THE EFFECT OF AUDIBLE SOUND WAVES ON THE GROWTH OF *Escherichia coli*

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ABSTRACT

The rationale of this study is to find a cheaper alternative other than ultrasound in order to control the growth of microbes. This could be beneficial to the practical microbiological technology development. In this study, sound treatments of 1000 Hz, 5000 Hz, 15 000 Hz and control (without sound) were used to assess the effect of audible sound waves on the growth of *Escherichia coli*. A standardized *E. coli* suspension of fixed concentration was used for inoculation throughout the experiment in nutrient agar and nutrient broth. The first incubation of the samples was done at 37°C for three hours. For the second incubation, the samples were incubated at room temperature (24 ± 2 °C) in an acoustic chamber, JedMark LV-1 at various sound treatments; five hours for nutrient broth and 16 hours for nutrient plates. The parameters in this study were cell mass (turbidity measurement) and cell numbers (direct cell count and plate count). Results showed that sound treatment of 5000 Hz was able to give a significant increase of 25.2 percent direct viable cell count of *E. coli* and also plate counts which nearly double the numbers of colonies formed compared to the 1000 Hz sound treatment. The sound treatment of 15 000 Hz indicated a significant increase of 16.7 percent of cell mass of *E. coli* compared to 5000 Hz. In conclusion, the results of this study revealed that audible sound waves do have effects on the growth of *E. coli*.

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KESAN BUNYI AUDIBEL TERHADAP PERTUMBUHAN *Escherichia coli*

ABSTRAK

Kepentingan kajian ini adalah untuk mendapatkan alternatif yang murah dalam kawalan pertumbuhan mikrob selain daripada rawatan ultrasonik. Kajian ini mungkin bermanfaat dalam perkembangan teknologi mikrobiologi. Dalam kajian ini, rawatan bunyi yang terdiri daripada 1000 Hz, 5000 Hz, 15 000 Hz dan kawalan (tanpa bunyi) telah digunakan untuk menilai kesan bunyi audibel terhadap pertumbuhan *Escherichia coli*. Konsentrasi sel *E. coli* yang diketahui digunakan sepanjang eksperimen ini bagi tujuan inokulasi pada nutrien agar dan larutan nutrien. Inkubasi peringkat pertama dilakukan bagi sampel-sampel pada suhu 37 °C selama tiga jam. Inkubasi peringkat kedua bagi sampel tersebut kemudiannya diteruskan di dalam sebuah kebuk akustik, JedMark LV-1 dengan rawatan bunyi pada suhu bilik (24 ± 2 °C); 5 jam untuk larutan nutrien dan 16 jam untuk nutrien agar. Parameter yang telah digunakan untuk mengukur pertumbuhan *E. coli* adalah pengiraan turbiditi sel dan bilangan sel hidup *E. coli* (penghitungan secara langsung dan tidak langsung). Keputusan eksperimen ini menunjukkan bahawa rawatan bunyi pada 5000 Hz mampu meningkatkan bilangan sel hidup *E. coli* sebanyak 25.2 peratus dan bilangan koloni pada nutrien agar sebanyak dua kali ganda apabila dibandingkan dengan rawatan bunyi 1000 Hz. Rawatan bunyi pada 15 000 Hz pula didapati mampu meningkatkan bacaan turbiditi sel *E. coli* secara signifikan sebanyak 16.7 peratus jika dibandingkan dengan rawatan bunyi 5000 Hz. Kesimpulannya, kajian ini menunjukkan bahawa bunyi audibel memang mempunyai kesan terhadap pertumbuhan *E. coli*.

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LIST OF SYMBOLS

$\pm$	plus or minus
$>$	more than
$<$	less than
$=$	equal to
F	force
W	work
d	distance
p	pressure
I	intensity
v	velocity
λ	lambda, wavelength in meter
f	frequency
T	time period in a second
P	power
w/v	weight per volume
r	distance

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LIST OF UNITS

Hz	Hertz
μ	micro
μm	micrometer
mm	millimeter
ms^{-1}	meter per second
N	Newton
°C	degree Celsius
W	watt
Wm^{-2}	watt per meter square
J	joule
ml	milliliter
μl	microliter
cfu	colony forming unit
g	gram

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CHAPTER 1

INTRODUCTION

1.1 Preface

Sound is a form of energy which is associated with the sense of hearing and with the physiology of ears and the psychology of the brain. Humans are able to interpret the sensations that reach their ears (Giancoli, 2000). Sound is also known as the physical phenomenon as 'a time varying disturbance of the density of a fluid medium, which is associated with very small vibration movements of the fluid particles' (Fahy, 1989).

