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The Role of Ultrasonic Generators as Killers Gram + and Gram – Bacteria

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A R T I C L E I N F O

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A B S T R A C T

It has created an ultrasonic generator as a destroyer of E.Coli bacteria.The range of frequency that produced by this ultrasonic generator is around 40 kHz up to 50 kHz. From the observation of death of E.Coli bacteria which is impacted by the ultrasonic wave radiation to the frequency variant used at the closed rank of 10-8 cfu/ml (coloni forming unit / ml) has obtained that the percentage of E.Coli bacteria death everage is increasing as well as the increasing frequency had been used. At the frequency of 40 kHz able to destroy E.Coli bacteria at the amount of 9,3 %, at the frequency of 42 kHz able to destroy at the amount of 14,1 %, at the frequency of 45 kHz able to destroy at the amount of 26,1 %, at the frequency of 48 kHz able to destroy at the amount 29,7 % and at the frequency of 50 kHz able to destroy at the amount of 38,8 %.By checking the result ot colouring gram of bacteria, we found that the cell of E.Coli bacteria has changed after we doing a radiation to that bacteria by using ultrasonic wave , such as ; the shape of bacteria is getting smaller, partition of the cell is cracked and lysis is occured on it.

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INTRODUCTION

Along with the development of science in the field of science, now ultrasonic waves can be applied to control the development of bacteria. The ultrasonic waves radiated on the bacteria will be able to damage the protein molecules and nucleic acids, resulting in the death of the bacteria.

Bacteria based on their cell wall structure are differentiated into Gram-positive bacteria and Gram-negative bacteria. Staphylococcus aureus is a Gram-positive bacterium member of the micrococcaceae family. These bacteria generally form a golden-yellow, rounded pigment (coclu) with a diameter of 0.5-1.25 micrometers and its cells are found in groups such as angur fruits. [7]Meanwhile, salmonella is one of the genus of Enetrobacteriaceae that is Gram-negative. These bacteria are in the form of facultative and aerogenic anaerobic rods. Salmonella can grow at an optimal temperature of 35-37oC

Problem Formulation

Based on the background that has been stated above, several problems can be formulated, namely:

1. How to design an ultrasonic generator that can be used to kill Gram-positive and Gram-negative bacteria?
2. What is the percentage of Gram-positive and Gram-negative bacterial deaths against the frequency variation used?

Research Objectives

The objectives of this study are:

1. Can design ultrasonic generators that can be used as Gram-positive and Gram-negative bacteria killers
2. It can be found out what percentage of Gram-positive and Gram-negative bacteria die against the frequency variation used.
3. It can determine the cell shape of Gram-positive and Gram-negative bacteria after being treated with ultrasonic waves.

THEORETICAL REVIEW

Transduser Ultrasonik

In electronic systems, ultrasonic waves are usually generated by a device called a transducer. These ultrasonic transducers are usually made of piezoelectric materials. Piezoelectric materials are capacitors with a certain dielectric, so that when they get an electric voltage, there will be pressure on both walls. On the other hand, when there is pressure, then an electric charge will arise on both walls. The ultrasonic transducer will work as a transmitter when the piezoelectric material is put under tension. On the other hand, ultrasonic transducers work as receivers when piezoelectric materials are under pressure.

Ultrasonic Waves

Sound frequencies that exceed 20 kHz are categorized as ultrasonic waves. In this frequency area, it is no longer able to be heard by the human ear. Some state that quiet/quiet sounds are expressed as ultrasonic frequencies, this statement cannot be used because there is no basis. The characteristics of ultrasonic waves passing through the medium cause the particles to vibrate and take place along the direction of longitudinal propagation of the waves, thus causing the particles of the medium to form tightness and strain. The continuous process that causes tightness and strain in a medium is caused by periodic vibration of particles during ultrasonic waves passing through it.

Bacteria

The name bacteria comes from the word 'bacterium' (Greek) which means stick or rod. Now the name is used to refer to a group of microorganisms that are single-celled, multiply by dividing, small in size so that they can be seen using a microscope. Based on the structure of the dindig cells, bacteria can be classified into two, Gram-positive and Gram-negative bacteria. The cell wall itself functions to give a certain shape to bacteria, to regulate the entry and exit of chemicals, and to play a role in cell division.

