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Review: Synthesis of Silver Nanoparticles in Several Methods

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AbstractOne of the metal nanoparticles that is widely used is silver nanoparticles (AgNPs) because of their potential applications in various scientific fields. Silver is a material that is abundant in nature, attractive, and inexpenive. This paper aims to conduct a literature review from 12 papers on the synthesis of AgNPs from 2007 to 2020. Based on several articles, there are 3 methods for synthesizing AgNPs: physics, chemistry, and biology. Physical methods use laser ablation and arc-discharge, chemical methods use gamma irradiation and chemical reduction, and biological methods use bacteria, plants, and fungi. The best method is biosynthesis because it is facile, safe, cost-effective, and environmentally friendly. AgNPs produced from biosynthesis using plants is better than using bacteria and fungi because it produces pure and small particle of AgNPs.

Keywords: *Silver nanoparticles, Nanoparticles synthesis, Physics method, Chemical method, Biosynthesis*

1. Introduction

Currently, metal nanoparticles (MNPs) are widely used in science and technology because they exhibit new and improved properties compared to their bulk form [1]. Their small size and large surface area are some of the advantages of MNPs, so that they quickly penetrate the human body through the lungs and intestines [2,3]. Silver nanoparticles (AgNPs) are one of the MNPs that are commonly used [4]. Silver is a material that is abundant in nature, attractive, and inexpenive. Previous research has shown that the physical, optical, and catalytic properties of AgNPs are greatly influenced by their shape, size, particle distribution, and surface which can be carried out by a variety of synthesis methods, reducing agents, and stabilizers [5]. Several studies have reported that AgNPs has strong antimicrobial, antibiotic, anti-inflammatory, and anticancer activity, so it is widely used in various scientific fields, such as optoelectronics, optics, pharmaceutical sciences, medical, and others [6,4].

AgNPs can be synthesized using several methods, namely physics, chemistry, and biology. Each method has common problems such as cost, scalability, particle size, size distribution, etc. Synthesis of AgNPs by physical methods usually requires large energy, high temperature, and expensive equipment [7]. The examples are laser ablation [8,9] and arc-discharge [10,11]. The most commonly used methods for synthesisizing AgNPs are chemical methods, such as gamma irradiation [12,13], and chemical reduction [14,15]. This method can produce pure AgNPs by using a simple tool [16], however, the materials used are dangerous. The biological method of synthesis AgNPs (biosynthesis) is an environmentally friendly, safe, inexpensive, and easy method. Several plant extracts and

microorganisms such as *Novosphingobium sp* THG-C3 [2], *Pseudoduganella eburnea* MAHUQ-39 [6], Psycrophilic and mesophilic [17], *Bacillus cereus* [18], *Aspergillus tereus* [19], *Trichoderma Reesei* [20], *Sambucus nigra L* [21], *Lantana camara L* [22], *Myrmecodia pendans* [23], and cinnamon [24] has been used successfully for the biosynthesis of AgNPs. The use of plant extracts shows a shorter biosynthetic process compared to microorganisms [25]. There are several review paper that discuss the synthesis of AgNPs, such as Jorge de Souza [26]. However, this review only covers biosynthetic methods. Although many researchers have reported on the synthesis of AgNPs, there have been limited reviews of the synthesis of AgNPs.

The purpose of writing this review is to discuss several methods of synthesizing AgNPs with various silver sources. In this review paper, 12 articles from 2007 to 2020 were used. The synthesis methods discussed are laser ablation, arc-discharge, gamma irradiation, chemical reduction, and biosynthesis using plants, bacteria, and fungi, along with their respective advantages and disadvantages. Based on the information in this paper, it is hoped that a safe, inexpensive, and easy AgNPs synthesis method can be widely used in various industrial fields.

2. Synthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) are the most studied and used nanoparticles [27]. Synthesis methods of AgNPs can be divided into three categories, namely chemical methods, physical methods, and biological methods [28]. These categories can be seen in Figure 1. Several methods will be discussed to determine which method is most effective in synthesizing AgNPs.

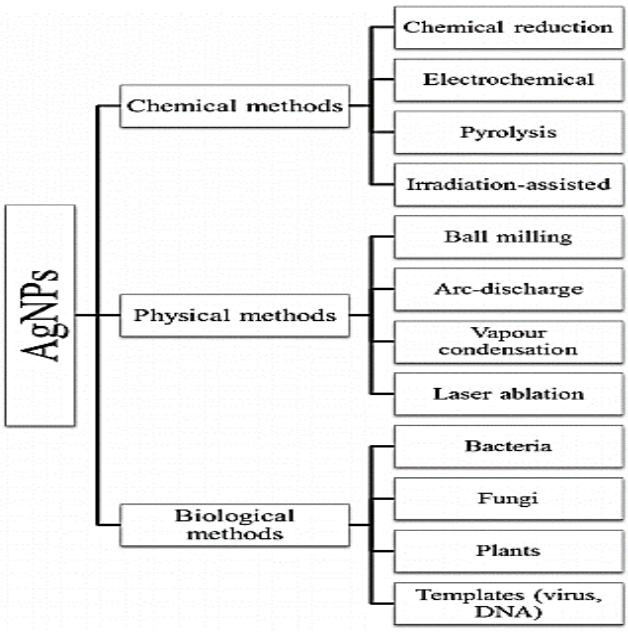

Figure 1. AgNPs synthesis methods [26].

2.1 Gamma Irradiation

AgNPs can be synthesized by several physico-chemical methods, such as microemulsion [29], electrochemistry [30], chemical reduction [31], irradiation [12], and metal ablation [32]. The irradiation method has the following advantages [33,34]:

1. The reducing agent produced is uniform in the medium.
2. The metal nanoparticles produced are purer and more stable.
3. No impurities, such as metal oxides.
4. Synthesis can be carried out at ambient temperature.

Eghbalifam et al. [12] reported the synthesis of AgNPs by the gamma irradiation method using polyvinyl alcohol (PVA), sodium alginate, isopropanol, and $AgNO_3$. Sodium alginate is a type of natural polymer consisting of α-L-guluronate and β-D-manuronate with excellent biocompatibility [35]. The irradiation process was carried out at different doses, namely 5, 10, and 15 kGy using γ ^{60}Co rays with a dose rate of 3.19 Gy/sec at room temperature [12].

This method produces AgNPs measuring 43.8 to 164 nm with an average size of 69.8 nm. At the 5 kGy irradiation dose the average size of AgNPs was 100 nm, while at 15 kGy it was about 80 nm. This indicates that the particle size of AgNPs decreases with increasing irradiation dose. At higher irradiation doses, the hydrogel formation increases so that the solution becomes thicker. The addition of PVA serves to stabilize AgNPs and prevent agglomeration of silver particles [12]. Some of the advantages of using PVA are that it is inexpenive, non-toxic, water-soluble, biocompatible, and biodegradable. Furthermore, the regular linear structure of PVA with a large number of hydroxyl side groups on the main chain shows excellent hydrophilicity and reactivity [36].

