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Effect Of Gamma Irradiation On Growth Of Escherichia Coli And Salmonella Sp.

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Abstract: *Escherichia coli was still detected in treated water and Salmonella sp. filled 90% of the pathogenic bacteria content in the Wastewater Treatment Plant (WWTP) sludge. This research aimed to know the effect of gamma irradiation on the growth of E. coli and Salmonella sp. Experimental bacteria were in the form of un-raw material, bacterial isolates. The experimental method of this research gave gamma irradiation doses 1, 2, 3, 4, and 5 kiloGray (kGy) to bacterial suspensions. The isolates were cultured on Nutrient Agar (NA) and followed by cultured on Nutrient Broth (NB) to get the suspensions. The suspensions were put in microtubes for irradiation then followed by enumeration on Plate Count Agar (PCA) in Total Plate Count (TPC) based on SNI-2897-2008. This research proved that the higher dose of gamma irradiation had been given, the lower growth of bacteria (or were the higher death number of bacteria) resulted. Decimal Reduction Dose (D_{10}) value of E. coli and Salmonella sp. were 0,3 kGy and 0,35 kGy, and totally dead by ≥ 3 kGy and ≥ 4 kGy. This research was expected to be referenced as secondary data for research whose the experimental bacteria is in the form of raw material such as WWTP sludge, wastewater, drinking water, river water, soil water, or organic fertilizer.*

INTRODUCTION

According to the urgency of new-antibiotic, pathogenic bacteria is divided into 3 (three) priority: serious, high, and medium. One of the pathogenic bacteria in crisis-priority and high-priority is *Escherichia coli* and *Salmonella sp*. (1). *E. coli* and *Salmonella sp.* could be found in domestic waste because their habitat is in the gastrointestinal tract of human and warm-blooded animals (2). *E. coli* is still detected in treated water (3). *Salmonella sp.* fills 90% of pathogenic bacteria content in the Wastewater Treatment Plant (WWTP) sludge (2,4).

Waste treatment must be concerned due to nature is the start and the end of everything. Most of the human intakes come from land and water which is possible to be exposed by waste. Therefore, it is not only the treatment of waste is needed but also the prevention like sterilization of consumable natural products.

Various methods have been used for the disinfection of pathogenic bacteria such as ultraviolet, chlorination, and heating. However, these treatments are energy-intensive processes and tend to be costly. In contrary, irradiation requires much less energy and incurs a smaller change in substance (4).

A comparative study of secondary wastewater processing conducted by Lee *et al.* (5) showed that the electrical power consumption of the UV and ozone method required 2—3 orders of magnitude higher than that of ionizing radiation. Ionizing radiation can be applied as an effective and economical alternative technique to conventional disinfection processes.

Utilizing irradiation technology could be as an alternative for chemical treatments in wastewater process regarding anthropogenic chemicals is still detected in treated wastewater (6). In addition, compared to the thermal treatments, this brings significant with great potential for food sterilization or preservation in order to control foodborne hazard, and increase the shelf life and safety of food (7).

Irradiation is radiation utilizing to object deliberately and directly. Irradiation uses ionizing and electromagnetic-wave energy. Because of that, irradiation is safe, clean, and environmentally friendly, with no residual (8). Furthermore, the operation

procedures for irradiation are simple, continuous, and completely disinfected (4)

Ionizing radiation is needed because its energy level can make a neutral atom to be positive ion and negative ion. Ionizing radiation can be in the form of gamma, electron beam (e-beam), or x-ray (9). Additionally, gamma irradiation can be obtained from Cobalt-60 (60Co) and Cesium-137 (137Cs) (8).

The mechanism of irradiation is divided into 2 (two) effects: directly and indirectly. Directly, ionizing-radiation leads either *Deoxyribonucleic Acid* (DNA) molecules or other crucial components break off. Indirectly, ionizing radiation leads radiolysis to the water molecule that induces free-radical. Reactivity of free-radical causes oxidation, reduction, and break off molecule bonds such as Carbon chain and DNA (10).

In living biological system, radiation works by removing electrons in the molecules of DNA. As a result, the organism is either prevented from growing, reproducing, or performing normal metabolic activities. Unless the organism able to repair itself, the result is either the death of the organism or its inability to produce offspring (11).