One example of gram+ bacteria is Staphylococus aureus. The name of this bacterium is derived from the word "Staphele" which means collection of angur, and the Latin word "aureus" which means gold. The name is given based on the shape of the bacterial cells when viewed under a microscope, and the golden color that is formed when the bacteria are grown on the surface of an Agar.

One example of gram-negative bacteria is Salmonella bacteria. which is one of the genus of Enetrobacteriaceae, rod-shaped, facultative and aerogenic anaerobic. It is usually motile in nature and has peritric flagella.

METHODOLOGY

Ultrasonic Generator Design

This circuit consists of an ultrasonic transducer that is fed a box wave with a frequency ranging from 50-65 kHz. The box waves are generated by a series of astable multivibrators using IC 555. The ultrasonic generator design is compiled based on the network scheme below.

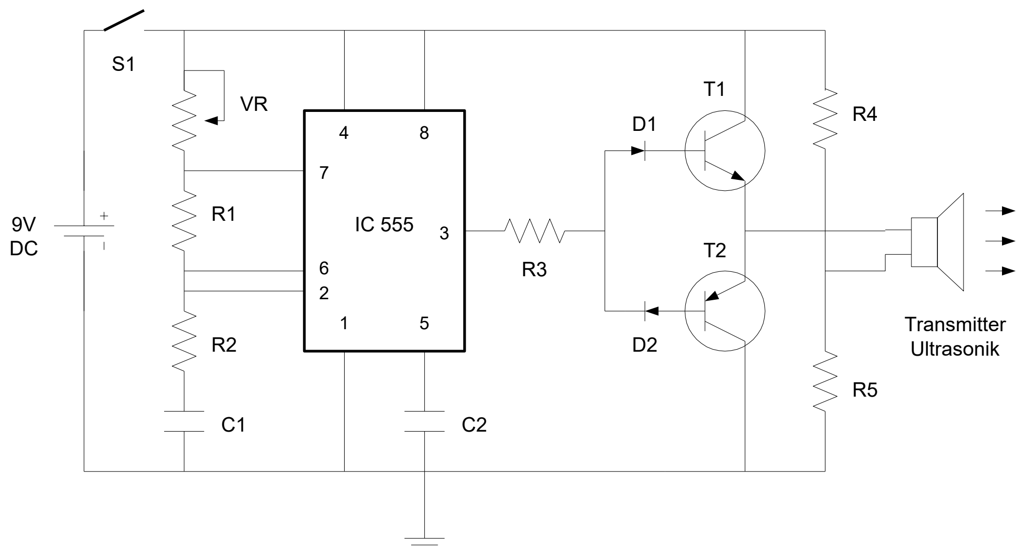

Figure 1. Ultrasonic generator design

How the Tool Works

When the power supply as a power plant is turned on, the stabilized multivibrator will work to produce a series of square waves. Since the waves produced by this astable multivibrator are still very weak, an operational amplifier is needed to produce large output waves.

Sample Treatment Preparation

Preparation steps include sterilization of tools and materials, manufacturing of media, rejuvenation of bacteria, and making suspensions of Gram-positive and Gram-negative bacteria.

Sterilization is carried out using an autoclaf at a temperature of 1210 C and a pressure of 2 atm (atmosphere) which aims to kill all microorganisms present in all materials and tools to be used in the research. This is to avoid being contaminated with other microorganisms.
The data retrieval flowchart is shown by the following figure.