Based on cyclic voltammetry and IR spectrophotometer, the hydroxyl groups of the PVA molecule can coordinate with AgNPs and produce stable colloids. Thus, the AgNPs/PVA colloid dispersion can be used for the production of Ag/PVA hydrogels in various forms (thin films, discs and sheets). The hydrogel can be used in biomedical applications as an antimicrobial treatment [37]. While sodium alginate is used because it is environmentally friendly and suitable for pharmaceutical and biomedical applications [35]. The release of silver at the variation of the gamma irradiation dose is shown in Figure 2. Synthesis of AgNPs in the study of Hosny et al. [13] using several materials which are $AgNO_3$, NaOH, tryptic soy broth, and honey. This experiment compared the synthesis results of AgNPs obtained from variations in pH and gamma irradiation. To determine the effect of differences in pH, the atmosphere of the solution was made different using NaOH. As for the pH variations of the solution were 6.00; 6.50; 7.00; 7.50; 8.00; 8.50; 9.00; 9.50; and 10.00. The formation of AgNPs is characterized by a change in the color of the solution from transparent to yellowish. In another experiment, gamma irradiation was applied in various doses (1, 5, 10, 15, 20, 25, and 30 kGy) using γ ^{60}Co rays, while the pH of was maintained at 3.9. The synthesis of silver nanoparticles produced from 5 kGy gamma irradiation is about 2.60-10.10 nm with a dominant particle size of 4.187 nm. Meanwhile, AgNPs obtained from the pH variation test showed the best results at pH 10 with a particle size range of 7.5–21 nm and a dominant particle size of 11.7 nm.

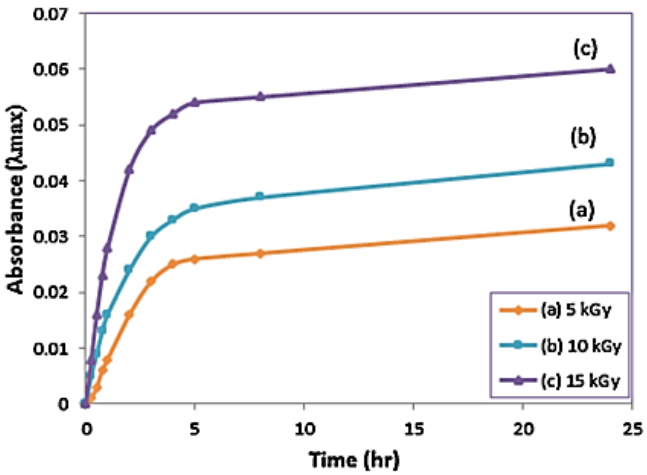

Figure 2. The release of silver from the composites at various doses of gamma irradiation and an Ag ion concentration Ag^+ 1.33 wt.% [13].

The gamma irradiation reduction method provides several advantages over conventional methods, which is the method is simple, the metal nanoparticles produced are very pure and stable, and there are no unwanted side products, so they are harmless and environmentally friendly

[38]. However, other studies have shown that gamma irradiation is proven to produce free radicals in the form of hydroxyl and radiotoxic. The mutagenic effect caused can lead to cancer [39].

2.2 Chemical Reduction

2.2.1 $NaBH_4$ in graphite powder

In the synthesis of AgNPs with the chemical reduction method, reducing agents, stabilizers, and silver nitrate solutions are used as precursors. This method is the most effective method for producing AgNPs, because the synthesis process is easy, fast, inexpensive, and uses low temperatures [40].

Graphene is a new material in the field of nanotechnology that is currently being developed, because it has good electrical conductivity, thermal conductivity, and tensile strength, and is thin in size. Graphene Oxide (GO) has the ability to inhibit bacterial growth by forming AgNPs/GO nanocomposites [41]. The use of GO as a stabilizer able to prevent the agglomeration of AgNPs particles.

In 2011, Das et al. [14] reported the results of a study on the synthesis of AgNPs using GO as a substrate and stabilizer. While $NaBH_4$ is used to reduce $AgNO_3$ on the surface of the GO matrix with in situ reduction. To 25 mL of GO solution was added with $AgNO_3$ solution with various concentrations, namely $1x10^{-3}$, $2x10^{-3}$, $4x10^{-3}$, dan $8x10^{-3}$ mol dm^{-3}. The solution mixture is stirred for 30 minutes at ambient temperature. Then 10 mL of 0.01 mol dm^{-3} $NaBH_4$ was added slowly and again stirred for 6 hours at room temperature for a total reduction.

The mixture of GO suspension and $AgNO_3$ solution changes color to dark brown until gray, depending on the concentration of $AgNO_3$. The size and shape of the AgNPs are influenced by the concentration of the $AgNO_3$ solution. Analysis on the UV-Vis Spectrophotometer showed the presence of a plasmon surface band at 400 nm when the $AgNO_3$ concentration was $1x10^{-3}$ and $2x10^{-3}$ mol dm^{-3}. However, at higher $AgNO_3$ concentrations, the band shifted to larger wavelengths to 407 nm for $4x10^{-3}$ mol dm^{-3} and 417 nm for $8x10^{-3}$ mol dm^{-3}. XRD pattern at the diffraction peak 2θ, showed value at 38.1°; 44.3°; 64.5°; and 77.5° corresponding to the FCC planes of the AgNPs which are (111), (200), (220), and (311). TEM analysis of the silver nanoparticles in Figure 3 shows the distribution of particles ranging from 5–25 nm in diameter. Figure 3 (a) shows that the smaller particles are spherical, while the larger nanoparticles are elongated. The elongated shape is the result of the aggregation of two or more particles shown in Figures 3 (b) and (d). $AgNO_3$ $1x10^{-3}$ mol dm^{-3} solution mostly produces spherical nanoparticles. As observed in Figures 3 (c) and (d), the synthesis of AgNPs using a salt concentration of Ag $8x10^{-3}$ mol dm^{-3} resulted in various particle shapes. At concentrations of $AgNO^3$ above $2x10^{-3}$ mol dm^{-3}, the resulting nanoparticles appear to clump together to form larger particles. Figures 3 (c) and (e) show the SAED pattern of AgNPs with $AgNO_3$ concentrations of 8×10^{-3} and 1×10^{-3} mol dm^{-3}. The analysis using SAED showed that the crystalline nature of AgNPs was crystalline [14].

2.2.2 Trisodium citrate and ascorbic acid

Suriati et al. [15] synthesized AgNPs by reducing $AgNO_3$ with trisodium citrate and ascorbic acid as surfactants. The chemical reduction method in the manufacture of AgNPs has several advantages, which are quite stable, economical, versatile, and the size and shape of the nanoparticles can be controlled, and can also be used on a large scale. The properties and capabilities of the reducing agents affect the size, shape, and size distribution of the resulting particles [15]. A strong reducing agent such as $NaBH_4$ can produce small particles that are well dispersed [42]. In the synthesis process, a reaction rate that is too fast causes the formation of large amounts of metal nuclei and results in small particles. On the other hand, particle agglomeration will occur if the reaction rate is too slow. Other than that, the choice of surfactant is very important because it determines the stability, solubility, reactivity, dispersibility, size, and shape of the nanoparticles obtained [15].

In the research conducted by Suriati et al. [15] The materials used to synthesize AgNPs are silver nitrate ($AgNO_3$), trisodium citrate ($C_6H_5O_7Na_3$), and ascorbic acid ($C_6H_8O_6$). The effect of variations in the concentration of trisodium citrate and ascorbic acid on the size and morphology of AgNPs was

observed. During the synthesis process, the trisodium citrate reducing agent directly reduces Ag^+ ions and produces Ag^0. The resulting Ag acts as a nucleation center and catalyzes the remaining metal ions in solution. The coalescence of atoms causes the formation of metal groups which are normally stabilized by ligands, surfactants, or polymers. In this study, ascorbic acid acts as a surfactant which is adsorbed onto the surface of the Ag atom, so that agglomeration of the nanoparticles can be prevented. The color change in the solution indicates the formation of AgNPs. Initially, the solution is light yellow, then turns yellow, and turns greenish before stabilizing [15].