The level of cell damage is affected by the resistance of the microorganism to irradiation, this is also known as Decimal Reduction Dose (DRD or also called as D_{10}). The higher of the D_{10} value it has, the more resistance to irradiation it will be. The value of D_{10} is needed dose in Gray (Gy) to decrease bacteria from 50.000 to be 5.000 or 90% inactivation of viable Colony Forming Unit (CFU) (1 log cycles decreasing). One Gray is the absorbed dose of the ionizing radiation corresponding to one Joule per kilogram (1 Gy = 1 J/kg) (12).

Adapted from Jeong *et al.* (13), the D_{10} was calculated as follows.

$$D_{10} = \frac{d}{\log\left(\frac{N_0}{N_d}\right)}$$

Where d is the irradiation dose in kGy, N_0 is the initial cell population, and N_d is the number of surviving cells in irradiated samples by dose d. The N and N_0 were declared in CFU per mililiter (CFU/ml).

The main purpose of this research is to know how the effect of gamma irradiation on the growth of *E. coli* and *Salmonella sp.* The purpose has been answered by the result of D_{10} value and the needed dose to sterilize/eliminate experimental bacteria. This research could be used as secondary data to other research especially that use raw material as the experimental object.

Experimental bacteria were in the form of un-raw material that had obtained from the Faculty of Medicine, Universitas Brawijaya (FK-UB). Bacterial isolates were cultured on Nutrient Agar (NA) and followed by cultured on Nutrient Broth (NB). Bacterial suspensions on NB were put in microtubes for irradiation then followed by enumeration on Plate Count Agar (PCA) in Total Plate Count (TPC) based on SNI-2897-2008.

Gamma irradiation doses given to samples were 1, 2, 3, 4, and 5 kGy. The source of gamma irradiation was obtained from Irradiator Gamma Cell 220 (IGC 220) Centre for Application of Isotopes and Radiation, National Nuclear Energy Agency (PAIR-BATAN). IGC 220 uses 60Co with radioactivity 6.421 Curies (Ci) and dose absorbed by object rate 4.630,3 Grays per hour (Gy/h).

EXPERIMENTAL SECTION

This research used 3 (three) variables: independent, dependent, and control. The independent variable is gamma irradiation in addition to the dependent variables are *Escherichia coli* and *Salmonella sp.* Besides, the control variables are temperature, duration, growth phase, inoculum absorbance, growth media, doses, and sterilization to contamination. These variables were controlled to minimalize the probable interference relationship between dependent and independent variables. Moreover, these were equally given to both pathogenic bacteria. Hence, the data result of both could be fairly compared. The research work steps consist of preparation, gamma irradiation, and enumeration.

Inoculum Preparation

The inoculum preparation was consisted of culturing, spectrophotometry, and sample-making. The bacterial isolates that had been preserved at < 16 °C were cultured on Nutrient Agar (NA) by streak agar slant inoculation technique with an inoculating loop. Then, incubated in the incubator at 37 °C for 18—24 hours. Thereafter, cultures on NA were cultured on Nutrient Broth (NB) by dip inoculation technique with an inoculating loop. Then, incubated in the water bath shaker 120 linear-rotations per minute (rpm) at 37 °C for 18—24 hours.

Inoculums, as the result of culturing, were measured by spectrophotometer that set on 660 nm with 1,5 ml cuvette volume and using NB as the blank. Spectrophotometry aimed to know the absorbance value or the concentration of bacteria to get the growth phase. The measurement was carried out while the bacterial suspension was continuously incubated in the water bath shaker. Bacteria were measured every 2 hours.

After the logarithmic phase had been detected, the inoculums were planned to be sampled for irradiation. When the absorbance value of both bacteria was equal, as much 1 (one) ml inoculum was inoculated

into microtubes with micropipette and tip. There was a triplet for each dose unit of each bacteria. One rack loaded two triplets consisted of each dose unit of both bacteria. Therefore, these are 5 (five) racks to load all experimental samples.

Gamma Irradiation

All loaded samples in the rack were placed in the irradiator tube. The dose-rate was 4.630,3 grays per hour (Gy/h), it meant the irradiator needed 777 seconds or 15 minutes 57 seconds to accelerate 1 Gy. Irradiator was set to automatically to be open after increment 1 (one) Gy. One by one, the sample was taken from the tube to immediately get into the cool box for transportation (< 16 °C).

