The effect of Co-60 Gamma Rays irradiation on chemical properties of biofertilizer carrier material

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Abstract. This study was conducted to test the method of sterilization using autoclave and Co-60 gamma rays irradiation on chemical properties changes of biofertilizer carriers (Jasinga Latosols, Pasar Jumat Latosols, compost, Rawa Pening peat, Kalimantan peat). This research was conducted in July 2016 to April 2017 at Center for the Application of Isotopes and Radiation National Nuclear Energy Agency (PAIR BATAN) laboratory and Ilmu Tanah Sumber Daya Lahan Insitut Pertanian Bogor (IPB) laboratory. The design used in this study is completely randomized design, with seven treatment and five replications. The treatments provided include the control (without sterilization), sterilization using autoclave, and sterilization using gamma ray of Co-60 with doses of 10 kilo Gray (kGy), 20 kGy, 30 kGy, 40 kGy and 50 kGy. The parameters observed in this research was available Mn²⁺, available Fe²⁺, available Zn²⁺, and effectiveness of sterilization method. This research also showed chemical character changes in materials as shown by Fouier-Transform Infrared Spectrometer (FTIR) spectra. The results showed that the methods sterilization was gave significant effect on pH changes, the levels of available P, the levels of available nitrates, available Mn²⁺, available Fe²⁺, and available Zn²⁺. Autoclave has led to a higher metal increase compared to Co-60 gamma irradiation. A dose of 30 kGy is an effective dose to sterilize the carriers material.

1. Introduction
In Indonesia, biofertilizers have been widely used in the agricultural practice to reduce the use of chemical fertilizers, pest and plant diseases control, and to accelerate the composting processes [1]. The estimation of the use of biofertilizer in Indonesia is less than 10% if compare with chemical fertilizer [2]. The main problems in application of biofertilizer is that so many low quality biofertilizers established in the farmer, so that many ineffective biofertilizers because of low total number microbes. Besides consist of effective functional microbes, biofertilizer is also determined by the type of nutrients such as carbon and minerals, manufacturing process such as pack and storage, and also the carrier materials such as peat, soil, and compost. Biofertilizers should be constructed with good carrier materials such as soil with high organic materials contain that have both physical and chemical characteristics which is suitable for the inoculants [3]. Carriers must provide a safe environment for microbes during storage, protect microbial population, and improve microbial performance when applied to soil[4].
Generally, carrier material sterilization uses autoclave technique. Autoclave is an effective tool for sterilizing materials using wet heat. Carrier material can also be sterilized effectively by using radiation. The problem is autoclave sterilization on tropical soils can increase the availability of some metal elements such as Mn$^{2+}$ [5]. This can be a problem for microbes that are sensitive to environmental changes, like Rhizobium which is sensitive in iron metals or Azotobacter is sensitive in Mo metals, especially the metals solubility in the carrier material. Irradiation with Co-60 gamma rays can be a good sterilization technology because it is easy, can be used on a large scale, and can sustain the inoculant population during storage[6–8]. Bartonicek and Pipota [9] mentioned that sterilization with gamma irradiation does not cause toxic side products. Irradiated doses at 15 kilos Gray (kGy) can kill fungus and actinomycetes. While at higher doses that is between 20-70 kGy algae and bacteria will be killed. Several studies have considered chemical characteristics a required irradiation dose of 70 kGy to sterilize organic soil such as peat [10].

Carrier sterilization methods should consider the effect that will be present on the carrier components. Sterilization methods with autoclave or by gamma ($\gamma$) irradiation may be changes on the physico-chemical properties of the carrier materials. McLaren [11] reported that irradiation with gamma rays 50 kGy on soil and humus can induce small release of ammonium (NH$_4$), soluble carbon (C), organic nitrogen (N), phosphorus (P) which are derived from soil microbes and humus. According to Rao et al. [12] Gamma-ray irradiation in lignin may increase the conjugate structure by decreasing particle size. The chemical stability of lignocellulose is reduced with irradiated doses. This reaction can be occured because radiochemistry changes in lignocellulosic molecules [13] and may affect on chemical properties of the carrier. Research on the effects of gamma has been widely used in materials but very limited to be applied in materials for biofertilizer carriers [9]. Although gamma ray irradiation has long been recognized as an effective sterilization method, but it is important to study the changes of chemical properties which will occur on the carrier. The first purpose of this research was to study some chemical properties changes (available Mn$^{2+}$, available Fe$^{2+}$, and available Zn$^{2+}$) in five types of carrier material using Co-60 gamma rays irradiation and autoclave. The second one is to find the best sterilization methods for carrier materials in biofertilizer industry in Indonesia.

