



## Experimental Investigation on the Effects of Audible Sound to the Growth of *Escherichia coli*

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### Abstract

In this paper, we report an experimental result regarding the effects of audible sound on the growth of *Escherichia coli* (*E. coli*). Standardized *E. coli* suspensions of fixed concentration were used for inoculation throughout the experiment in nutrient agar (NA) and nutrient broth (NB). First, the samples were incubated at 37°C for three hours in a water bath-shaker for NB and in a conventional oven for NA. The samples were then transferred to an acoustic chamber JedMark LV-1 with given sound treatment at controlled temperature of 24±2°C for five hours for NB and 16 hours for NA. Three different tonal frequencies were selected for sound treatment in this experiment which is 1 kHz, 5 kHz and 15 kHz. The growth of *E. coli* was assessed by their cell number through indirect viable cell counts (*E. coli* on NA) and direct viable cell counts (*E. coli* on NB), after the incubation with sound in the acoustic chamber. We found that all selected frequencies were able to promote the growth of *E. coli*. In particular, the tonal sound of 5 kHz gave significant increase in cell number of *E. coli* for both growth media.

**Keywords:** *Escherichia coli*, Audible sound wave, Plate count, Direct microscopic count

### 1. Introduction

Mechanical waves have been shown to have effect on microbes. Ultrasound for example, has been used for sterilizing and killing unwanted bacteria due to thinning of cell membranes, localized heating and production of free radicals (Piyasena et al., 2003). Ultrasound is able to inactivate bacteria and deagglomerate bacterial clusters or flocks through a number of physical, mechanical and chemical effects arising from acoustic cavitations (Joyce et al., 2003). However, certain frequency of ultrasound was found to increase the growth rate of bacteria cells such as *E. coli*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* cells adhered to and grew on a polyethylene surface (Pitt and Ross, 2003). Ultrasound technology is relatively expensive to be used for large scale microbiological decontamination or production. The consideration of the amount of energy inputs and its cost must be first carried out if this technology is put to be used (Hao et al., 2004). A more affordable alternative way using audible sound wave may sound practical, and there have been some evidences for this possibility. Matsushashi *et al.* (1998) for example, had found that audible sound and ultrasound waves between 6 kHz to 40 kHz can induce colony formation of *Bacillus carbophilus* grown on non-permissive media. In this paper, the effect of audible sound of certain frequencies to *E. coli* is investigated as a compliment to the application of ultrasound. The effect is assessed based on the comparison in their cell number, through direct and indirect viable cell counts, with their respective control samples.

## 2. Materials and methods

### 2.1 *Esherichia coli*

The investigation on the effects of audible sound to *E. coli* was carried out in two modes - on the *E. coli* that was inoculated on NA and on the *E. coli* that was inoculated on NB medium. The *E. coli* on NA was used for indirect viable cell count using plate counts whereas the *E. coli* on NB was used for direct viable cell counts using a haemocytometer. *E. coli* was obtained from previous stock cultured at Microbiology Lab, Universiti Malaysia Sabah and maintained on NA plate (medium) at 37°C for 24 hours in an oven. The medium was prepared by suspending 10g of NA in 500ml distilled water and was autoclaved to sterilize at 121°C for two hours. This is the secondary cultured *E. coli* to be used to prepare samples in the experiment.

### 2.2 Inoculation

Eight NB and six NA media were prepared in this experiment. The NB medium was prepared by suspending 4g of NB in 500ml distilled water and autoclaved to sterilize at 121°C for two hours whereas NA medium was prepared in the same way described previously. Single colony of *E. coli* from the secondary culture was transferred to each NB medium which was prepared in screwed-capped bottles. The remainder of the secondary cultured *E. coli* was diluted ten-folds and then divided into eight for inoculation on NA which was prepared on petri dishes.

### 2.3 Incubation of the samples

The NB samples were incubated in water bath-shaker at 100 rotations per minute at 37°C for 3 hours and the NA samples with *E. coli* were incubated in conventional oven also at 37°C for 3 hours. After the incubation, the samples were then transferred to an acoustic chamber JedMark LV-1 (Figure 1) for sound treatment through the opening. The chamber which is made of thick glass, is air tight when in operation and equipped with three ultraviolet lamps for fully sterilizing the inner part of the chamber before the treatment carried out. The chamber was also installed with a loudspeaker connected to a function generator so that tonal frequency of sound can be generated. The samples were exposed to sound for five hours at frequency of 1 kHz, 5 kHz and 15 kHz for sound treatment. For each selected frequency, two NA and NB samples were exposed simultaneously whereas two control samples was maintained without sound treatment as reference as reference. During the treatment process, the temperature was maintained at 24±2°C.