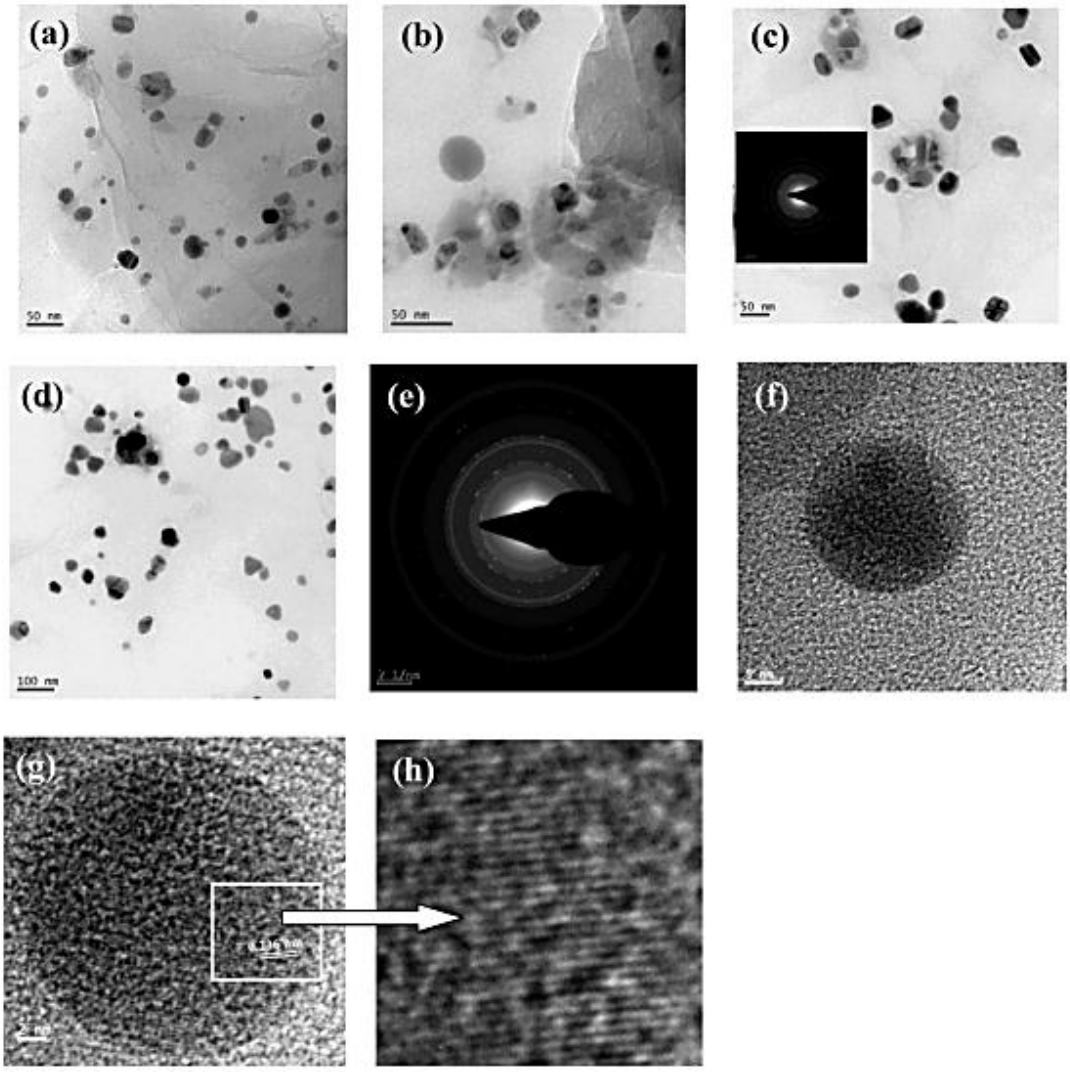

Figure 3. TEM image of synthesized AgNPs [14].

Based on TEM analysis, the AgNPs produced from trisodium citrate dihydrate has the form of quasi-spherical particles and other shapes, such as well-dispersed polygons. The mean size of AgNPs at the trisodium citrate dihydrate concentrations of 4.00 and 8.00 mM was 38.53 and 36.32 nm, respectively. The analysis results showed that when the concentration of trisodium citrate dihydrate increased from 4.00 to 8.00 mM, the histogram of the AgNPs particles experienced a narrowing of the size distribution, from 20–65 nm to 20–50 nm [15].

Whereas in the synthesis of AgNPs using ascorbic acid, the final color of colloidal silver changed from light yellow to greenish. The mean size of the AgNPs increased with increasing ascorbic acid concentration, from 37.24 nm at 1.00 mM to 47.28 nm at 4.00 mM [15]. The higher concentration of

stabilizers can reduce the dispersivity of the nanoparticles in solution, this is indicated by the increasing size of the AgNPs.

2.3 Laser Ablation

Laser ablation is an environmentally friendly method for synthesizing AgNPs by physical method. The result is a silver nano colloid which is stable in a variety of dispersing media without the use of metal precursors and reducing agents [43]. In the laser ablation method, a Neodymium-yttrium Aluminum Garnet (Nd: YAG) laser with a wavelength of 532 or 1064 nm is generally used and deionized water as the medium. Water is used in the ablation process because it is inexpenive, safe, exhibits a high heat capacity, and does not absorb laser light [44].

Zafar et al. [9] synthesized AgNPs using an Nd: YAG laser with a wavelength of 1064 nm. The materials used were AgNO3 and NaBH4. The laser operates at a repetition rate of 10 Hz with 1000 shots on the first sample and 2000 shots in the second sample. This synthesis produces round AgNPs with a size of 10–40 nm. AgNPs formed by 1000-shot laser ablation produces particle sizes in the 20 to 35 nm range with a maximum particle count of 25–30 nm. Meanwhile, AgNPs formed by laser ablation of 2000 shots produced particle sizes in the range of 10 to 35 nm with a maximum particle number of 20–30 nm. Based on the XRD test, the AgNPs produced have a polycrystalline structure.

In 2011, Darroudi et al. [8] used gelatin as a stabilizer. Gelatin is a good stabilizer because it prevents agglomeration [43]. The laser ablation method using gelatin stabilizers produced AgNPs with a size range of 8.90–13 nm. In general, the mean particle size increases with increasing laser fluency [5]. This is evidenced by the research results of Nancy et al. [45] shown in Figure 4. Based on the results of TEM analysis, the size of AgNPs in the lowest laser fluency (Figure 4a) was 5 nm, while in the highest laser fluency (Figure 4 f) was 35 nm [45].

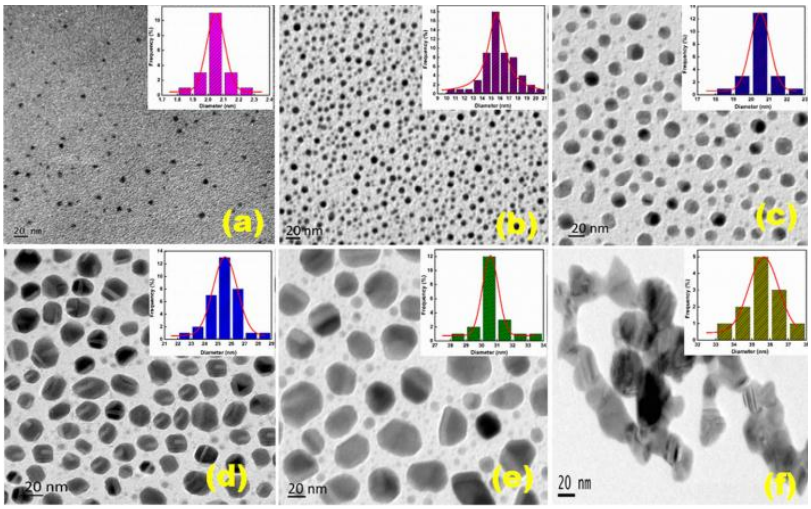

Figure 4. TEM image of the synthesized AgNPs in different laser fluency (a) 5.30 J cm^{-2}; (b) 26.30 J cm^{-2}; (c) 31.60 J cm^{-2}; (d) 36.80 J cm^{-2}; (e) 42.10 J cm^{-2}; dan (f) 47.40 J cm^{-2} [45].