2. Materials and methods
This research was conducted in Bogor Agricultural Institute (IPB) laboratory and Center for the Application of Isotopes and Radiation (PAIR) National Nuclear Energy Agency (BATAN). The research started from July 2016 until April 2017. The materials used in this study were taken from different locations in Java and Kalimantan (Figure 1). The materials used were Latosol Pasar Jumat which is taken from Lebak Bulus Village, Cilandak Sub-district, South Jakarta A (6°17'43.3"S106° 46'28.8"E), Latosol Jasiring which is taken from Jasina village, Jasina District, Bogor, West Java B (6°27'38.0"S106°26'59.0"E), Borneo peat from Alalak Village, District Alalak, Barito Kuala District, South Kalimantan C (3°10'45.1"S114°34'44.2"E) and Raw Pening peat taken from Asinan village, Bawen Sub-district, Semarang, Central Java D (7°15'48.7"S110° 27'01.9"E). Borneo peat from Alalak Village, District Alalak, Barito Kuala District, South Kalimantan (3°10'45.1"S114°34'44.2"E).
While the compost taken from Dramaga Bogor West Java (6°33'47.6"S106°43'29.7"E). The materials used in the laboratory are Merck Germany Nutrient Agar (NA), Merck Germany nitric acid 65%, Merck Germany ammonium vanadate, Merck Germany ammonium molybdate, Merck Germany sulfuric acid, Merck Germany hydrochloric acid, Merck Germany selenium mixture, and Merck Germany NaOH. Another chemical ingredient used for the determination of Mn$^{2+}$, Fe$^{2+}$, and Zn$^{2+}$ with diethylene triamine penta acetic acid (DTPA) extract.

Each material is smashed with stone mortar (Ø 10 cm) and then filtered with an 18 metal mesh screen Germany. Materials then weighed with a Precissa XT-220 digital balance of 30 grams for each and inserted into High Density Polyethylene (HDPE) plastic[14] with size of 12 x 25 cm. Each carrier material was then sterilized by autoclave TOMMY (sis-31215) Japan and gamma rays Co-60 using Gamma Chamber 4000 A irradiator according to the treatment. Sterilization using autoclave was performed two times application at 121°C for 60 minutes with a 24 hour application interval [4]. The gamma Co-60 irradiation dose were 0 (control without treatment), 10, 20, 30, 40, and 50 kGy and the replication was five times. The parameters measured were metal solubilities such as available Fe$^{2+}$, available Mn$^{2+}$, available Zn$^{2+}$ measured with AAS Shimadzu AA-6300 using AAS method of DTPA extract. The methods used for these parameters based to Eviati and Sulaiman [15]. Group clusters in the carrier material using Shimadzu IRPrestige-21 Fourier Transform Infra-Red (FTIR) with KBr pellet method [16]. The solid sample of the carrier was crushed in a mortar with a small mortar together with the solid with dry KBr crystals (2 mg sample + 100 mg dry KBr). The mixture was then pressed using a hydrolytic press to form a transparent pellet. The thin sample tablets were then neutralized in the cell holder of the Shimadzu IRPrestige-21 FTIR spectrophotometer. The carrier sterility rate is calculated by the Total Plate Count by the pour plate method[17]. Put 90 ml of 0.85% NaCl solution in an Erlenmeyer and 9 ml of 0.85% NaCl solution in a test tube according to the required dilution ratio. Then sterilized by autoclave at 121°C for 20 minutes. Suspension of 10 grams of the sample carrier in 90 ml of sterile physiological, 1 ml of the soil suspension is taken and put into 9 ml of physiological (10$^{-2}$), the step is repeated until the required dilution. Choose the right range in the tube, then pipette 1 ml of the dilution result and put it in petri and nutrient agar, which is still liquid at a temperature of ± 500°C, wait until the surface is dry and then incubate. A total of 3 plates of nutrient agar (NA) were used for each level of dilution. The number of microbial colonies in the plate which had 30-300 colonies was then counted. Add the microbes from 3 plates and then determine the average number. Multiply the average amount by the reciprocal of the diluent ratio and multiply again by 10. Statistical analysis using SAS 9.1 software. The analysis used analysis of variance (ANOVA) at the 95% confidence level. The results

**Figure 1.** Soil samples point A = Pasar Jumat; B= Jasinga, C= Kalimantan; D = Rawa Pening.
of the analysis that showed a significant effect on the treatment were further tested with the DMRT (Duncan's Multiple Range Test) follow-up test at a 95% confidence level.