Bacterial Enumeration

Irradiation was followed by enumeration on Plate Count Agar (PCA) in Total Plate Count (TPC) based on SNI-2897-2008. Enumeration is the method to obtain the number of bacteria. The irradiated sample must be used in ≤ 2 days since irradiated. Enumeration included serial-dilution, inoculation, incubation, and counting.

Every 1 (one) ml of sample was diluted by 0,85% NaCl solution. The sample was ensured mixed well before diluting. The range of a factor of dilution started 10^0—10^{12}. The factor of dilution for each unit dose was different. The more doses have been given the less factor of dilution would be given. All experimental samples were diluted in duplet for duple inoculation. Inoculation on PCA used the drop or Miles & Misra technique.

Aliquot, the volume of the inoculated sample was 0,01 ml. It was inoculated by micropipette and tip. Each plate loaded all dilution of each dose unit for every experimental bacteria. Afterward, incubated at room temperature in the sterile atmosphere, which was laminar room ± 27 °C for 18—24 hours. The colony form must be counted in ≤ 24 hours since incubated.

RESULTS AND DISCUSSION

1. Growth Curve

It is important to know the growth phase of bacteria. Fig 1. shows the growth curve of *Escherichia coli* and *Salmonella sp.* In 30 hours, the curves show that both bacteria were in the logarithmic phase. The logarithmic phase means bacteria in the condition of actively self-multiplicate.

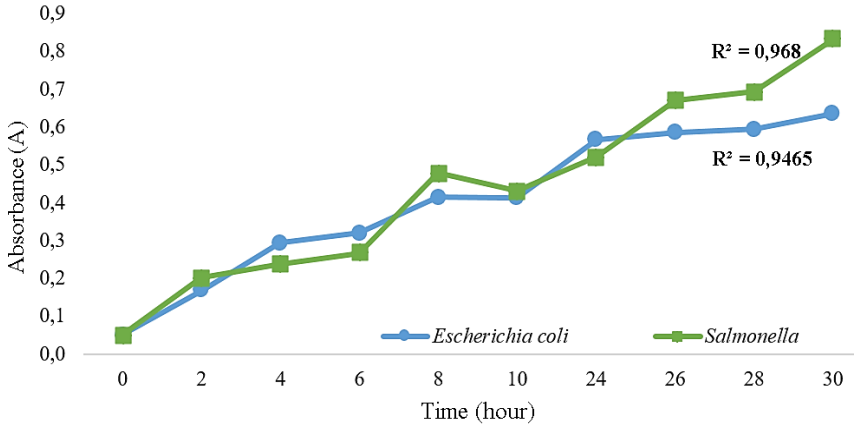

Fig. 1. Growth of incubated *E. coli* and *Salmonella sp.* suspension every 2 hours

Absorbance value is directly proportional to the substance value (bacteria). The bacteria were started in the value of absorbance by 0,05 A. By hour 30, the absorbance of *E. coli* was 0,636 A in addition to *Salmonella sp.* was 0,832 A. Absorbance of *E. coli* is lower than *Salmonella sp.* Therefore, the doubling-time needed by *E. coli* was slower than *Salmonella sp.* In general, experimental bacteria had doubled in 42 minutes for *E. coli* and 80 minutes for *Salmonella sp.*

2. Survival Bacteria

Colony Forming Unit (CFU) that appeared on agar corresponds to leftover bacteria (N_d) that has been irradiated by dose (d) in gray (Gy). Leftover bacteria are also known as survival bacteria, bacteria that survived from irradiation. The N denotes bacteria in CFU per milliliter (CFU/ml). Graphic of survival bacteria shows in Fig. 2.

