feature and unique properties of ZnO nano-micro particles are not limited with bio engineering it also offers wide applications in the area of agriculture field and as well as electronics such as UV light emitters, piezo electric devices, chemical sensors and spintronics [11,12]. Zn is one of the most essential micro elements for humans to study due to its vital role in human body such as growth and division of cells, ceratogenesis, osteogenesis, immune response and also included functioning of pancreases [13]. Binding of biological cells and/or proteins to the nanoparticles helps to deepen the insight into nanoparticle mediated biological effects. These findings can help in modifying the behaviour and structure of adsorbed biological cells [13]. Recently Wahab in 2010, Pugazhendhi in 2018 and Abdel-Raouf in 2017 found that interaction of nanoparticles with bacteria leads to its antimicrobial properties of nanoparticle [14-16]. These findings show that nanomaterials assisted microwave exposure can lead to selective elimination of pathogens. In order to understand the effect of external alternating electric field on the bacteria, Kunal Samantaray in 2017 worked on the rod-shaped pathogen *(Salmonella Typhimurium*) suspension using confocal microscopy [17] and found that in case of low electric field strength individual bacteria orients itself to align in the direction of electric field and at intermediate electric field bacteria align in such a way to form 1D chain in the direction of applied electric field. On increasing the field strength these 1D chain assembled to form 2D array. This effect is related to the quorum sensing of bacteria which affects the cell location and density of bacteria. This can be understood using the diffusion charge around the cell wall of bacteria and polarization effect on bacteria. One of the interesting areas of research is quorum sensing of bacteria which

allows bacteria to detect and resolve their social and physical environment. It also involves self-produced extracellular chemical signals, which can accumulate in a local environment and are capable to activate transcription of specific genes. The absorption of electromagnetic fields by the microbes depends upon the dielectric properties and chemical composition of microorganism [18]. A.S Anoux, Harris, Christine M. et al. presented permittivity measurement in the microwave range for understanding the lactic acid fermentation in bacteria. Permittivity can be a powerful physical quantity to detect metabolic events such as fermentation in microorganism [19,20]. In 2016, Aida Brahimi reported that when the droplet of bacterial sample is poured between two electrode channels (isolated from the electrical source) so there is difference in conductivity of live and dead bacteria droplet [21]. In our results, *E. coli* solution form electric ionic connectivity so permittivity is high. In presence of Zn the electrical ionic connectivity of *E. coli* solution get disturb and their permittivity goes down .The aim of the research work is not only to understand the high frequency characterization of *E. coli* bacteria but also the interaction of bacteria with ZnO nano-micro particles in order to understand its antimicrobial nature

2. Experimental Procedure

2.1 Preparation of bacterial strains

E. coli strain is cultured in tryptone soy broth (TSB) medium for 24 hours. The concentration of bacterial culture was estimated by standard plating technique by using plate count agar (PCA), to be 211 CFU in 10^{-11} dilution shown in Fig. 7. The serial dilutions for standard plating were made by using saline solution (0.85% NaCl). The serial dilution of 10^{-1} was done by transferring 1ml of *E. coli* into autoclaved broth and then 1 ml of 10^{-1} dilution was transferred to 9 ml of normal saline for getting 10^{-2} serial dilution and the procedure was repeated until we got a dilution of 10^{-12} shown in Fig.6. Thus, the serial dilution for *E. coli* was prepared and in parallel to understand the interaction of *E. coli* with ZnO so further serial dilution 10^{-1} was done by transferring 1 ml of *E. coli* and 5ml of ZnO tetrapods (concentration 15.5mg/5ml) in 100 ml of autoclaved broth. Then the serial dilution of *E. coli* and *E. coli* + broth is used for microwave characterization

2.2 Transmission method to measure the permittivity of E.coli bacteria

Characterization of *E. coli* bacteria performed using transmission method in the microwave range of 6.5 GHz to 12.5 GHz. Handling and culturing of bacterial sample is done very carefully. For making the results accurate, identical disposable sample holders are used for holding the bacterial sample in the transmission device shown in -Fig. 1. Procedure of calculating permittivity of bacteria is given in section III and Fig. 2. The microwave and agar plating studies were done in two days continuation. In first day, the serial dilution of *E. coli* up to 10^{-12} was performed. The second day to gain information on the interactions of ZnO tetrapods with bacteria, the serial dilutions on *E. coli* +Broth+ ZnO up to 10^{-12} was realized.