One of the advantages of laser ablation over other conventional methods is that it does not use chemical reagents. Therefore, the colloid produced is very pure and there are no by-products [46]. However, laser ablation requires a large amount of energy to operate [44].

2.4 Arc-discharge

One of the physical methods of AgNPs synthesis is arc-discharge by releasing the arc in solution. The arc-discharge method is simple, but it is difficult to obtain a well-structured result [11]. The arc-discharge method is affected by several conditions, such as the gap between the electrodes, the electrode diameter, the type of medium (liquid or gas) to store the electrodes, pressure, electric current, power supply type, voltage, temperature, and cathode shape [11]. Generally, this method uses two

silver electrodes that are heated at a high temperature and converted into AgNPs. However, the electrode used in Ashkarran's research [10] was a titanium electrode. The titanium electrode was chosen because it is inactive and has a high melting temperature. Using an electron-rich plasma release source to reduce $AgNO_3$ is a bottom-up approach that results in smaller particle sizes [10].

An arc current of 15 A is applied between the two titanium electrodes in the $AgNO_3$ solution with an arc duration of 6 minutes. The anode and cathode used were titanium wire, 2 mm in diameter, and a purity level of 99.99%. The reduction process of AgNO3 occurs because of the arc discharge between the titanium electrodes which are separated from each other [10].

The change in the color of the solution to yellow indicates that all Ag^+ ions are reduced and AgNPs have been formed. The number of electrons injected from the release zone into the solution affects the reduction of the $AgNO_3$ molecule. The results of the UV-Vis spectrophotometer analysis showed that the absorption peak was around 410 nm which is the absorbance characteristic of the AgNPs plasmon. To reduce Ag^+ to Ag^0, a source of electron-rich plasma regions is used, according to equation [10]:

$$AgNO_3 \rightarrow Ag^+ + NO_3^-$$

$$Ag^+ + e^- \rightarrow Ag$$

TEM analysis is used to visualize the size and shape of the nanoparticles produced. Based on the results of the analysis, the average size obtained is about 18 nm with an average diameter of 27 nm and a narrow distribution of about 14 nm. [10].

Figures 5 a and b show the SEM analysis results of AgNPs synthesized at an arc current of 15 A with an arc duration of 1 and 6 minutes. The number of electrons injected from the release zone into the solution with an arc duration of 6 minutes is more than the arc duration of 1 minute, resulting in more AgNPs.

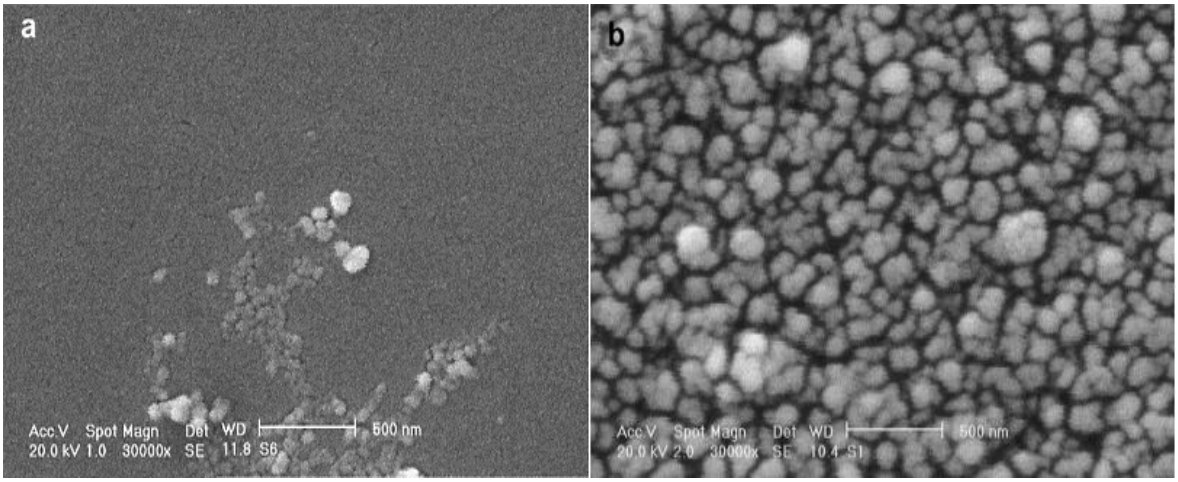

Figure 5. SEM image of AgNPs at 15 A arc current with arc duration (a) 1 minute and (b) 6 minutes [10].

El-Khatib et al. [11] also synthesized AgNPs using the arc-discharge method. In this study, ethanol was used as the medium and electrodes with different shapes, specifically cylindrical form (system A) and disc shape (system B). The transformation of silver metal vapor into nanoparticles goes through three stages, namely nucleation, group growth, and condensation in ethanol. Ethanol gives better AgNPs results, which are round and small in size. The resulting AgNPs have an average particle size in system A of 22 nm and system B of 26 nm. Based on the zeta potential analysis, system A is better than system B because the particle distribution is more homogeneous and the number of particles produced is higher (system A 46 mg/minute and system B 34 mg/minute).

The main advantage of the arc-discharge method using titanium electrodes is the ability to reduce $AgNO_3$ by titanium electrodes more effectively than silver electrodes. Also, this method uses simple equipment, no need for vacuum equipment, fewer synthesis steps, low impurities, cost-

effective, and produces good nanoparticles. The drawback of this method is that it takes a long time for the electrode to reduce $AgNO_3$ (Table 1).

2.5 Biosynthesis from Plants

A widely studied alternative method for the synthesis of AgNPs is biosynthesis. As already mentioned, the physical and chemical synthesis methods are require high energy and use hazardous chemicals. So those other techniques that are more environmentally friendly, such as involving plant extracts [47], microorganism [48], and natural polymers [49] are needed.

2.5.1 *Sambucus nigra* L.

In 2016, Moldovan [21] reported that the biosynthesis of AgNPs from the fruit of *Sambucus nigra* L. was environmentally friendly, fast, and inexpenive. The fruit extract of *Sambucus nigra* L. contains bioactive compounds that act as a bioreductor and capping agent to avoid the use of toxic chemicals.

In this study, $AgNO_3$ was reduced using 30 mL of *Sambucus nigra* L. fruit extract mixed with 70 mL of an aqueous solution of $AgNO_3$ 1 M. During the reduction process, the pH of the mixture was adjusted by adding 0.01 N NaOH solution. The reduction process is indicated by a change in the color of the solution to brown after 10 minutes. Then, the resulting nanoparticles were purified by centrifugation at 10000 rpm for 20 minutes, then washed with aquabidest, and stored at 4˚C [21]. The formation of AgNPs is indicated by a change in the color of the water extract from pink-purple to brown.

Figure 6 shows the obtained polydispersion AgNPs with a round shape. These AgNPs ranged in size from 8 to 33 nm in diameter with an average size of 26 nm from the TEM analysis. The TEM image shows the presence of a thin layer on the surface of the AgNPs which may be caused by the organic molecules from the extract which also acts as limiting and stabilizing agents. Based on FTIR analysis, the peak absorption of OH 3388 cm^{-1} and C-O 1251 cm^{-1} for phenol groups, which means that there are bioactive compounds which are derivatives of flavonoids in fruit extracts [21].