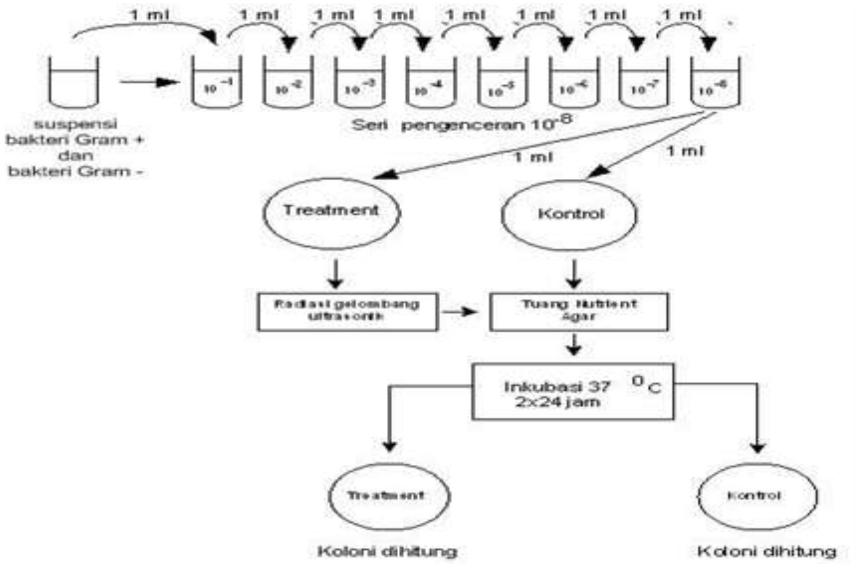

Figure 2. Data Capture Flow Diagram

Data Capture

To determine the percentage of mortality of Gram-positive and Gram-negative bacteria, Gram-positive and Gram-negative bacteria before and after irradiation were each poured with Nutrient Agar media with a temperature of ± 500 C. After solidification, it was incubated for 2x24 hours in an upside-down position. Furthermore, the number of colonies of Gram-positive and Gram-negative bacteria that grew was calculated. The percentage of Gram-positive and Gram-negative bacterial deaths is calculated using the formula:

$$P = \frac{A}{Y} x100\%$$

with:
P = Percent of Gram-positive and Gram-negative bacterial bacterial deaths

A = The difference between the number of colonies before and after radiation.
Y = Number of colonies before radiation.

Data Analysis
From the data obtained, it will be determined:

1. The average value of bacteria. The Gram-positive and Gram-negative bacteria affected for each frequency are:

$$\overline{X} = \frac{\sum X_i}{n}$$

With: $\overline{X}$ = Average value of observations.
Xi = Observation.
n = Number of samples.

2. Define the standard deviation with the formula:

$$S = \sqrt{\frac{\sum (X_i - \overline{X})^2}{n(n-1)}}$$

With: S = Standard deviation.

RESULTS AND DISCUSSION

The results of the study are shown by the graph below for various variations in distance and frequency. A graph of the relationship of the average mortality of Gram+ bacteria at a distance of 5 cm to the frequency variation used can be shown by the following figure.