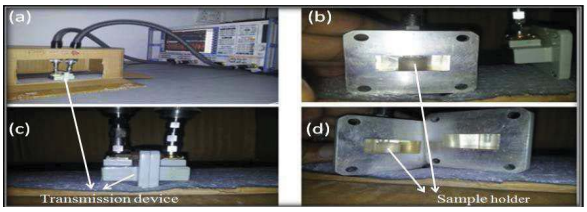

Figure1 (a) Transmission device connected with VNA (b) Sample holder inside the transmission device (c) Close view of transmission device (d) View of sample holder while opening the transmission device

3. Dielctric Measurements of Bacteria using Transmission Method

3.2 Theoretical model and equations

Consider a sample of thickness d is placed inside a transmission device of width 'a' and height 'b'. The separation of front and back face of material from the transmission device is d_1 and d_2

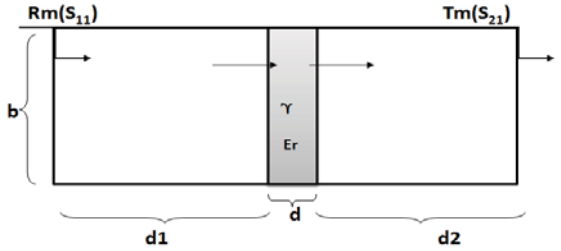

Figure 2 Theoretical modeling of the Transmission device

$$S_{11} = \frac{R(1-T^2)}{1-R^2T^2} e^{-2jd_1\gamma_o} \quad (1)$$

$$S_{21} = \frac{T(1-R^2)}{1-R^2T^2} e^{-2j(d_1+d_2)\gamma_o} \quad (2)$$

$$T = \frac{S_{11}+S_{21}-R}{1-R(S_{11}+S_{21})} \quad (3)$$

$$K = S_{11}^2 + S_{21}^2 + 1 \quad (4)$$

$$\gamma_o = \left(\frac{\omega}{c}\right)^2 - \left(\frac{\pi}{a}\right)^2 \quad (5)$$

$$\gamma = \alpha + j\beta = \frac{2jk\pi - lnT}{d} \quad (6)$$

$$\epsilon_s' = \frac{\beta^2 - \left(\frac{\pi}{a}\right)^2}{\omega^2 \mu_o \varepsilon_o} \quad (7)$$

Where S11 and S21 are reflection and transmission losses, R and T reflection and transmission coefficient. γ_o and γ is complex propagation coefficient in free space and sample. Ω is angular frequency and ε_s' is permittivity of sample.

Firstly, we have connected the transmission device of width a=23mm, height b= 10mm and total length d_1+d_2=40mm to vector network analyzer shown in Figure 1 and then fetch the magnitude and phase of S_{11}, S_{21} parameter in dB and degree units one by one for all media as air, sample holder and sample etc.. To achieve the propagation coefficient (γ) for all media we have inserted the S data into MATLAB program implemented by equation 1-9 [22-23]. Complex

propagation coefficient (γ) for air, sample holder, water and sample is calculated from the already implemented MATLAB program using by equation 1 to 7. Using the imaginary part of propagation coefficient (β), we have calculated the permittivity (ε_s) for all media. In our case sample is in liquid form, therefore we used plastic containers with internal radius 4mm, height 5mm and plastic thickness of 1mm. In our case d is 6mm.

For transmission device reflection (S11) and transmission (S21) losses by conductors, dielectric fills and radiation can contribute to unloaded loss. These losses can be individually accounted for by defining conductor loss (CL), dielectric loss (DL) and radiation loss (RL). So the unloaded loss present in the device itself is sum of these components.

$$UL=CL+DL+RL \quad (8)$$

In our case we have inserted sample holder in the device so DL will be contributed by sample holder. The loss due to the presence of sample loading result sample loss (SL) in a system. So the total loss due to the presence of sample in a system is defined by eq.9.

$$TL=UL+SL \quad (9)$$

So by eq.9 we can find the exact loss due to the sample in a system. This transmission analysis is used for achieving the exact response of sample by considering the additive nature of losses.

