Aquatic Bacteria Of Pseudomonas Aeruginosa Growth Model In Tube Ultrasonic

Syamsul arifin, Ni’mahzahroh, Sugianto, R apsari, Suhaningsih

Abstract: The phenomenon of fish farmers in reducing the high growth of bacteria in fish ponds of freshwater by using salt (krosok), but not all the bacteria killed (especially Pseudomonas aeruginosa). This objective research is proving to increase some knob counts (small ball) as ultrasonic transducer, there is a linear increase in the forward intensity of ultrasonic and an exponential increase the death of Pseudomonas aeruginosa colony. Pseudomonas aeruginosa (ATCC 27853) can be grown in a nutrient agar, a nutrient broth, a human blood agar plate, a koi blood plate. In this research especially diluted media in water of physiology as compared in control for viability of Pseudomonas aeruginosa. In the media, these bacteria exposed ultrasonic for 30 minutes. The exposure system of ultrasonic uses some knob (ie: 3, 6, 9, 12 knops) and the media would have own optimum frequency in the increased intensity (energy) and the increased death of Pseudomonas aeruginosa, which depends on the homogeneity when it created the media of Pseudomonas aeruginosa. This study shows each medium has own optimum frequency, each some knob of ultrasonic transmitter, has the intensity transmite increase of ultrasonic and in the death of Pseudomonas aeruginosa colony. Application of ultrasonic exposure is new knowledge related to energy ultrasonic and the ability to pass on skills lethal of Pseudomonas aeruginosa colony related differensis knops counts are derived from the same source intensity value.

Index Terms: Water of physiology of Pseudomonas aeruginosa, fresh-water ponds of Pseudomonas aeruginosa, optimum intensity transmits, frequency ultrasonic, the quantity knops, Pseudomonas aeruginosa colony death.

1 INTRODUCTION

Bacterial pathogens in aquatic environments that can cause infections in fish, where a growing number of pathogenic bacteria causing the infection and death of fish (especially Koi fish freshwater aquaculture). There are several bacterial pathogens that exist in the body of the fish and pond water which one of them is the bacterium Pseudomonas (P) aeruginosa the ATCC (Americans Type Colecton Culture) No. 27 853, which can cause nosocomial pathogens in fish, and humans [1]. Culturing P aeruginosa in water of physiology where mixing 8 g salt with 10 ml water, constitute a new experience. This research is updating the last research about technique of ultrasonic exposure, which ultrasonic exposure resource technique to do from outside container or from the upper surface of liquid, i.e. Ter mnsen, et al, (2008) [2], carmen JC, et al, (2006) [3], William G. Pitt, et al, (1994) [4], Williams KA et al (1995) [5], etc. and ultrasonic exposure method that uses special media, some bacterias, and actual must renewed research isn’t it.

This research in vitro to develop ultrasonic exposure of transducer, that had the criteria is: 1. water of physiology containing P.aeruginosa with optical density (turbidity) 0.01, 2. water of physiology containing P.aeruginosa with optical density 0.01 and dilution factor till 10^3, 3. The ultrasonic transducer from a knob of tin, which copper wire to holding knob and connected to positif condensor a piezoelectric speaker. 4. The ultrasonic transducer (by knobs) be inside water of physiology. 5. Using tube modification with speaker receiver, function generator and oscilloscope. 6. Biosafety and biosecurity in physics laboratory using a modification aquarium. And observation will be developed from this research is The optimization of the transmitted intensity from Knops to receiver speaker in media, The effectiv frequency and the distance between transducer speaker to reciver speaker in the media, The comparison of total plate count of bacteria (the death) after ultrasonic exposure between the water of physiology containing P.aeruginosa on OD = 0.01 and the water of physiology containing P.aeruginosa on OD = 0.01 with diluted factor till 10^6

2 MATERIALS AND METHODS

P aeruginosa

Morphological of P. aeruginosa is can move (have one flagella), rod shaped, sized from 0.6 to 2 mm, visible form of a single cell, double or short chains, easy to grow in many different types of culture media. In the nutrient agar the young bacteri have a lightgreen color (uneven), the old bacteria can be to change color blue (evenly), and at time of his death brown (evenly). at the nutrient broth, the bacteria are in the surface, the green coloring, no spore, but at the sugar media, no do action to fermentation and at the agar of blood that are very pathogenic. [2]

Water of phisiology and P aeruginosa

P. aeruginosa can survive in all condition i.e the land, the fresh water, the sea, the animal, the plant, human act. The plan in this research of P.aeruginosa to be grown in physiology water, that supposed can be surviving and growing.