Sounds are longitudinal waves that consist of many different frequencies. Sound waves are able to travel in solid, gas and liquid medium (Giancoli, 2000). Like most waves, such as light waves and radio waves, all waves possess certain common properties. All waves are able to carry information and transport energy from one point to another. As the sound wave travels through the air, the longitudinal movement of the sound will bring slight changes to the air pressure and this slight change in pressure allow our ears to hear (Rossing *et al.*, 2002). Humans are able to hear within the range of 20 to 20 000 Hertz (Hz).

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Sound is important because it enable humans to communicate with each other through speech or music. For animals such as bats, sound is used to acquire information about properties of the surrounding environment. This is called echolocation which enable them to move in the dark and to catch prey (Hickman *et al.*, 2006). Besides communication, sounds also have biological effects on most living things, for example, sound in the audible range of 200 Hz to 400 Hz were found to increase the germination rate of paddy rice seed (Wang *et al.,* 2003). Whereas for people, unwanted sound which is often referred to as noise is found to affect human mental health and hearing loss due to long noise exposure (Rossing *et al.,* 2002).

For bacteria, the application of ultrasound technology is often used for sterilizing and killing unwanted microbes. Ultrasounds are sound waves with frequencies over 20 kHz which is over the threshold of human hearing (Pitt & Ross, 2003). Ultrasound is able to inactivate bacteria and deaggolomorate bacterial clusters or flocs through a number of physical, mechanical and chemical effects arising from acoustic cavitations (Joyce *et al.,* 2003). According to Joyce *et al.* (2003), sonification using high ultrasonic intensities was able to achieve 100 percent death rate on cultured *Bacillus* species. The application of ultrasound technology also proved to be successful in preventing cyanobacterial bloom (Hao *et al.,* 2004). Besides decreasing bacteria growth, ultrasound was also found to increase the growth rate of bacteria cells attached to surfaces (Pitt & Ross, 2003). Unfortunately, ultrasound technology is far too much expensive to be used for general large scale-microbiological decontamination or production. The consideration of the amount of energy inputs and its cost must be done if ultrasonic technology is put to be used (Hao *et al.,* 2004).

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As ultrasound technology was expensive to use and the capability to create it was high, a cheaper alternative using audible sound may be practical. The earliest research of the response of bacterial cells towards sound waves in the audible range and ultrasound range had been studied by Matsuhashi *et al.* (1998). They were able to detect sound production emitted by *Bacillus subtilis* at frequencies between 8000 and 43 000 Hz by using a sensitive microphone system. Besides that, they were able to induce colony formation of *Bacillus carbophilus* grown in non-permissive media by using artificial generated sound waves at 6000 to 40 000 Hz. The generated sound waves which were able to induce colony formation on *B. carbophilus,* had similar broad peaks frequencies with the sound frequency produced by *B. subtilis* (Matsuhashi *et al.,* 1998).

Researches on the effect of audible sound wave towards the growth of bacteria have been little studied. So, in this study, audible sound with frequencies ranging from 20 to 20 000 Hz were used as sound treatment to study their effect on the growth of *Escherichia coli* (*E. coli*).

1.2 Research objectives

The objectives of this research were to determine the effect of audible sound waves on bacteria growth and to determine the frequency of the audible range that promotes or decreases the growth rate of *E. coli.*

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CHAPTER 2

LITERATURE REVIEW

2.1 Sound

2.1.1 Types of sound waves

There are three types of sound waves with different frequency ranges. Infrasonic sounds have frequencies below 20 Hz which are lower than the audible range and could not be heard by humans. Infrasound is produced by natural sources such as earthquake and ocean waves (Faughn *et al.,* 2006). Ultrasonic waves have frequencies above the audible range which is more than 20 000 Hz. Ultrasound waves are emitted by cleaning devices, jet engines and high speed drills. Ultrasound does not travel very far from its source since they are absorbed very strongly by air (Rossing *et al.*, 2002). Humans are only able to hear within the range of 20 to 20 000 Hz which is called the audible range (Giancoli, 2000).

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2.1.2 The properties of sound

Sound, like light and electromagnetic radiation, travels in the form of waves (Faughn *et al.,* 2006). Sound waves in the air are longitudinal waves whereas light waves and radio waves are transverse waves. Longitudinal waves are waves in which the individual particles of the medium are motioned in a direction that is parallel to the direction of energy transport (Roederer, 1973). All waves have common properties regardless of their nature. They all have energy and their waves can be reflected, refracted, diffracted or adsorbed.

One of the common properties of waves is that they transmit energy and information through a medium but the medium itself is not moving with the wave (Rossing *et al.*, 2002). A disturbance, or change in the physical quantity, is passed on from one point to the next as the waves propagate and the medium is left undisturbed. Only the amount of energy transmitted through the medium and the mechanism involved are different. For example, ocean waves carry greater energy than the energy carried by a sound wave generated by a single human voice (Faughn *et al.*, 2006).