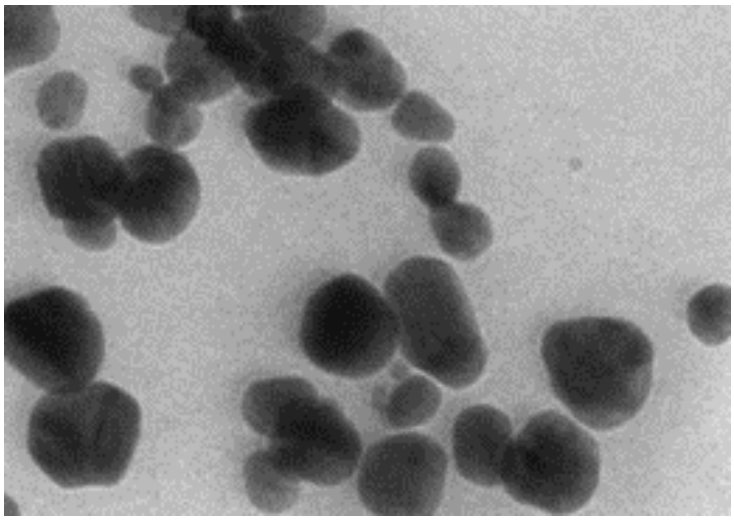

Figure 6. TEM results of the round AgNPs polydispersion [21].

Zeta potential analysis of photosynthesized AgNPs showed a sharp peak with a negative value of -20.90 mV. The negatively charged surface of the nanoparticles showed that anionic capping agents such as polyphenols from the fruit extract of *Sambucus nigra* L. were coordinated with the outer surface of the AgNPs. The Zeta potential value is used to ensure the stability of the colloidal silver dispersion [50].

2.5.2 *Lantana camara* L.

In 2016, VP and Muthukumar K [22] reported the biosynthesis of AgNPs from *Lantana camara* L. using ultrasound. Ultrasound is used to damage cells so that cells can release intracellular contents.

Biosynthesis using ultrasonic can reduce reduction time, increase the reaction rate of AgNPs synthesis, and environmentally friendly.

Table 1. The advantages and disadvantages of each AgNPs synthesis methods

Method	Material	Result	Advantages	Disadvantages	Reference
Biosynthesis using bacteria	*Novosphingobium sp* THG-C3	AgNPs with the size 8-25nm and spherical morphology.	Does not use chemicals and toxic solvents (safe), simple synthesis steps, environmentally friendly, and inexpenive.	The purity of the resulting nanoparticles is unknown.	[2]
	Pseudoduganella eburnea MAHUQ-39	AgNPs with the size 8-24nm, particle size is 141.2 nm and spherical morphologically	The synthesis process is easier, faster, environmentally friendly and inexpenive so that can be used for high production.	The purity of the resulting nanoparticles is unknown.	[6]
	Five types of bacteria psyrochilic (*Pseudomonas antarctica*, *Pseudomonas proteolytica*, *Pseudomonas meridiana*, *Arthrobacter kerguelensis*, and *Arthrobacter gangotriensis*), two types of bacteria mesophilic (*Bacillus indicus* dan *Bacillus cecebensis*)	AgNPs with a minimum size of 6.1 ± 2.8 nm to a maximum of 12.2 ± 5.7 and spherical morphology.	Environmentally friendly, does not use dangerous chemicals, inexpenive, the synthesis time is shorter than previous studies using *B. licheniformis*, *B. subtilis*, and *F. oxysporum*, and the stability of synthesized AgNPs is quite good, which is in more than 24 hours.	Not all synthesized AgNPs were stable enough, the AgNPs synthesized by *P. Antarctica* was thought to be unstable due to aggregation.	[17]
	Bacillus Cereus	AgNPs with an average size of about 15 nm.	Environmentally friendly and the resulting AgNPs canbe used as antibacterial.	The time it takes is long.	[18]
Biosynthesis using fungi	*Aspergillus tereus*	AgNPs with an average size of 4.3 nm, spherical morphology and polydispersion.	Fungi produces more bioactive compounds when compared to bacteria so they are more suitable for large-scale production and easier synthesis.	The results of AgNPs were not pure because they were still mixed with biomass (observed by XRD).	[19]
	Trichoderma Reesei	AgNPs with an average size of 5-50 nm with varies morphology.	The formation of extracellular enzymes *Trichoderma reesei* is very large, up to 100 g/L, much higher than other fungi, and is an environmentally friendly and	The morphology of the silver nanoparticles was irregular.	[20]

			biologically safe fungi. Also, it requires low cost, and the resulting nanoparticles are quite stable.		
Biosynthesis using plants	*Sambucus nigra L*	AgNPs with a mean size of 26 nm, a slightly spherical morphology, and aggressed.	Environmentally friendly and inexpenive.	The time it takes is long.	[21]
	Lantana camara L	AgNPs with an average size of 33.8 nm and spherical morphology.	The application of ultrasound effectively increases the reduction process in several folds. The sonication method can speed up the contact time between the sample and the solvent even at room temperature so that it shortens the time, requires less solvent, and results are more accurate and precise.	Expensive and need a process purification.	[22]
	ant nests (*Myrmecodia pendans)*	AgNPs with a mean size of 78.3 nm (with stabilizers) and 76.1 nm (without stabilizers).	Environmentally friendly, fast reaction, simple synthesis step, and inexpenive.	The availability of ant nests is running low and the resulting particle size is quite large.	[23]
	Cinnamon	AgNPs with a size of 50-70nm.	The AgNPs produced are stable and environmentally friendly.	Produce a large particle size.	[24]
Chemical reduction	Grapheine Oxide Suspension (GrO)	AgNPs with a size of 5-25 nm.	The resulting AgNPs can be used as antimicrobials against *E. coli and P. aeruginosa* whose effectiveness depends on the size and shape of the Ag nanoparticles.	The size and shape of the nanoparticles depend on the concentration of the Ag salt. The shape of the nanoparticles was irregular at the higher concentration of the Ag salt (8 × 10 mol dm).	[14]
	$AgNO_3$, Trisodium Citrate ($C_6H_5O_7Na_3$) dan Ascorbic Acid ($C_6H_8O_6$)	AgNPs using trisodium citrate reducers with different concentrations produced nanoparticles ranging in size from 36.32 to 38.53 nm, meanwhile,	The method is stable, economical, versatile, and easy to control the size and shape of the nanoparticles.	Produces a large size of silver nanoparticles.	[15]

		AgNPs using ascorbic acid reducers with different concentrations produced nanoparticles ranging in size from 37.24 to 47.28 nm.			
Laser ablation	Ag^+, $NaBH_4$, dan Deionized water	AgNPs with a size 10–40 nm and spherical morphology.	Does not use chemical reagents, so the resulting colloid is very pure with unique surface characteristics without side products.	Need a large amount of energy.	[9]
	Ag^+, Deionized water, dan Gelatin.	AgNPs range in size from 8.9 to 13 nm.	Small particle size and pure.	Need a large amount of energy.	[8]
Arc-discharge	Titanium electrodes in $AgNO_3$ solution.	AgNPs with an average size of 18 nm.	Using simple equipment without a vacuum. Steps of reduction produce low impurity and inexpenive.	The time it takes is long for the electrodes to reduce $AgNO_3$.	[10]
	Ethanol as medium and electrodes in cylindrical and Disc shapes.	AgNPs have spherical with an average particle size of 22 nm (system A) and 26 nm (system B).	A simple method, free of chemical agents, small particle size, and inexpenive.	It is difficult to get high yields with a good structure.	[11]
Gamma irradiation	Polyvinyl Alcohol (PVA), Natrium Alginat, $AgNO_3$, and Isopropanol	AgNPs range in size from 43.8 to 164 nm, and an average diameter of 69.8 nm.	The simple method, produces metal nanoparticles in a fully reduced state, is very pure and stable, and there are no reducing substances or side products, so it is harmless and environmentally friendly.	The resulting nanoparticles are quite large in size.	[12]
	$AgNO_3$, Soy broth *tryptic* and Honey	AgNPs have spherical in shape with a size range of 2.69-10 nm and a dominant particle size of 4.187 nm.	The resulting particle size is small, uniform, and stable.	Radiation produces radiotoxic which can trigger mutagenic effects.	[13]