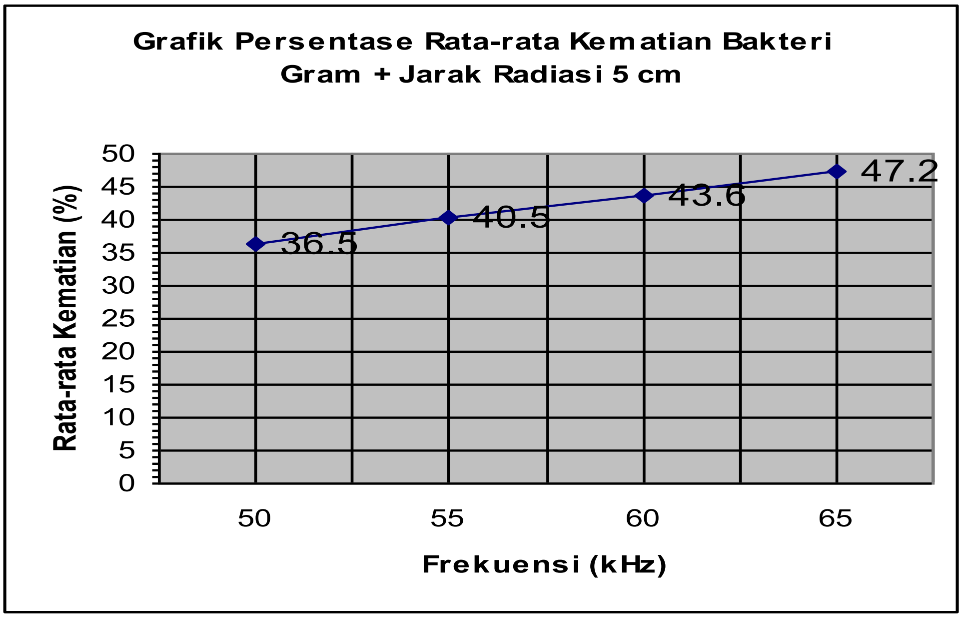

Figure 3. Graph of Average Percentage of Bacterial Mortality

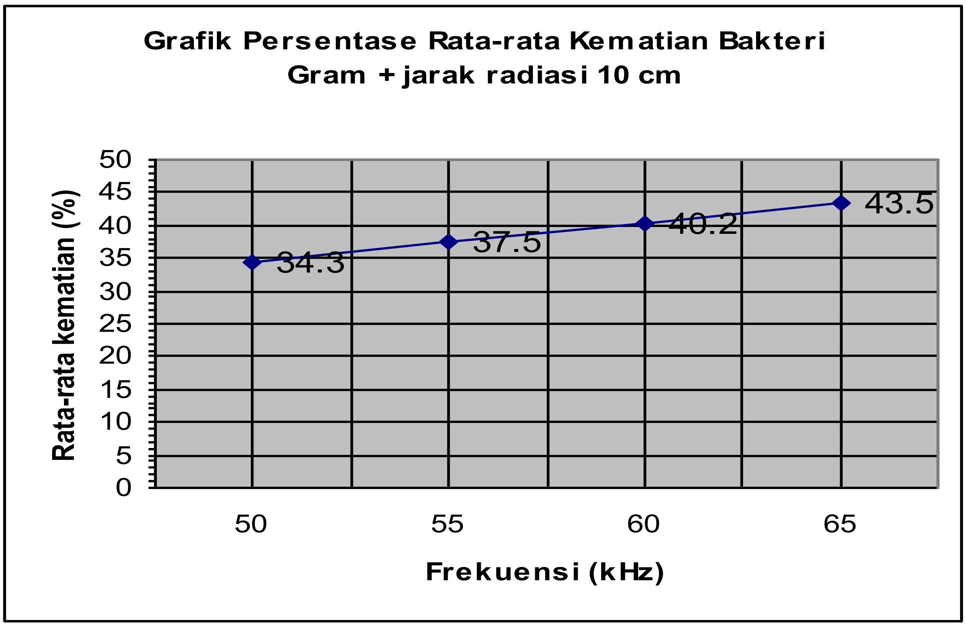

Figure 4. Graph of Average Percentage of Bacterial Mortality

From the results of observation of the death of Gram+ bacteria due to ultrasonic wave radiation at a distance of 10 cm to the variation of frequencies with a density of 10-8 cfu/ml, it was found that the percentage of Gram+ bacterial mortality at the 50 kHz frequency was 34.3%, at the 55 kHz frequency was 37.5%, at the 60 kHz frequency was 40.2%, and at the 65 kHz frequency was 43.5%, In the table, the average mortality of Gram+ bacteria at the 50 kHz frequency amounted to 42.6 cfu/ml. the 55 kHz frequency amounted to 46.3 cfu/ml, the 60 kHz frequency amounted to 52.6 cfu/ml, and the 65 kHz frequency amounted to 65 cfu/ml.

From the results of observation of the death of Gram bacteria - due to radiation of ultrasonic waves at a distance of 5 cm to the variation of frequencies with a density of 10-8 cfu/ml, it was obtained that the percentage of Gram bacterial mortality - at the frequency of 50 kHz was 50.3%, at the frequency of 55 kHz it was 54.2%, at the frequency of 60 kHz it was 59.1%, and at the frequency of 65 kHz was 63.6%, In the table it was also obtained that the average mortality of Gram bacteria - at the frequency of 50 kHz amounted to 49.3 cfu/ml, the 55 kHz frequency amounted to 62.3 cfu/ml, the 60 kHz frequency amounted to 87.6 cfu/ml, and the 65 kHz frequency amounted to 96.3 cfu/ml.