3. Results and discussion

3.1. The effect of sterilization method on available Mn$^{2+}$, Fe$^{2+}$, and Zn$^{2+}$

Table 1 showed the effect of sterilization method on the availability of Mn$^{2+}$ part per million (ppm). The autoclave method gave the highest increase on available Mn$^{2+}$. The increase in available Mn$^{2+}$ is significant and almost two fold in Jasinga Latosol, Pasar Jumat Latosol, compost, and Rawa Pening peat. While the increase available Mn$^{2+}$ in Kalimantan peat ranges from 1.63-fold. This research is support that sterilization with autoclave previous reported [18], that the sterilization using heat methods can increasing available Mn$^{2+}$ solubility in the soils. These results indicate that the change in the Mn$^{2+}$ available in the carrier material was higher by temperature (heat) if compared with gamma-ray irradiation of Co-60.

| Treatment | Jasinga Latosol ppm (Mn$^{2+}$) | Pasar Jumat Latosol | Kompos | Rawa Pening peat | Kaliman-tan peat |
|-----------|---------------------------------|---------------------|--------|-----------------|-----------------|
| Kontrol   | 55.38 c                          | 61.57 d             | 55.80 c | 60.7 b          | 19.71 c         |
| D10       | 55.78 bc                         | 61.58 d             | 56.53 c | 61.65 b         | 19.99 c         |
| D20       | 55.61 c                          | 61.48 d             | 61.85 b | 62.27 b         | 20.83 bc        |
| D30       | 56.05 bc                         | 63.50 b             | 59.23 bc| 62.57 b         | 21.43 bc        |
| D40       | 57.1 bc                          | 63.05 bc            | 61.50 b | 62.83 b         | 21.62 bc        |
| D50       | 57.77 b                          | 62.32 cd            | 59.42 bc| 62.18 b         | 22.97 b         |
| Autoclaved| 114.22 a                         | 127.00 a            | 121.32 a| 130.92 a        | 32.13 a         |

*The numbers followed by the same letter in the same column are not significantly different based on the 5% Duncan Multi Range Test (DMRT)

Available Fe$^{2+}$ in the carrier material also increased (Table 2) as well as the increase in available Mn. This experiment showed that available Fe$^{2+}$ increased in all carrier types, but the autoclave treatment gave the highest increase. Autoclave sterilization on Jasinga Latosol was able to increase available Fe$^{2+}$ as much as 145.67% from control, whereas with irradiation dose 50 kGy could be increased 103.84% from control. Autoclave sterilization on Pasar Jumat Latosol and compost was increased available Fe$^{2+}$ respectively as much as 289.03% and 6.41% of control. This study showed that sterilization method either through autoclave or by gamma Co-60 irradiation on low clay organic content in material can cause the increase of available Fe$^{2+}$.

As well as the increased of available Mn$^{2+}$ and available Fe$^{2+}$, available Zn$^{2+}$ is not consistent with increasing irradiated doses. This occured because available Zn$^{2+}$ in the soil solution is generally in the form of free ions and therefore sensitive to pH change [19]. However, the treatment of autoclave gave the highest increase in available Zn$^{2+}$. Autoclave treatment is more damaging to the material, this is indicated by more available metal elements such as Mn$^{2+}$, Fe$^{3+}$, and Zn$^{2+}$. The results of this study indicate that gamma irradiation of Co-60 relatively affects fewer chemical properties when compared with autoclave treatment. Research from Gournis et al. [20] and Plotze et al. [21] found that the effect of γ-irradiated doses on various minerals occurred due to the partial reduction of Fe (III) to Fe (II). This is what causes the Fe$^{2+}$ solubility to increase.
**Table 2.** The effect sterilization method on available Iron (Fe$^{2+}$).