### 2.4 Measurements

Soon after the exposure, direct *E. coli* cell counts was performed on NB samples using haemocytometer whereas indirect cell counts was carried out on NA samples using plates counts technique. The same *E. coli* cell counting method was performed on the respective control medium type. In the direct cell counts, trypan blue (0.4% w/v) was used to stain dead cells (stained dark blue and immobile). In indirect cell counts, the number of viable colonies growth on NA plates was counted with the assumption that the number of *E. coli* cells is proportional to the number of colonies formed on the media which is measured in colony forming unit per milliliter, CFU.

## 3. Results and discussion

### 3.1 Direct viable cell count

As previously mentioned, direct viable cell counts method was used to determine the number of viable *E. coli* cell on the NB samples using a hemocytometer. Throughout the experiment, less than one percent of dead cells were found in each NB samples. It was found that sound treatment at all selected frequencies have increased the number of viable cells compared to the control samples. Sound treatment at 1 kHz for example was found to increase the average number of viable cells to  $3.97 \times 10^8$  cells per ml,  $4.98 \times 10^8$  cells per ml for the sound at 5 kHz and  $4.83 \times 10^8$  cells per ml for the sound at 15 kHz. These increases are equivalent to 7%, 34% and 30.5% for the sound treatment at 1 kHz, 5 kHz and 15 kHz, respectively, compared to the average number of viable cells found on the control sample which is only  $3.70 \times 10^8$  cells per ml. The cell number for this direct viable cell counts is given in Figure 2. From the observations, sound treatment at 5 kHz found to give the most significant increases in the number of viable cells followed by the sound at 15 kHz and 1 kHz.

### 3.2 Indirect viable cell count

Indirect viable cell counts (or plate counts) was performed to determine the viable *E. coli* cells on NB samples using ordinary microscope. The assumption is each *E. coli* cell give rise to one colony, and therefore the indirect viable cell count is measured in colony forming unit per milliliter, CFU. As in the direct viable cell count, it was found that sound treatment at all selected frequencies also have increased the CFU of *E. coli* (and so the number of *E. coli* cells). Sound treatment at 1 kHz for example increased the average CFU to  $1.75 \times 10^9$  compared to only  $1.69 \times 10^9$  CFU for the control sample. On the other hand, sound treatment at 5 kHz and 15 kHz gave rise to uncountable colony. However, visual inspection shows that sound treatment at 5 kHz gave higher value of CFU compared to frequency 15 kHz. The resultant increased for the sound treatment at 1 kHz is 3.5% compared to the control samples. The mean of *E. coli* CFU is shown in Table 1 whereas Figure 3 represents the visual inspection of the samples. In Table 1 and Figure 3, the mean

number of CFU for 5 kHz and 15 kHz sound treatments were simply given so that they can be distinguished.

#### 4. Conclusion

In this paper, we presented the result of an experimental investigation on the effects of audible sound to the growth of *E. coli* inoculated on NA and NB media. We found that all selected frequencies (1 kHz, 5 kHz and 15 kHz) have increased the number of viable cells of the bacteria. This shows that the bacteria react positively to the given sound treatment which results in the growth of the number of the bacteria cells. However, the degree of their respond is different to a different sound frequency. This is consistence with the finding reported by other researchers. In this experiment, we found that *E. coli* on NA and NB responded more to sound at frequency 5 kHz compared to other frequencies. This shows that *E. coli* has selective frequency response towards sound treatment.

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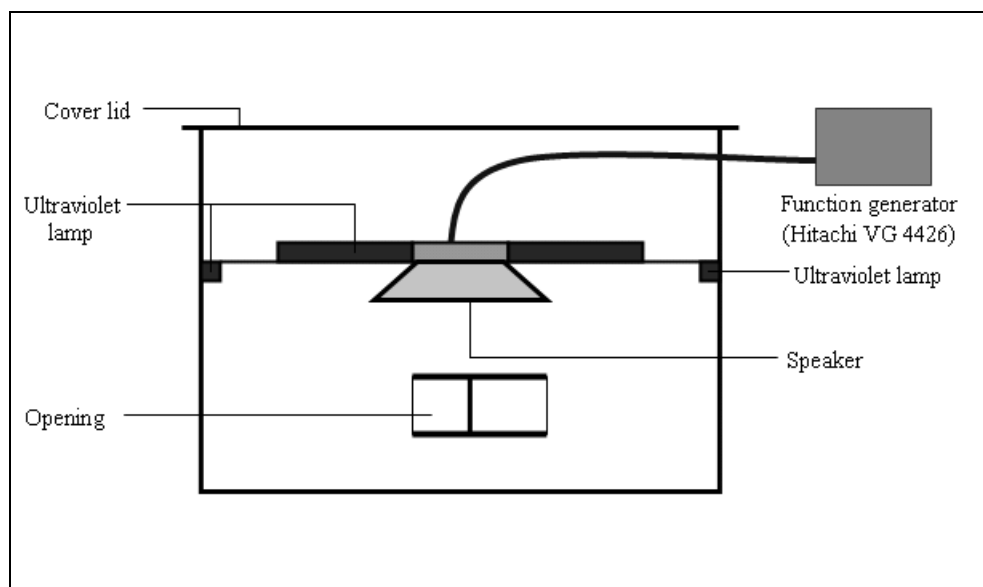