2.1.3 The propagation of sound

Sound travels through the particles in the medium of transport such as air, liquid or solid matter. Sound cannot travel in the absence of matter such as vacuum. When sound propagates through a medium, the particles or atoms in the medium vibrate. Sound waves are elastic waves which will cause the particles in the medium to start to push or pull through elastics forces onto other neighbouring particles. This will caused

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the particles to vibrate as the elastic forces of the sound waves propagate away from the initial region of the sound source. The propagation of this wave is caused by energy which is needed to put motion in each particle in order to pass information from one point to another (Roederer, 1973).

Sound waves are produced by vibrating source such as the vibrating strings of a piano or by the vibrating vocal cords of a singer. The vibrations cause the displacement of the air molecules around them. A sound producing source such as a speaker creates sound waves by the vibration of its diaphragm. When the diaphragm of the speaker moves outward, the air molecules near the speaker are forced closely together than normal creating high air pressure and high molecular density called compression. This compression will propagate away from the speaker. When the speaker's diaphragm moves inward, a low pressure and low molecular density is created which also propagates away from the source. This region is called rarefaction. As the source continues to vibrate, a succession of compression and rarefactions forms and spread out of it (Faughn *et al.,* 2006). The propagation of sound with its compressed and rarefied regions in air is shown in Figure 2.1. As the sound wave propagates away from the source, they will be detected by a human ear.

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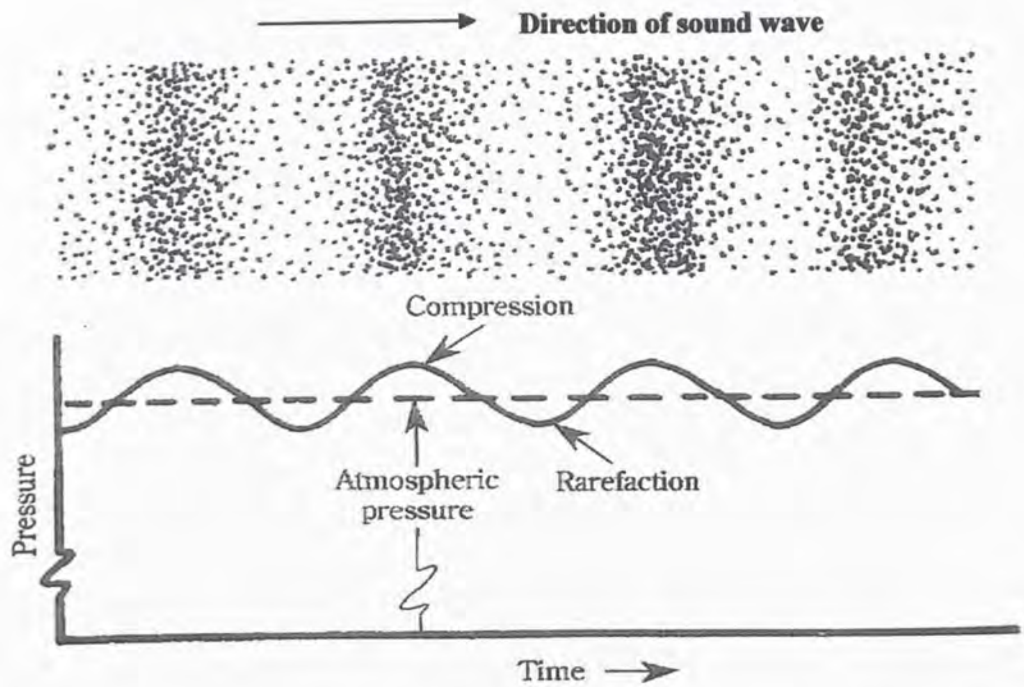

Figure 2.1 The propagation of sound wave in air. The compressed regions are very slightly above and the rarefaction regions are very slightly atmospheric pressure (Source: Everest, 1994).

2.1.4 The Speed of Sound

The speed of sound v, differs in the aspects of matter and increases with temperature. The speed of sound refers to how fast the disturbance is passed from one particle to another particle. Sound travels faster in liquids and solid than in air. The speed of a sound wave in air depends upon the properties of the air, such as the temperature and the pressure. The speed of sound wave (v) in normal atmospheric pressure is given by the following equation (2.1) where t is the temperature in degree Celsius in meter per second (Rossing *et al.*, 2004).

$$v = 331.3 \text{ ms}^{-1} + 0.6t \text{ ms}^{-1} \tag{2.1}$$

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At normal atmospheric pressure, the speed of sound at room temperature is 343 ms^{-1} (Faughn *et al.,* 2006). A sound wave will propagate away from the source in a well defined speed in a straight line until it is absorbed or reflected.