| Treatment     | Jasinga Latosol | Pasar Jumat Latosol | Compost | Rawa Pening peat | Kalimantan peat |
|---------------|-----------------|---------------------|---------|------------------|-----------------|
| Control       | 47.38 e         | 12.13 d             | 58.35 e | 314.00 a         | 584.38 c        |
| D10           | 59.98 de        | 24.57 bc            | 62.73 de| 302.08 bc        | 643.98 b        |
| D20           | 72.40 cd        | 49.85 a             | 64.10 de| 305.77 b         | 667.09 ab       |
| D30           | 72.42 cd        | 19.81 c             | 73.35 bc| 297.45 cd        | 677.18 ab       |
| D40           | 83.65 bc        | 21.30 bc            | 66.97 cd| 295.68 d         | 680.81 a        |
| D50           | 96.58 ab        | 25.28 b             | 75.15 b | 297.77 d         | 670.96 a        |
| Autoclave     | 116.40 a        | 47.19 a             | 97.10 a | 310.85 a         | 688.32 a        |

*The numbers followed by the same letter in the same column are not significantly different based on the 5% Duncan Multi Range Test (DMRT)*

**Table 3.** The effect sterilization method on available Zinc (Zn$^{2+}$).

| Treatment     | Jasinga Latosol | Pasar Jumat Latosol | Compost | Rawa Pening peat | Kalimantan peat |
|---------------|-----------------|---------------------|---------|------------------|-----------------|
| Control       | 17.83 c         | 34.55 c             | 73.92 c | 25.29 d          | 3.20 c          |
| D10           | 27.99 c         | 83.90 b             | 76.18 c | 60.71 b          | 4.40 bc         |
| D20           | 19.08 c         | 91.14 ab            | 113.18 ab| 44.15 c          | 7.34 a          |
| D30           | 28.12 c         | 96.33 ab            | 99.91 b | 48.30 bc         | 7.20 a          |
| D40           | 42.01 b         | 93.87 ab            | 119.35 a| 84.30 a          | 6.61 ab         |
| D50           | 44.17 b         | 99.81 a             | 102.87 b| 85.35 a          | 9.25 a          |
| Autoclaved    | 64.17 a         | 89.67 ab            | 120.72 a| 89.82 a          | 9.36 a          |

*The numbers followed by the same letter in the same column are not significantly different based on the 5% Duncan Multi Range Test (DMRT)*

The effect of the sterilization method on the increase available of Fe$^{2+}$, Zn$^{2+}$, and Mn$^{2+}$ is caused by the damage that occurs in the structure of materials, especially degradable materials such as soil organic matter. Berns et al. [22] showed that sterilization with autoclave on some clay and organic effect on the damage of soil organic matter. Autoclave sterilization is not only capable of releasing organic carbon chelates that exist between soil particles but also on the surface of soil particles. Stevenson [23] reported that chelate by organic material can regulate the availability of metals in the soil. Organic materials function in the formation of chelat (organic bond) to the micro elements Fe$^{2+}$, Zn$^{2+}$, Mn$^{2+}$ [24]. Gamma Rays irradiation interaction with material are known to produce electron excitation, ionization, recombination, and fragmentation reactions.

According to Borrelly [25] the main species resulting from water radiolysis in the form of H·, OH·, and e-($aq$) can alter organic molecules. H· and OH· radicals react with organic molecules (RH) to produce free radicals (R·) and hydrogen gas (water) molecules. The reaction can be described as follows:

$$RH + H^· \rightarrow R^· + H_2$$
$$R^· + H^· \rightarrow RH$$
$$R^· + R^· \rightarrow RR$$
$$R^· + OH^· \rightarrow ROH$$
$$R^· + O_2 \rightarrow RO_2^·$$

This study showed that gamma irradiation of Co-60 influences the availability of Fe$^{2+}$, Zn$^{2+}$, Mn$^{2+}$, but the availability of these metal elements is inconsistent with the increased dose of Co-60 gamma-ray irradiation. Gournis [26] reported that there has been damage to the clay mineral structure due to gamma...
ray irradiation. This is due to the breaking of Si-O, Fe-O, and Al-O bonds, thereby changing the electronegativity in the iron gradient, and thus divide the quadrupol in the Mössbauer spectrum. In addition, gamma rays in clay can cause migration of small amounts of inter-layer cations, such as H\textsuperscript{+} and Li\textsuperscript{+}, leaving a gap in the hexagonal layer of oxygen in the clay layer.