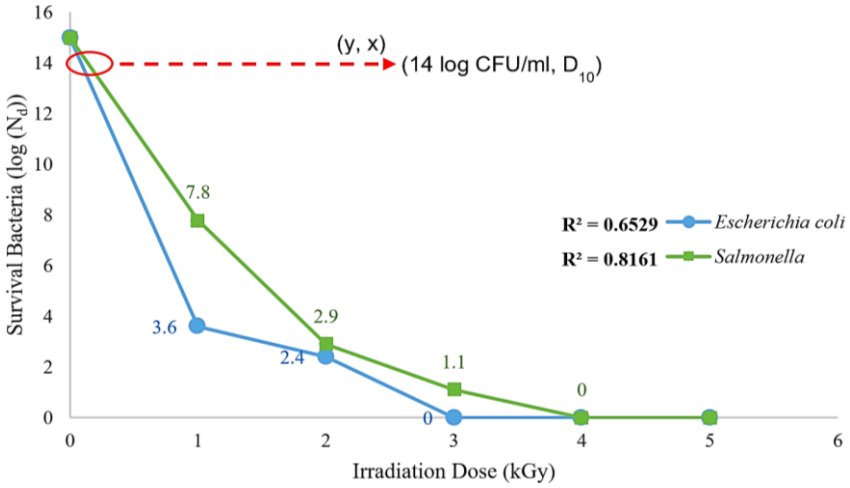

Fig. 2. Effect of Gamma Irradiation treatment on the Survival Bacteria of *E. coli* and *Salmonella sp.*

By 0 kGy, the non-irradiated control sample of both was in the same number, 1,0 x 10^{15} CFU/ml (N_0 = 15 log). By 1 kGy, survival bacteria of *Escherichia coli* were 4,0 x 10^3 (N_1 *E. coli* = 3,6 log) and *Salmonella sp.* were 6,8 x 10^7 CFU/ml (N_1 *Salmonella sp.* = 7,8 log). By 2 kGy, survival bacteria of *Escherichia coli* were 2,8 x 10^2 (N_2 *E. coli* = 2,4 log) and *Salmonella sp.* were 8,1 x 10^2 CFU/ml (N_2 *Salmonella sp.* = 2,9 log). By 3 kGy, there was no survival bacteria of *E. coli* but *Salmonella sp.*, 1,4 x 10^1 CFU/ml (N_3 *Salmonella sp.* = 1,2 log). By 4 and 5 kGy, there are no survival bacteria of both at all.

3. Death Bacteria

The survival bacteria decreased as the irradiation doses increased. Contrary, the death bacteria increased as irradiation doses increased. The number of N_{death} was not generated from this research directly instead of got from the formulation. However, the number of the death bacteria is defined as the number of initial bacteria (N_0) diminished by survival bacteria (N_d). In the form of logarithmic, the formulation is written as log (N_{death}) = log (N_0/N_d). Graphic of death bacteria shows in Fig. 3.

By 1 kilogray (kGy), the death of *Escherichia coli* is 11,4 log and *Salmonella sp.* is 7,2 log. By 2 kGy, the death of *E. coli* is 12,6 log and *Salmonella sp.* is 12,1 log. By 3 kGy, *E. coli* has total death, 15 log, but the death *Salmonella sp.* is 13,9 log. Otherwise, *Salmonella sp.* has total death by 4 kGy. Base on the definition of Decimal Reduction Dose (D_{10}), the needed dose in Gray (Gy) to decrease bacteria from 50.000 to be 5.000 or 90% decreasing (1 log cycles decreasing). Therefore, in this research, the needed dose to decrease bacteria from 10^{15} to be 10^{14} or from 15 log to be 14 log. From the results, the D_{10} value of both must be less than 1 kGy. By 1 kGy, both bacteria have died more than 10^1 or 1 log.

D_{10} value of both is obtained from regression linear in the figure above. By using Y = 3,36x + 4,08, hence the D_{10} of *E. coli* is 1/3,36 = 0,3 kGy. By using Y = 2,86x + 3,38, hence the D_{10} of *Salmonella sp.* is 1/2,86 = 0,35 kGy. These prove that the D_{10} must be less than 1 kGy. To conclude, the D_{10} of *E. coli* is less than D_{10} of *Salmonella sp.* This result relates to the needed dose for the total death of experimental bacteria. *E. coli* needed dose is ≥ 3 kGy, which is less than *Salmonella sp.* needed, ≥ 4 kGy. Therefore, the D_{10} value is directly proportional to the needed dose for total death.