In this study, 1 mL of *Lantana camara* L. extract was reacted with 9 mL of 1 M $AgNO_3$ at ambient temperature, then sonicated for 10 minutes separately. The completion of the AgNPs synthesis process is indicated by the appearance of the reddish-brown color on the reaction medium. The EDX results show an absorption peak at 3 keV that is typical for the nanocrystalline absorption of silver metal. Based on the results of TEM analysis, the morphology of AgNPs was round with an average size of 33.80 nm. XRD analysis shows the diffraction position at 2θ are 38.04°; 44.24°; 64.37°; and 77.31°

corresponding to the FCC fields of AgNPs, namely (111), (200), (220), and (311). Through the Scherrer equation, the particle size of the AgNPs is 25 nm. Meanwhile, the analysis from HRSEM showed that the polydispersion particles had an average diameter of 45 nm without any aggregation. Based on FTIR analysis, it is known that the *Lantana camara* L. leaf extract contains polyphenols, flavonoids, and protein compounds. Polyphenols function as a bioreductor and capping agents for silver ions, while proteins and flavonoids serve to stabilize and prevent agglomeration of AgNPs. The analysis using XPS proved that AgNPs are in a reduced state in their metal ions [22].

The main focus of this research is the synthesis of AgNPs in a short time, which is 10 minutes. The use of ultrasound in this method can increase the reduction process many times. AgNPs were synthesized purely without impurities, but very few AgNPs were produced in the monodispersed form [22].

2.5.3 *Myrmecodia pendans*

Ant nests (*Myrmecodia pendans*) are plants originating in Papua and are known to contain kaempferol (13.767 mg/g), luteoline (0.005 mg/g), routine (0.003 mg/g), quercetin (0.030 mg/g) and apigenin (4.700 mg/g) which belong to the flavonoid group [13]. Flavonoids serve as reducing agents in the synthesis of AgNPs. The use of ant nest extract as a bioreductor is expected to minimize the use of inorganic material that is harmful to the environment.

Maarebia et al. [23] synthesized AgNPs using *Myrmecodia pendans* extract as a bioreductor and $AgNO_3$ solution as a metal precursor. This analysis was performed using two factors, namely without stabilizers and with a 2 mL PVA (Polyvinyl Alcohol) stabilizer [23]. PVA serves as a stabilizing agent to avoid particle agglomeration. The principle of this method is to minimize Ag^0 from Ag^+ ions obtained from $AgNO_3$ solution and *Myrmecodia pendans* extract. AgNPs synthesis was initiated by the reaction of 2 mL of Myrmecodia pendans extract with 20 mL of 1 mM of $AgNO_3$ solution using a multi stirrer for 2.5 hours.

The results of the AgNPs synthesis are distinguished by a change in the color of the solution mixture to brownish-yellow. Characterization using an UV-Vis spectrophotometer for AgNPs using a PVA stabilizer reached a maximum peak of 408.50 nm. In the meantime, the value obtained for AgNPs without stabilizers is 408.00 nm. The results of PSA identification showed that the particle size of AgNPs without PVA was 76.1 nm with a polydispersity of 0.324, while the particle size of AgNPs with PVA was 78.3 nm with a polydispersity of 0.303. Based on the results of the SEM study, AgNPs without PVA were globular with a diameter of 50–64 nm and AgNPs with PVA were not agglomerated. EDS study results showed that the mass percent of the silver element in AgNPs without stabilizers was 83.70% and for AgNPs using stabilizers was 82.14%. The XRD findings at an angle of 2θ showed three peaks for PVA-free AgNPs, namely (111), (202), and (311). Meanwhile, for PVA-based AgNPs, there are four peaks, namely (111), (200), (202), and (311). The distribution of the synthesized AgNPs particles showed different sizes ranging from 8.86–32.59 nm [23].

2.5.4 Biosynthesis from Cinnamon Extract

One of the chemical reduction methods for the synthesis of AgNPs which is environmentally is the use of cinnamon extract as a reducing agent [51]. AgNPs tend to undergo aggregation in order to form large sizes. The stability of the AgNPs plays a very important role when it is characterized and applied to a product [52].

Premkumar et al. [24] reported the synthesis of AgNPs using cinnamon extract with $AgNO_3$ solution as a metal precursor and cinnamon extract as a bioreductor and stabilizer. Cinnamon extract was obtained by dissolving 1 g of cinnamon in 25 mL of distilled water. After being mixed and heated at 60–70°C for 25–30 minutes, the solution is filtered, then the filtrate obtained is used for the synthesis of AgNPs. Cinnamon extract filtrate with dilution in water of 0.5:4.5 was mixed with 10 µL of 1 mM

of $AgNO_3$ solution. Then incubated at 37°C for 48 hours. The synthesis findings were characterized using UV-Vis spectrophotometer, FTIR, FESEM, and XRD.

The formation of AgNPs is marked by reddish-brown discoloration. Characterization using UV-Vis spectrophotomety resulted in a peak at 412 nm. FTIR analysis is used to identify biomolecules that cause the reduction of Ag^+ ions. Characterization using FTIR resulted in a several spectra, namely 3259.29; 1634.60; 564.12; 520.32; and 510.10. FESEM analysis shows that the resulting AgNPs are spherical and the diameters range from 50–70 nm. On the basis of the XRD study, the synthesized AgNPs had a peak 27,743˚; 32,161˚; 38,025˚; 46,159˚; and 54,720˚ [24].

2.6 Biosynthesis from Bacteria

2.6.1 Psycrophilic and Mesophilic

Shivaji et al. [17] synthesized AgNPs using five types of psychrophilic bacteria, that are *Pseudomonas antarctica*, *Pseudomonas proteolytic*, *Pseudomonas meridiana*, *Arthrobacter kerguelensis*, and *Arthrobacter gangotriensis*, as well as two types of mesophilic bacteria, which are *Bacillus indicus*, and *Bacillus cecebensis*. Bacterial cultures were grown in nutrient broth media using an orbital shaker at 180 rpm with a temperature of 22°C. In the supernatant culture of the psychrophilic bacteria, there was a brown discoloration which indicated the formation of AgNPs. Characterization using a UV-Vis spectrophotometer resulted in a maximum peak at 410 nm. However, the addition of $AgNO_3$ to the seven bacterial culture media did not produce AgNPs. In other findings, the effects of the synthesis of AgNPs using a cell-free supernatant from *A. kerguelensis* and *P. antarctica* were stable for up to 8 months if stored in dark conditions. Meanwhile, the nanoparticles produced using culture media are less stable. Morphology and size of the AgNPs of *B. indicus* and *P. antarctica* showed spherical particles with mean sizes varying from 6.10 ± 2.80 nm to 12.20 ± 5.70 at TEM and 4.60–13.30 nm at AFM.

The results of the *A. Kerguelensis* cell-free supernatant synthesis has shown the best stability of AgNPs. This is because of the components secreted by *A. kerguelensis* bacteria have the potential to increase the stability of AgNPs. The advantages of using this method are that it is environmentally friendly, does not use harmful chemicals, and inexpensive. In addition, the synthesis time is relatively shorter than that of previous studies using *B. licheniformis*, *B. Subtilis*, and *F. oxysporum*. However, not all AgNPs produced had good stability, such as the synthesis of P. Antarctica which was unstable due to aggregation [17].