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Ultrasonic
Resource of ultrasonic is from the function generator type VOM VFG 3020 DDS, merk PROTEK, made in South Korea. This research was setting frequency about 20 – 60 kHz and setting intensity in 1 Vpp and 20 Vpp, at 1 Vdc. And measuring instruments for frequency and intensity ultrasonic signal is oscilloscope type 3025 merk PROTEK made in South Korea.

The Speaker piezoelectric
Specification of the speaker piezoelectric type 40 T – 16B made in Taiwan is: Center frequency (kHz) = 40.0 ± 1.0 kHz, Sound pressure level (0 dB = 0.0002 µ bar) > 119 dB, Band width (kHz) = 4.0 – 112 Db, Beam angle – 6 dB = 55°. Driving voltage (RMS) = 30 V, Working temperature = – 30 ~ 80°C. The modifying speaker is speaker piezoelectric that newly purchased and was destroyed shell so stay condenser piezoelectric (diameter = 1.0 cm, thick condenser = 0.04 cm). And modifying speaker is diameter plastic holder condenser to till = 1.2 cm.

The transducer knobs
The knob in this research is hand made. The material of knob is from tin. That is like a smooth ball in diameter less than 3 mm and over 2 mm and no painted. The knob has a wire holding from copper (the length of copper wire on holding knobs = 3 cm, diameter = 0.011 cm and black painted), that connected to plat condenser piezoelectric and the condenser connect on function generator. When the speaker using one frequency, and the knob too. This research has three groups each group has a section which consists of: 3 Knobs, 6 Knobs, 9 Knobs, 12, Knobs. The best in exposure ultrasonic is making a small ball (knob), because ultrasonic radiation is perfectly, that formula is [6]:

\[ I = \frac{1}{\mu} (\varepsilon_2 - \varepsilon_1) \]

The tube and receiver speaker
Specification of the test tube is a glass, type short, diameter of inside about 1.3 cm and depth 9.9 cm that are modified in a way to make a hole the bottom of the tube so that it can be entered from the cable polarities of receiver speaker, and glued so not leak. The receiver speaker to be connected on the oscilloscope PROTEK type 3025. The tube containing a transducer (with and without knobs), receiver speaker and volume of media PZ (with and without P.aeruginosa) =3 ml and In this experiment have four treatment, ie:

1. To observe frequency and transmitted intensity optimum in physiology water without P.aeruginosa. Aim is to knowing in media PZ have a transmitted intensity optimum in one frequency

2. To observe effect the quantity of knobs in value transmitted intensity on physiology water with P.aeruginosa. Aim is to knowing the quantity of knob have trek line (or not) in transmitted intensity of value in media PZ with P.aeruginosa.

3. To observe effect the quantity of knobs and the quantity colony P.aeruginosa on physiology water with P.aeruginosa, Aim is to knowing the quantity of knob have trek line (or not) in the quantity colony in media PZ with P.aeruginosa

The researches have different activity, but its have similarity prospect.

The transmitted intensity
The formula of transmitted intensity in ultrasonic is deviding between value transducer intensity and value receiver intensity that sabine formula in the air [7], this research using liquid (physiology water), so this is the result.

3 Result
Result per experiment is: P. aeruginosa can survive in Physiologi water.
In order to quantity express the result in this study; P. aeruginosa can survive in Physiologi water is:

<table>
<thead>
<tr>
<th>No</th>
<th>Media</th>
<th>0</th>
<th>+ 6</th>
<th>+ 9</th>
<th>+ 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NB + P.a</td>
<td>0.13</td>
<td>0.25</td>
<td>0.32</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Mean from observation data</td>
<td>0.14</td>
<td>0.23</td>
<td>0.33</td>
<td>0.425</td>
</tr>
<tr>
<td>2</td>
<td>NB + P.a diluted in PZ (OD = 0.01)</td>
<td>0.01</td>
<td>0.05</td>
<td>0.1</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Mean from observation data</td>
<td>0.01</td>
<td>0.04</td>
<td>0.11</td>
<td>0.14</td>
</tr>
<tr>
<td>3</td>
<td>NB + P.a diluted factor (10^-10) in PZ</td>
<td>0</td>
<td>0</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mean from observation data</td>
<td>0</td>
<td>0</td>
<td>0.02</td>
<td>0.005</td>
</tr>
<tr>
<td>4</td>
<td>NB + P.a diluted factor (10^-12) in PZ</td>
<td>0</td>
<td>0</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mean from observation data</td>
<td>0</td>
<td>0</td>
<td>0.0075</td>
<td>0.0025</td>
</tr>
</tbody>
</table>