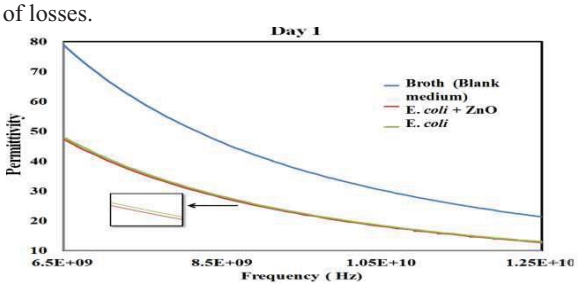

Figure 1Dielectric constant of solution in day1

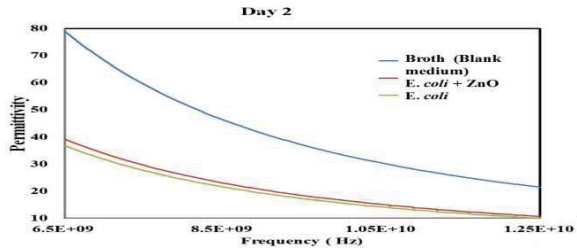

Figure 2Dielectric constant of solution in day2

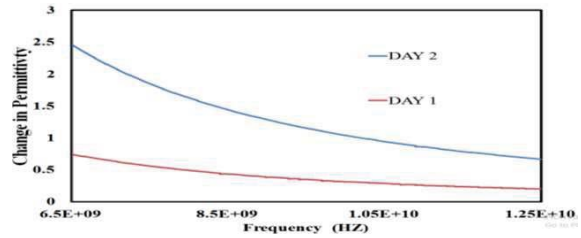

Figure 3Measured changes in permittivity of E. coli + ZnO dispersion with respect to E. coli solution.

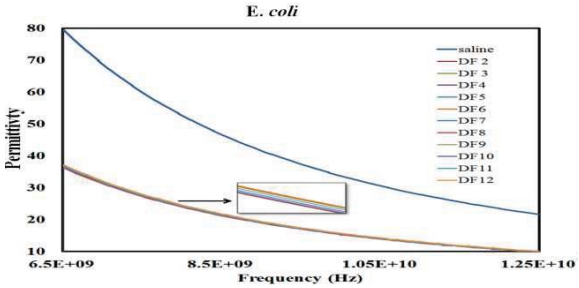

Figure 4Permittivity of sSaline and different dilution factors (DF) of E. coli sSolution.

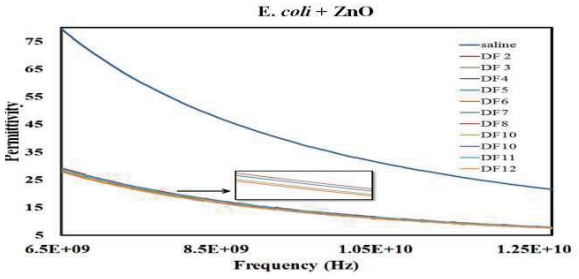

Figure 5 Permittivity of Saline and different dilution factors (DF) of E. *coli* + ZnO dispersion.

Figure 6 Colonies forming of E. coli in different dilutions (a) 10^{-7} (b) 10^{-8} (c) 10^{-9} (d) 10^{-10} after 24 hrs iIncubation process

Figure 7Dilution Factor 10^{-11} is used for plate counting

Figure 8colonies is affected by presence of ZnO after 48 hours of incubation

4. Results and Discussion

Figure 3(a&b) show that the dielectric permittivity of Broth is more than *E. coli* and *E. coli* + ZnO dispersion and in day 1 there is no measurable change between *E. coli* and *E. coli*+ Zn (can be contributed because we had just mixed the

ZnO in *E.coli* solution). After incubating for 24 hours these two solutions and the next day (day 2) we have observed that there is a measurable change in their dielectric properties (Fig. 3b). Fig. 4 is used for clearly distinguish the measurable change in *E. coli* solution with respect to *E. coli* + ZnO in day 1 and day 2 and it suggests that the presence of ZnO is changing the dielectric properties of *E. coli* of lower dilutions also. From the Fig. 5 the rate of changing dielectric properties of *E. coli* + Zn solution was more in day 2 compare to day 1. We had also tried to characterize the *E. coli* and *E. coli* + Zn for lower dilution (10^{-2} to 10^{-11}) shown in Fig. 5(a&b). one can differentiate saline and dilutions easily using the dielectric properties. Due to the limitations and accuracy in the transmission method we cannot easily differentiate all dilutions. So, we have presented zoomed portion in plotted figure 5 and it showed that permittivity of the dilutions are little changing. The dielectric constant of *E. coli* solution is more compared to *E. coli* + ZnO solution shown in Fig.5. In 2016 Aida E. brahimi et. al performed experiment for electrical conductivity analysis of bacteria induced by stimulating concentration difference across cell membrane due to evaporation, through which they could successfully differentiate dead and live cells [21]. The same concept reflects in our results that *E. coli* solution form electric ionic connectivity so permittivity of *E. coli* solution is high in comparison to *E. coli*+ZnO dispersion. In presence of zinc oxide the electrical ionic connectivity of *E. coli* solution gets disturb and their dielectric constant goes down as well as during agar plating analysis the colonies *E. coli* in *E. coli*+ZnO dispersion was low in compare in *E. coli* solution shown in fig. 8. The studies carried out explore ZnO tetrapodal structures as anti microbial agent.

5. Conclusion

This study experimentally studies the interactions of ZnO tetrapodal structure with *E. coli* and the interactions are studied by measuring the change in dielectric permittivity. It is seen that with time the permittivity of the medium decreases which indicates suppression of signaling pathways for population growth.

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