2.6.2 *Novosphingobium sp.* THG-C3

Biosynthesis of AgNPs using bacteria is an environmentally friendly and inexpenseive method. Du et al. [2] synthesized AgNPs from *Novosphingobium sp*. THG-C3. Bacteria are obtained by soil insulation since the soil is a source of microbial diversity that can be used for human welfare. Bacteria were cultured in 100 mL nutrient broth for 48 hours. Next, cells were removed by centrifugation at 9000 rpm for 10 minutes. 0.1 mL of 1 M $AgNO_3$ solution was added to 100 mL of supernatant and incubated for 2 days in an orbital shaker at 120 rpm and 25°C.

Synthesis of AgNPs by *Novosphingobium sp*. THG-C3 is carried out extracellularly. The formation of AgNPs was characterized by a color change from light yellow to dark brown on the culture supernatant within 48 hours. Characterization using a UV-Vis spectrophotometer resulted in a peak at 406 nm which corresponds to the plasmon surface resonance band of AgNPs. Based on the FE-TEM analysis, the morphology of the synthesized AgNPs is spherical with a size of 8–25 nm. XRD results showed the diffraction peaks at 2θ, namely 38.20˚; 44.37˚; 64.43˚; and 77.47˚ which correspond to the FCC lattice planes of the AgNPS, namely (111), (200), (220), and (311). The results of the SAED analysis showed that the crystal lattice plane matched the XRD results and confirmed the crystalline nature of the AgNPs. Elemental mapping analysis reveals that silver is the dominant element in AgNPs. The

composition and purity of the synthesized AgNPs were analyzed using EDX with a yield close to 3 keV, which is the silver atom signal [2].

2.6.3 *Pseudoduganella eburnea* MAHUQ-39

Through previous reports, it was found that secreted enzymes from bacteria are essential for nanoparticle biosynthesis. The mechanism that occurs is the trapped metal ions on the surface or within the bacterial cell and the trapped metal ions are converted into nanoparticles in the presence of secreted enzymes by bacteria [44]. The advantages of extracellular biosynthesis are easier, more cost-effective, and produce nanoparticles that can be purified easily and quickly. In the meantime, the intracellular purification steps are more difficult and complex [6].

Huq [6] reported the results of an AgNPs biosynthesis study using *Pseudoduganella eburnea* MAHUQ-39. The bacteria were cultured in 100 mL of the R2A medium. Then incubated in a shaking incubator at 140 rpm and a temperature of 30 C for 2 days. Next, 0.1 mL of 1 M $AgNO_3$ solution was added to 100 mL of culture supernatant and incubated at 140 rpm and 30˚C for 24 hours.

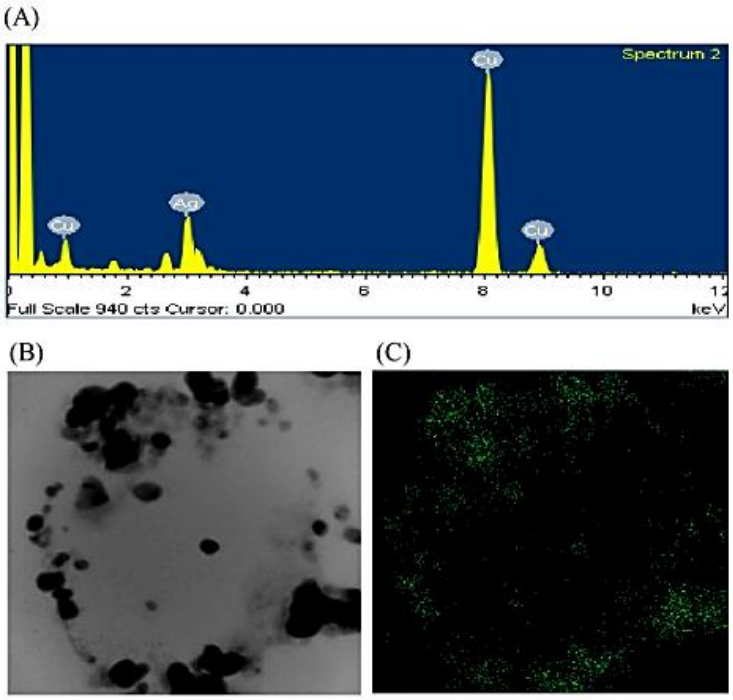

Figure 7. EDX Spectrum of AgNPs synthesized (A) FE-TEM for element mapping (B) and distribution of Ag elements in element mapping (C) [6]

The formation of AgNPs resulted in a color change in the culture supernatant from yellow to brown within 24 hours. This is due to the surface plasmon resonance. Characterization of AgNPs using a UV-Vis spectrophotometer resulted in a peak at 448 nm. The composition and purity of the AgNPs in Figure 7A were obtained with an EDX spectrometer showing a homogeneous distribution of AgNPs with an elemental silver signal of about 3 keV. Characterization using FE-TEM is shown in Figure 7B, which shows the morphology of round AgNPs with sizes between 8 - 24 nm. Meanwhile, the XRD spectrum shows a diffraction peak at 2θ, namely 38.13˚; 44.31˚; 64.44˚; and 77.42˚ which correspond to the grid planes of the AgNPs, namely (111), (200), (220), and (311). The mapping results and the SAED pattern shown in Figure 7C indicate that the structure of the AgNPs is crystalline. The findings of the FTIR study indicate the presence of functional groups of biomolecular compounds involved in the stabilization of AgNPs. The observed absorption bands are 3439.16; 2922.29; 2854.13; 2360.07; 2342.06; 1734.23; 1636.01; 1457.49; 1057.74 cm^{-1}. Using DLS, the mean particle size of synthesized

AgNPs was 141.2 nm with a polydispersity value of 0.369 [20]. Synthesis of AgNPs using *Pseudoduganella eburnea* MAHUQ-39 is a method that is environmentally friendly, fast, easy, economical, and can be used for high production [6].

2.6.4 *Bacillus cereus*

Prakash et al. [18] synthesized AgNPs from the bacteria *Bacillus cereus*. In this research, the bacteria were cultured using 250 mL MGYP medium. Then homogenized using a rotary shaker at 100 rpm and incubated at 37.5˚C for 24 hours. This synthesis was conducted extracellularly by adding $AgNO_3$ to the inoculum, then placing it in a shaker for 24 hours. The concentration of $AgNO_3$ solution used was ranged from 50, 100, 500, 1000, 1500 to 20000 ppm. After 24 hours, the culture was filtered and a cell-free supernatant was generated. Further research was carried out with a UV-Vis spectrophotometer with a wavelength of 200-600 nm. To avoid the presence of impurities from the medium in the nanoparticles, wash them 3-4 times using distilled water.

The culture switches color after 24 hours, which becomes dark orange. This indicates the extracellular reduction of silver salts to AgNPs. The results of the UV-Vis analysis showed that the sharp plasmon peaks at 435 nm suggested that silver was in the nanoscale range. While the XRD analysis results show the XRD pattern in the AgNPs lattice planes of the FCC, which are (111), (200), (220), and (311), and the AgNPs structure is crystalline. TEM analysis results from AgNPs show that the morphology is round with a size of 10–30 nm, most of which are 15 nm in size. Based on the HRTEM analysis, an interplanar distance (d) of 2.02Å was obtained where this result corresponds to the crystallization area (200) of the AgNPs. The synthesis of AgNPs using Bacillus cereus bacteria takes a long time but does not have a detrimental effect on the environment. Therefore, this approach can be used as an alternative method that is environmentally friendly [18].