There is an increase in the quantity of colonies on each the age scale of bacteria with the scale of turbidity (OD) in more diluting factor in physiology water on diluting factor till 10^-12
The equation of exponensial in:

1. Media NB and P.aeruginosa is [7]

Independent: Hour (X)
Dependent: Mth Rsq d.f. F Sigf b0 b1
Colony (Y) EXP 980 2 98.87 .010 ,1436 .0815

\[ Y = (0.1436) \cdot e^{(0.0815 \cdot X)} \]

with koefisien determination (Rsq) = 98% and the influence physiology water to populasri P.aeruginosa in tube is very good, why sigf 0.01 < 0.05 the meaning is true physiology water have a sense.
Estimation data and error

<table>
<thead>
<tr>
<th>Observation data</th>
<th>0.01</th>
<th>0.045</th>
<th>0.105</th>
<th>0.145</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimasi data</td>
<td>0.1436</td>
<td>0.2342387</td>
<td>0.299165</td>
<td>0.4497773</td>
</tr>
<tr>
<td>error</td>
<td>-0.0036</td>
<td>-0.00424</td>
<td>0.030835</td>
<td>-0.02478</td>
</tr>
</tbody>
</table>

2. NB + P.a diluted in PZ (OD = 0,01)

Independent: Hour (X)
Dependent Mth Rsq d.f. F Sigf b0 b1
Colony (Y) LIN .953 2 40,13 .024 .0033 .0101
Colony (Y) EXP .935 2 28,75 .033 .0122 .1974

The fact Rsq linier is better than Rsq linier, but in microbiology the exponential is true, so the equation is

\[ Y = (0.0122) \cdot e^{(0.19754 \cdot X)} \]

with koefisien determination (Rsq) = 98%
with Rsq exponential = 93,5% and sigf = 0,33 (> 0,05)

3. NB + P.a diluted factor (10^-10) in PZ

This variable contains non-positive values. Log transform cannot be applied. Models COMPOUND, POWER, S, GROWTH, EXPONENTIAL and LGSTIC cannot be calculated.

No equation

1. NB + P.a diluted factor (10^-12) in PZ

This variable contains non-positive values. Log transform cannot be applied. Models COMPOUND, POWER, S, GROWTH, EXPONENTIAL and LGSTIC cannot be calculated.

No equation

Effect of distance knob transducer to speaker recier on physiology water without P.aeruginosa using the difference reactor (reactor1, reactor2, reactor3) Tolerance of intensity transmitted in distance between the knobs transducer – receiver speaker (0.6 cm) in physiology water without P.aeruginosa, at the same frequency (48 kHz) is:

<table>
<thead>
<tr>
<th>No</th>
<th>Knobs</th>
<th>2013 April 4</th>
<th>2013 June 9</th>
<th>2013 June 13</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Transmitted intensity (%)</td>
<td>Transmitted intensity (%)</td>
<td>Transmitted intensity (%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Non US</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>70.48</td>
<td>47.42</td>
<td>47.42</td>
<td>0.593 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>74.76</td>
<td>56.57</td>
<td>56.57</td>
<td>0.667 ± 0.16</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>82.16</td>
<td>60.28</td>
<td>60.28</td>
<td>0.734 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>90.91</td>
<td>78.57</td>
<td>78.57</td>
<td>0.827 ± 0.12</td>
</tr>
</tbody>
</table>

1. The tolerance in three knobs is:
   \[ \text{Tol.}_3\text{knobs} = 0.593 \pm 0.2 \]

that an up borders is 0.793 and a down border is 0.393. Result the values three knobs to be inside are correct.

2. The tolerance in six knobs is:
   \[ \text{Tol.}_6\text{knobs} = 0.667 \pm 0.16, \]

that an up borders is 0.827 and a down border is 0.507. Result the values six knobs to be inside are correct.

3. The tolerance in nine knobs is:
   \[ \text{Tol.}_9\text{knobs} = 0.734 \pm 0.2, \]

that an up borders is 0.934 and a down border is 0.534. Result the values nine knobs to be inside are correct.