2.1.5 Frequency and Wavelength

While speed refers to the distance which the disturbance travels per unit of time, frequency refers to the number of cycles an individual particle makes per second which is measured in the unit Hertz (Everest, 1994). For example, if a particle of air undergoes 500 longitudinal vibrations in 2 seconds, then the frequency of the wave would be 250 vibrations per second. As a sound wave propagates through a medium, each particle of the medium will vibrate at the same frequency.

$$1 \text{ Hertz} = 1 \text{ vibration per second} \tag{2.2}$$

Wavelength is referred to as the distance a wave travels in the time it takes to complete one cycle. A wavelength is measured between the two successive points that behave identically on a cycle (Faughn *et al.*, 2006) as shown in Figure 2.2. Wavelength (λ), frequency (*f*) and the speed (*v*) of sound are related as shown in the following equation:

$$Wavelength, \lambda = \frac{v}{f} \tag{2.3}$$

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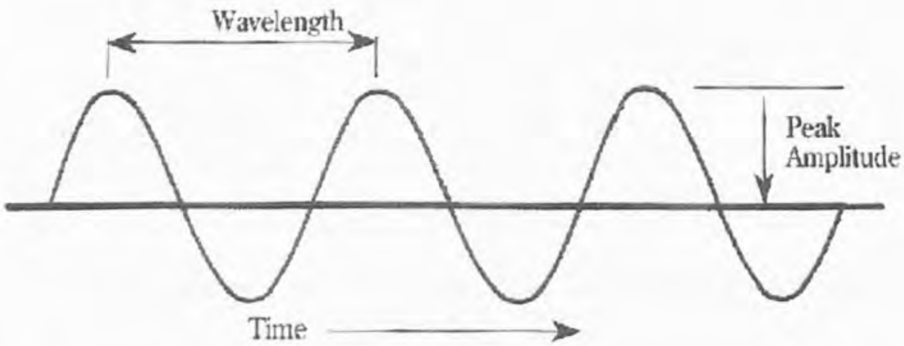

Figure 2.2 A simple sine wave. A wavelength is measured between the two successive points that behave identically on a cycle (Source: Everest, 1994).

A human ear can hear sound ranging from 20 to 20 000 Hz. The difference between sound waves with high frequency and sound waves with low frequency could be best explained graphically using a pressure-time plot (Figure 2.3). Higher frequency sound wave would have a pressure-time plot with a small period of time between successive high pressure points compared to a low frequency sound wave (Henderson, 2004).

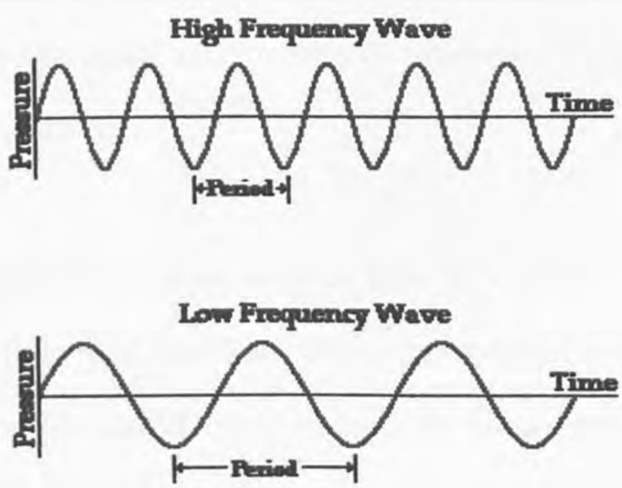

Figure 2.3 Pressure-time plots of sound wave with different frequency (Source: Henderson, 2004).

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2.1.6 Sound as a mechanical energy

Energy is present in a variety of forms, including mechanical, chemical, electromagnetic and nuclear energy (Faughn *et al.*, 2006). Sound is considered one of the many forms of energy. In physics, work is considered done when a force is applied to an object that moved through some displacement. The work (*W*) done is the product of the average force (*F*) and the distance (*d*) moved parallel to the force (Rossing *et al.*, 2002). The formal mathematical expression of this statement can be represented by:

$$W = Fd \tag{2.4}$$

Work, *W* is expressed in joules (*J*). In the study of acoustics, sound is closely related to the mechanical energy which is closely related to work (Rossing *et al.*, 2002). Mechanical energy is the sum of potential and kinetic energy of a body. By doing work, the transfer of energy is done. Sound energy is one of the vibrating systems which have mechanical energy where the mechanical energy is carried by the moving molecules in matter.

In many physical situations, a given force is applied or "spread" over an extended surface of the body (Roederer, 1973). Sound waves in air are air pressure oscillations and when the sound waves travel to the human ear, the given force or pressure will be the oscillating force, *F* acting on the eardrum.

$$F = (p - p').S \tag{2.5}$$

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