The results showed that either sterilization using autoclave or by Co-60 gamma-ray irradiation was effective in decrease the microbial number. Microbes can still survive at the dose of 20 kGy on Jasinga Latosol, Pasar Jumat Latosol, and Kalimantan peat. Sakr et al. [27] showed that *Streptomyces canarius* can still survive at a dose of 25 kGy, *S. chibaensis* and *S. albidosfuscus* still survive at a dose of 20 kGy while *S. ambofaciens* at a dose of 15 kGy. In this study the materials became sterile when the dose was increased from 30 kGy to 50 kGy. The number of microbes that died at doses of 30, 40, and 50 kGy reached 100%. This result is consistent with Stunda et al. [28] who reported that no more microbes were found in Baldone's peat after being given Co-60 gamma rays irradiation at the dose of 30 kGy.

The resistance of each microb is different, so only microbes with high tolerance are able to withstand exposure to high temperatures and Co-60 gamma-ray irradiation. Yardin [29] reported that microbes resistance to irradiation is highly dependent on the ability of microbial physiology, spore-forming ability, substrate availability, environmental factors, temperature, and humidity. The mechanism of decreasing the number of microbes by autoclave is through the direct destruction of microbial cells by water molecules in steam form. Hot steam enters and break the cell wall, its causes hugh damage to the organelles and also to the cell wall so that the cell undergoes lysis. Heat formed from autoclave can denature the protein in living organisms and thus the microbial metabolic process stops and results in microbial death.

| Treatment | Jasinga Latosol | Pasar Jumat Latosol | Compost | Rawa Pening peat | Kalimantan peat |
|-----------|----------------|---------------------|---------|-----------------|-----------------|
| D0        |1.33x10\textsuperscript{6} |2.39x10\textsuperscript{7} |1.34x10\textsuperscript{7} |1.25x10\textsuperscript{7} |2.73x10\textsuperscript{7} |
| D10       |4.83x10\textsuperscript{4} |2.88x10\textsuperscript{5} |1.41x10\textsuperscript{4} |4.87x10\textsuperscript{4} |5.13x10\textsuperscript{4} |
| D20       |6.10x10\textsuperscript{4} |6.47x10\textsuperscript{4} |0         |0                |1.13x10\textsuperscript{4} |
| D30       |0            |0                 |0         |0                |0                |
| D40       |0            |0                 |0         |0                |0                |
| D50       |0            |0                 |0         |0                |0                |
| Autoclave |0            |0                 |0         |0                |0                |

Directly Co-60 gamma rays can damage DNA chains in microbes through the breakdown of sugar phosphate (through carbon chain breaking). Microbes exposed to Co-60 gamma rays may repair damage to one of the DNA chains, survive, and live by mutation. If microbials are unable to repair damaged DNA chains, especially the breakup of 2 pairs of DNA chains the microbials will not survive and will be dead.

Indirectly, the water contained in sterilized materials with Co-60 gamma rays plays a role in damaging microbial cells. The effect of irradiation on microb can occur because energy from Co-60 gamma rays is able to ionize water molecules. Water molecules exposed to gamma-ray Co-60 can produce free radical ions that can bind to various compounds [30]. Compounds contained in the microb when binding to free radicals are likely to produce compounds that are toxic. According to Borrely et al. [26] if the gamma rays contact to pure water, then every 100 eV of absorbed energy will produce 2.7 OH radicals, 2.6 e aq- (hydrated electrons), 0.6 H radicals, 0.45 molecules of H\textsubscript{2}, and 0.7 molecules of...
H$_2$O$_2$. The compounds formed from these free radicals can kill microbial, including the possibility of H$_2$O$_2$ formation. Microbes that do not have genes with the ability to neutralize hydrogen peroxide will not survive and will die.

In this study it is known that the sterilization method can change the chemical properties of materials such as Fe$^{2+}$, Mn$^{2+}$ and Zn$^{2+}$ (see Table 1, 2, and 3), these can also one of the factors that could be the cause of microbial death. Microbes that are not resistant to environmental changes are particularly sensitive to changes in composition and the amount of available Fe$^{2+}$, Mn$^{2+}$ and Zn$^{2+}$ will die. Porcheron et al. [31] reported that in all microorganisms, metal ions are essential for survival in the environment in which they live. Metallic ions are required in many biological processes as components of metalloproteins and serve as cofactors or structural elements for enzymes. However, it is very important for bacteria to ensure absorption and availability of incoming metals according to physiological needs, otherwise there is an imbalance of homeostasis so that the metal is toxic.