2.7 Biosynthesis from Fungi

2.7.1 *Aspergillus tereus*

Li et al. [19] used *Aspergillus tereus* fungi isolated from the soil to synthesize AgNPs. Aspergillus tereus fungi were grown on Potato Dextrose Broth (PDB) medium at 28°C in a rotary shake at 120 rpm for 96 hours. A total of 25 g of biomass is stored in a flask containing 100 mL of Milli-Q water. Then, the flask is incubated for 24 hours and filtered to produce cell filtrate. After that, 50 mL of cell filtrate was mixed with 10 mL of $AgNO_3$ solution 10 mmol/L. Furthermore, incubated at 28°C in a dark conditions for 24 hours. The results of the synthesis of AgNPs were characterized by a UV-Vis spectrophotometer at a wavelength of 240–570 nm, XRD analysis, and TEM analysis.

In the research of Li et al. [19] formation of AgNPs was indicated by a color change in the crude cell filtrate of *Aspergillus terreus* after the addition of the $AgNO_3$ solution. After incubation for 24 hours, the color intensity of the cell filtrate with $AgNO_3$ remained brown. This indicates that the particles are well dispersed in the solution. The UV-Vis spectrophotometer results of the $AgNO_3$ cell filtrate showed the presence of AgNPs with a strong peak at 440 nm. From the TEM analysis results, it was obtained that spherical particle with an average size polydispersion of 4.30 nm as shown in Figure 8. The results of intense XRD peaks were observed and obtained plane patterns (111), (200), (220), and (311). at an angle of 2θ of 38.28 °; 44.38 °; 64.54 °; and 77.64 °. The size of the AgNPs according to the XRD analysis results was around 5.2 nm. These results are consistent with the TEM analysis. When compared with bacteria, the synthesis of AgNPs with fungi produces more bioactive compounds so that the reduction time is shorter. Also, the synthesis process is much easier and can be used for large-scale production. Meanwhile, the deficiency of AgNPs synthesis with fungi is that the AgNPs obtained are not pure because they are still mixed with biomass. This was observed when analyzing using XRD [18].

2.7.2 *Trichoderma reesei*

Vahabi et al. [20] synthesizes AgNPs from the fungi *Trichoderma reesei* to produce AgNPs on a large scale. Inoculation of *Trichoderma reesei* fungi was carried out in Potato Dextrose Agar (PDA) medium at 28˚C in a petri dish. Fungi was grown in bottles containing 100 mL GC medium (consisting of 0.50% glucose and 0.40% casein hydrolyzate) at a temperature of 25-28˚C and stirred using a magnetic stirrer at 150 rpm for 72 hours. Then the mycelium mass (the vegetative part of the fungi) is separated from the culture. The mass of 10 g of mycelium was mixed with 100 mL of 1 mM $AgNO_3$ solution. The mixture was placed in a 100 rpm rotating shaker at 28˚C for 120 hours. In this process, silver nanoparticles are produced by reducing silver ions to silver metal. Silver ion reduction was characterized by a UV-Vis spectrophotometer [20].

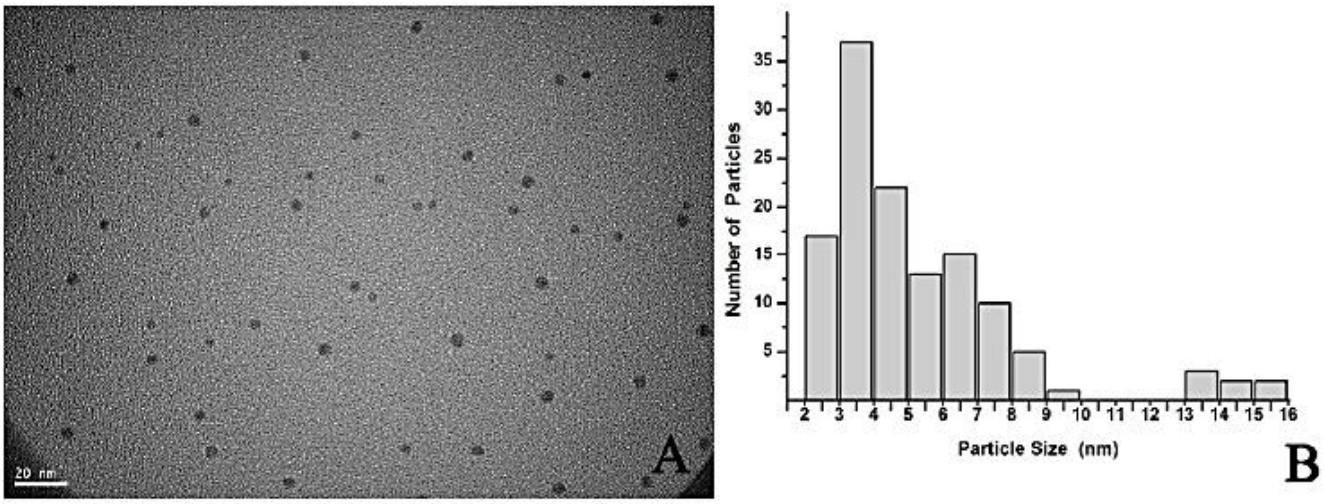

Figure 8. (A) Representative image of synthesized AgNPs by reducing $AgNO_3$ solution with crude cell filtrate from *Aspergillus terreus* (B) Distribution of the size of the AgNPs from the TEM analysis [19].

The results of the UV-Vis spectrophotometer analysis showed an increase in the intensity of the silver solution with increasing time which was marked by an increase in peaks from 414 to 420 nm. This indicates an increase in the amount of AgNPs formed in solution. The reaction of AgNPs formation takes about 1 month and the resulting solution is stable without any particle aggregation. The resulting AgNPs have a size of 5–50 nm with various morphology and are crystalline, based on the diffraction pattern of the analysis [20].

The synthesis of nanoparticles using fungi has several advantages, such as high production of specific enzymes or metabolites, high growth rates, easy handling in large-scale production, and low cost. *Trichoderma reesei* fungi is famous for its formation of extracellular enzymes which are very large, up to 100 g /L. The result is much higher than other fungi. Also, *Trichoderma reesei* is a fungi that is environmentally friendly and biologically safe, because it is not a pathogenic fungi.

Based on several methods of AgNPs synthesis that have been studied, there are several deficiencies and advantages of each method. The deficiencies and advantages of each synthesis method are presented in table 1. Synthesis of AgNPs by biosynthesis is the best method because the method is environmentally friendly, does not use harmful chemicals, so it is safe and cost-effective. Even some biosynthesis requires a fairly short time and the synthesis steps are simple. Although, not all AgNPs produced are small and stable. The sources used in biosynthesis are various, including bacteria, fungi, and plants, all of which have their advantages and disadvantages. Biosynthesis using bacteria requires the isolation of microorganisms, needed an aseptic conditions, and maintenance which costs a lot. Meanwhile, biosynthesis using fungi produced less pure AgNPs. Therefore, biosynthesis using plants

is the most effective method for large-scale production, because it is easy, fast, environmentally friendly, inexpensive, and produces smaller AgNPs compared to other methods.

3. Conclusion

Synthesis of Silver Nanoparticles (AgNPs) can be made from various methods, such as physics, chemistry, and biology. Biological methods are usually biosynthetic using plants, bacteria, and fungi. In physics methods through laser ablation and arc-discharge. Meanwhile, chemical methods can be done through chemical reduction and gamma irradiation. The chemical synthesis method produce pure AgNPs, but they have dangerous side effects for the environment. Laser ablation has the same advantages as chemical reduction, but it requires a large amount of energy. Meanwhile, for gamma irradiation, the resulting AgNPs have a relatively large size, although they are pure and have no side products. When comparing the risks posed, the biosynthetic method is considered to be the best, because it is environmentally friendly and cost-effective. Furthermore, it can be concluded that the best method for the synthesis of AgNPs is biosynthesis using plants. Because biosynthesis using plants produces AgNPs which are smaller in size than other methods. Also, biosynthesis using bacteria requires maintenance which costs a lot. Meanwhile, biosynthesis using fungi resulted in less pure AgNPs.

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