4. The tolerance in twelve knobs is:
   \[ \text{Tol.}_12\text{knobs} = 0.827 \pm 0.12, \]

that an up borders is 0.947 and a down border is 0.707. Result the values twelve knobs to be inside are correct.

The equation of linear regression between each experiment in intensity transmitted to receiver speaker on quantity of knobs is:

\[ Y_1 = 0.624 + 0.0229 X_1 \]
\[ Y_2 = 0.364 + 0.0324 X_2 \]
\[ Y_3 = 6.94 + 0.425 X_3 \]

R-Sq = 97.9%
The equation of the linear regression in:
Experiment 2013 April 4-5 has trend positif with R-Sq= 97.9%,
Experiment 2013 june 9 has trend positif with R-Sq = 92.0% dan experiment 2013 june 13 has trend positif with R-Sq= 96.8%, where from several trials have a very strong relationship level. Effect of ultrasonic with knobs on physiology water with P.aeruginosa The microbiology test in the physiology water in the Reactor with the population of P. aeruginosa, In OD = 0.01, Non Diluted, distance knobs group – receiver speaker = 0.6 Cm, frequency = 48 kHz, Intensity resources = 20 V pp, at 2 V dc, is:

<table>
<thead>
<tr>
<th>No</th>
<th>Knobs</th>
<th>Transmitted intensity</th>
<th>The quantity colony</th>
<th>Specific colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Non US</td>
<td>0</td>
<td>139</td>
<td>Big colony</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.66279</td>
<td>∞</td>
<td>dot / (very small colony)</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.68072</td>
<td>3345</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>0.85326</td>
<td>2250</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>0.97356</td>
<td>1530</td>
<td></td>
</tr>
</tbody>
</table>

The equation of the linear between the knobs quantity and the transmitted intensity is

Independent: Knobs (X)

Dependent Mth Rsq d.f. F Sigf b0 b1

Trans. Int. (Y) LIN ,990 1 94,27 ,065 ,5430 ,1464

\[ Y = 0.543 + 0.1464 X \]

with Rsq = 99% and sigf = 0.065 (> 0.05)

The equation of exponential between the knobs quantity and the colony quantity is

Dependent Mth Rsq d.f. F Sigf b0 b1

Colony (Y) EXP 1,000 1 15523,1 ,005 4936,98 - ,3911

\[ Y = (4936,98) \cdot e^{-0.3911 \cdot X} \]

with Rsq = 100% and sigf = 0.005 (< 0.05)

The quality colony without exposure ultrasonic have structure more big than the quality of colony with exposure ultrasonic The microbiology test in the physiology water in the Reactor with the population of P. aeruginosa, In OD = 0.01, Diluted factor = 10^6, Distance group of knobs – receiver speaker = 0.6 Cm, Frequency = 48 kHz, Intensity resources = 20 V pp, At 2 V dc, is:

The equation of linier between the knobs quantity and the transmitted intensity is Indepenend knobs

Dependent Mth Rsq d.f. F Sigf b0 b1

Tran Int. LIN ,955 1 21,28 ,136 ,7708 ,0562

\[ Y = 0.7708 + 0.0562 \cdot X \]

with Rsq = 100% and sigf = 0.136 (> 0.05)

The equation of exponential between the knobs quantity and the colony quantity is Indepenend knobs

Dependent Mth Rsq d.f. F Sigf b0 b1

Colony EXP ,781 1 3,57 ,310 706,979 - ,6313

\[ Y = (706,979) \cdot e^{-0.6313 \cdot X} \]

with Rsq = 78% and sigf = 0.310 (> 0.05)

Result:
1. The quality colony without exposure ultrasonic have structure more big than the quality of colony with exposure ultrasonic
2. Show in Rsq between the ultrasonic effect in population P. aeruginosa OD = 0.01, not diluted and the ultrasonic effect in population of P. aeruginosa OD = 0.01 by the dilution factor = 10-8 on the quantity of knob is no difference,
3. Show in sigf between the ultrasonic effect in population P. aeruginosa OD = 0.01, not diluted and the ultrasonic effect in population of P. aeruginosa OD = 0.01 by the dilution factor = 10-8 on the quantity of knob is two treatment having effect, that ultrasonic in population OD = 0.01 not diluted more big than in population OD = 0.01 diluted 10^-8.

4. CONCLUSION

Although there are similarities in the form of equation on death of P.aeruginosa on each the influence of the quantity of knob, but the quality of death also occur from the intensity of transmitted, so two phenomenon are cause and effect that very influential
5. REFERENCES