Some researches also conducted gamma irradiation in *E. coli* and *Salmonella* isolates. The D_{10} of *E. coli* gained by Christopher *et al*. (14) has not much different result. It was 0,433 kGy. Besides, Sihaloho *et al.* (15) needed 0,07 kGy to reduce one log cycle of *E. coli* and 0,27 to reduce one log cycle of *Salmonella aureus*. In addition, Munir *et al.* (12) needed 0,45 kGy to reduce one log cycle of *E. coli* and 0,65 kGy to reduce one log cycle of *Salmonella*. Those researches showed the inactivation of microbial populations is considerably influenced by conditions of the environment during irradiation. For instance, gaseous composition, temperature, and medium (volume, mass, and compound). Microorganisms are most resistant when irradiated in dry conditions. Presence of water molecules when irradiation leads to the existence of free radicals. Its reactivity is going to cause indirect effects in the cell even more indirectly damage the *Deoxyribonucleic Acid* (DNA) (18).

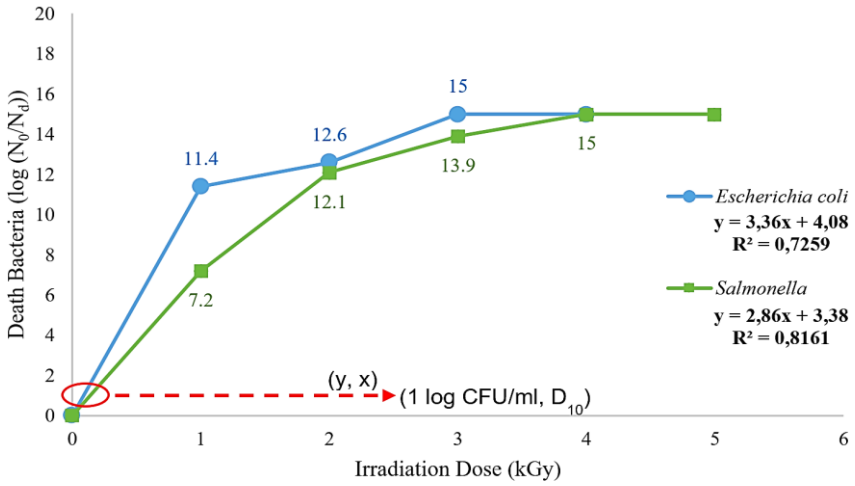

Fig. 3. Effect of Gamma Irradiation treatment on the Death Bacteria of *E. coli* and *Salmonella sp.*

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Additionally, every species has its uniqueness that being a discrepancy to other species either physically or chemically. Some substances have certain responses to radiation differently (20). The major discrepancies between these Gram-negative bacteria are the cell wall and the outer membrane. Probably, the condition of the outer membrane of *E. coli* was easier to be penetrated by gamma irradiation than *Salmonella sp.* Destruction of the outer membrane would be followed by the destruction of the cell wall. The external stresses are going to lead the cell lysis (21,22).

The X-axis of graphics refers to the given dose. The Y-axis of graphics refers to the survival bacteria and the other one refers to as the death bacteria. These result in a relevancy between the less survival and the more death. Base on the result, the number of bacteria had the most significant decrease in the first increment of one dose. Nonetheless, the decrease diminishes as the increment of one dose be given. The reason is due to the D_{10} leads to massive death, 90% of initial bacteria, and a lot more death by 1 kGy. Consequently, the rest bacteria are less than 10% that causes lesser gradient on and on. Additionally, the reasons why the graphics show a discontinuous trend are the logarithmic scale of the Y-axis and the determinant of measured-dose number.

CONCLUSION

Gamma irradiation affected the growth of *Escherichia coli* and *Salmonella sp.* bacteria. The relationship between gamma irradiation dose with the survival bacteria is inversely proportional. Gamma irradiation dose to death bacteria is directly proportional. *E. coli* is more sensitive to gamma irradiation than *Salmonella sp.* In other words, *Salmonella sp.* is more resistant to gamma irradiation than *E. coli*. D_{10} of *E. coli* is smaller than *Salmonella sp.*, 0,3 kGy < 0,35 kGy. The dose that is needed to eliminating *E. coli* is smaller than *Salmonella sp.*, (≥ 3 kGy) < (≥ 4 kGy).

For further research, the experimental object might be conducted in raw material to be easier applied in life. Raw material might be related to environmental problem solving such as WWTP sludge, wastewater, water for drinking, soil water, river water, and organic fertilizer.

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