A graph of the relationship of the average mortality of Gram bacteria - at a distance of 5 cm to the frequency variation used can be shown by the figure below.

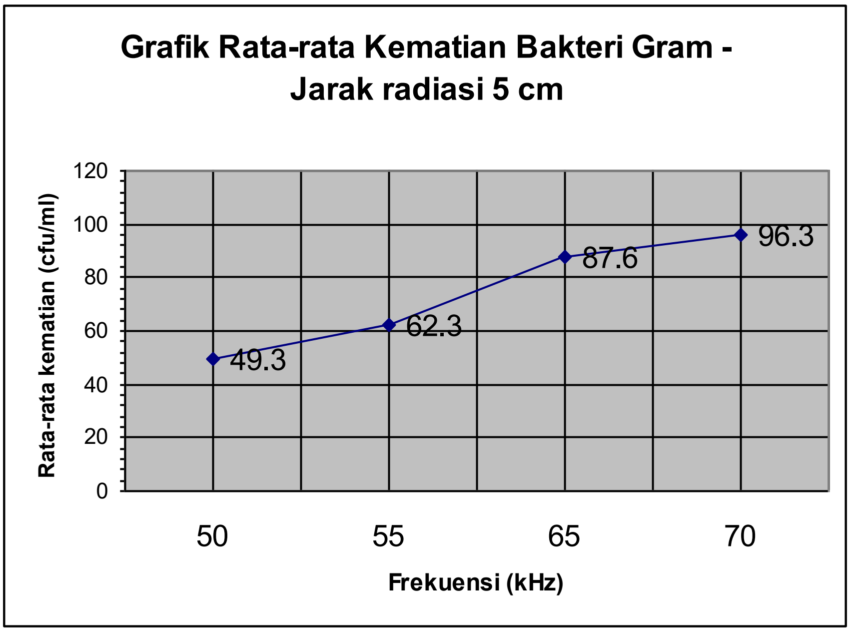

Figure 5. Graph of Average Percentage of Bacterial Mortality

From the results of observation of the death of Gram bacteria - due to radiation of ultrasonic waves at a distance of 10 cm to a frequency variation with a density of 10-8 cfu/ml) it was obtained that the percentage of Gram bacterial mortality - at the frequency of 50 kHz was 47.7%, at the frequency of 55 kHz it was 50.5%, at the frequency of 60 kHz it was 54.1%, and at the frequency of 65 kHz was 57.9%, In the table it was also obtained that the average mortality of Gram bacteria - at the frequency of 50 kHz amounted to 47.6 cfu/ml, the 55 kHz frequency amounted to 61 cfu/ml, the 60 kHz frequency amounted to 80.3 cfu/ml, and the 65 kHz frequency amounted to 90.6 cfu/ml.