Figure1. Schematic diagram of the JedMark LV-1 acoustic chamber

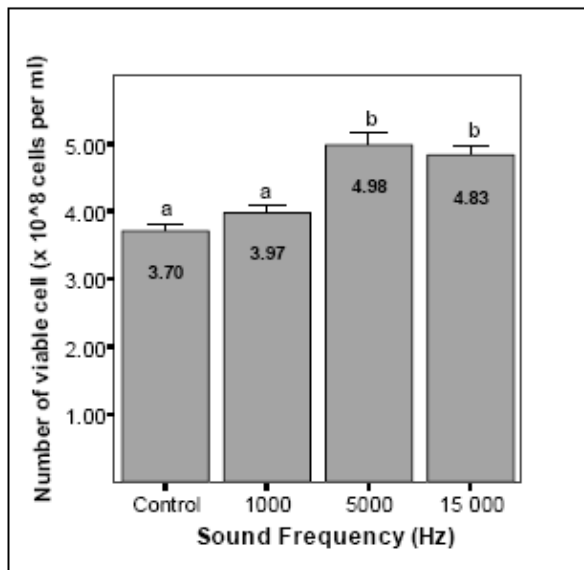


Figure 2. Viable *E. coli* cell count (x10<sup>8</sup> cells per ml) for different sound treatments

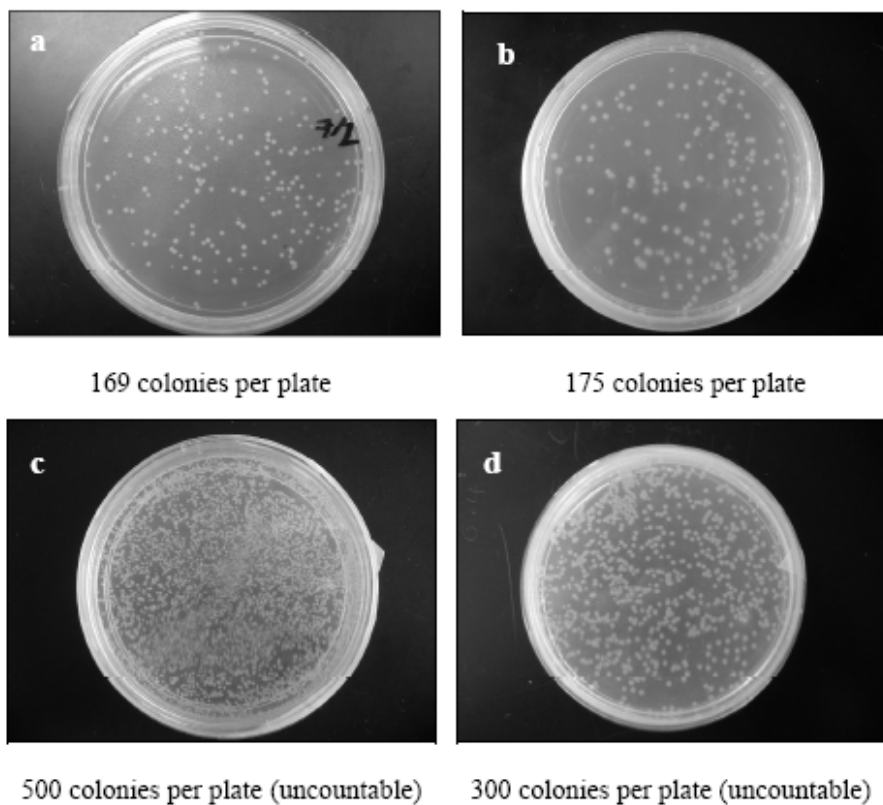


Figure 3. Number of colony per plate for the different sound treatments.  
 (a) Control, (b) 1kHz, (c) 5 kHz and (d) 15kHz