3.2. Fourier Transform Infra-Red (FTIR) on carrier materials
The raw infrared spectrum of peat soils is presented in Table 8 [46] below:

| peak (cm$^{-1}$) | Functional groups                                           |
|-----------------|-------------------------------------------------------------|
| 1265.3          | C-O from ester, ether, phenol                               |
| 1381.03         | Salt of carboxylic acid                                     |
| 1435.04         | Aliphatic C-H deformation group                             |
| 1620.21         | C = C in cyclic and acyclic components, benzene rings       |
| 1712.79         | C = C from carboxylic acids, cyclic and acyclic aldehydes, ketones, and quinones |
| 2854.65         | Carboxylic ions                                            |
| 2924.09         | Aliphatic groups C-H, CH2, C-H3                            |
| 3425.58         | H-OH bond group, OH free                                   |

The FTIR observations in Figure 2 showed that in all types of carrier tested have a carbonyl group (C = O) in the 1800-1600 cm$^{-1}$ region, this is indicated by the appearance of the peak graph in the area. The presence of a peak in the region of 1800-1600 cm$^{-1}$ indicates that the carrier sample has the possibility of containing carboxylic acids, amideesters, anhirides, aldehydes, and ketones. There is a difference in each peak of carrier material which sterilized process either by Co-60 gamma ray irradiation or by autoclave.

All tested materials have undergone a change in chemical properties. This is indicated by the change in the peak area, especially in the area of 3400-3700 cm$^{-1}$, which is the area associated with the H-group, OH group, and OH-free (radical) bonds. Fig. 2a (Jasinga Latosol) and Fig. 2b (Pasar Jumat Latosol) showed that the 3400-3700 peak area decreased in irradiation treatment at a dose of 40 kGy, 50 kGy, and autoclave when compared with the control. The appearance of peak area changes in this hydroxyl group indicates a change in the OH-composition. The changes in OH-composition can trigger reactions with other functional groups. These observations confirm that Co-60 gamma-ray irradiation and autoclave affects the change of functional group composition in the carrier especially in the hydroxyl group composition. This is in accordance with Ward [30] which reported that free radical formation can occur through contact of Co-60 gamma radiation with water contained in the material.

It generally showed that 2,800-3,000 cm$^{-1}$ area also shows changes in the peak area, changes may occur in the composition of the carboxylic group and the aliphatic group in the carrier. In this study, the peak-analysed carrier material had almost the same, but after the chromatogram was compared with combining it, there were new peaks that were missing and / or emerging. Although the missing / emerging peak in Autoclave treatment (JAS A, LPJ A, KOM A, GRP A, and GK A) is not the dominant
peak but the intermediate peaks, it is sufficient to describe that the carrier chemical structure and composition changes. The peak size in the control (JAS 0, LPJ 0, KOM 0, GRP 0, and GK 0) spectrum is a direct indicator of the number of clusters in the material. The changes in the chemical structure indicate that the process can change compounds, especially organic compounds. These changes may cause changes in chemical properties in the carrier as a whole.

Figure 2. Analysis of functional groups using IR solution software of carrier materials which have been treated by Co-60 gamma irradiation and autoclave. Remarks: (a) Jasinga Latosol (JAS) treated with sterilization dose 0-50 kGy and autoclave (JAS 0 - JAS 50; JAS A); (b) Pasar Jumat Latosol (LPJ 0 - LPJ 50; LPJ A); (c) Compost (KOM 0 - KOM 50; KOM A); (d) Rawa Pening peat (GRP 0 - GRP 50; GRP A); (e) Kalimantan peat (GK 0 - GK 50; GK A).
4. Conclusions
This research showed that treatment of sterilization both Co-60 gamma ray irradiation and autoclave can change chemical properties of the carrier material. However, the effect of temperature 121°C through autoclave sterilization is more destructive to the material compared to irradiation sterilization with the Co-60 gamma rays. Sterilization with autoclave is able to increase metal solubility reaches two times from control. This chemical change is also indicated by the results of material analysis using FTIR. Co-60 gamma irradiation doses of 30 kGy can effectively 100% sterilized to five carrier types. Sterilization with Co-60 gamma rays is better than autoclave, due to less chemical composition changes.

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