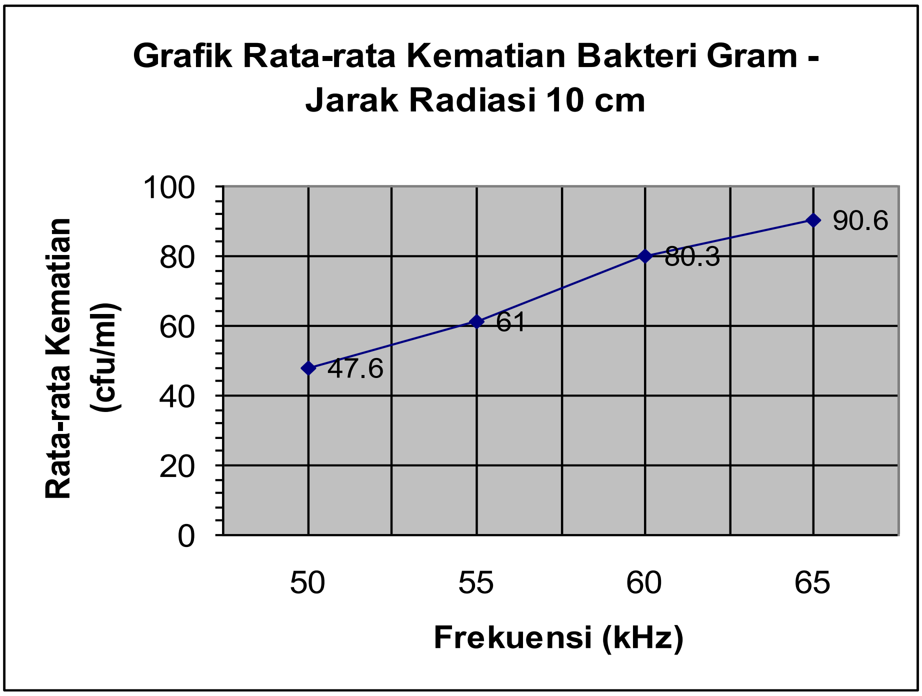

Figure 6. Graph of Average Percentage of Bacterial Mortality

In the image above it can be seen that the average percentage of Gram+ and Gram-bacterial deaths increases in proportion to the increase in frequency. Likewise with the average death of Gram+ bacteria, the greater the frequency used, the greater the average bacterial death caused. The effect of the radiation distance of the ultrasonic waves to the sample, the closer the radiation distance, the higher the percentage rate of bacterial death. This is because the intensity received by the sample is quite high. The intensity of the sound received by the sample is proportional to the sound energy received by the sample, so the higher the frequency of sound used, the higher the sound energy received by the sample.

With the high energy of sound, the percentage of deaths of Gram+ and Gram-bacteria - is also increasing. This causes the energy effect of ultrasonic waves on the bacteria to be stronger. The effect of this energy can likely be in the form of vibrations or strong mechanical stress on the cells of Gram+ and Gram-based bacteria - causing the coagulation of proteins and damaging the nucleic acids of the bacteria. The presence of damage to these bacterial cells will cause the bacteria to die. From the results of the observation of the number of bacterial colonies that grow on the media and the results of the staining of the Gram of bacteria, it can be seen that there is a significant difference between bacteria that are treated with ultrasonic wave radiation and bacteria that are not given radiation treatment. From the results of Gram staining, Gram+ and Gram bacteria - which have been irradiated with ultrasonic waves show that in bacterial cells there are cell abnormalities, namely shrinkage of cell shape, rupture of the cell wall and undergo lysis (loss of fluid in the cell).

CONCLUSION

From the results of the research and observations carried out, several conclusions can be drawn, namely:

1. An ultrasonic generator has been designed that can be used as a killer of Gram+ bacteria and Gram bacteria – which has a frequency range ranging from 50 kHz – 65 kHz.
2. From the observation results of the death of Gram+ bacteria caused by ultrasonic wave radiation with variations in distance and frequency variation used for 10 minutes at a density of 10-8 cfu/ml, it was obtained that the average percentage of Gram+ bacterial deaths caused increased in proportion to the increase in frequency used.

RECOMMENDATION

Based on these conclusions, it is recommended that future studies further optimize ultrasonic frequency settings above 65 kHz and exposure duration to enhance bacterial inactivation efficiency while ensuring safety and energy efficiency. Additional research should also evaluate the effectiveness of the ultrasonic generator on different bacterial concentrations and other microbial groups under varied environmental conditions. Moreover, integrating this technology into practical disinfection systems and assessing its long-term operational stability will be important steps toward real-world biomedical and industrial applications.

FURTHER STUDY

Further research can be directed to the development and optimization of ultrasonic generators by expanding the frequency range and variation of exposure time to determine the limits of the effectiveness and efficiency of bacterial inactivation, both Gram positive and Gram-negative. In addition, further studies are needed on the influence of power intensity, propagation medium, and distance of ultrasonic sources on the rate of bacterial mortality quantitatively and microscopically. Further research is also recommended to test the effectiveness of this system on other types of pathogenic bacteria as well as evaluate its potential application in the medical, food, and sanitary fields by considering the safety and stability aspects of the system in long-term use.

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