Effective Deagglomeration in Biosynthesized Nanoparticles: A Mini Review

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Abstract. Materials with nanoscale particle size have different properties from its bulk phase, which allows for wider application of the material. There are various methods to synthesize nanoparticles, namely physical, chemical, and biological method. Nowadays, nanoparticle synthesis method is focused on biological method because of its advantages, such as environmentally friendly, relatively simple procedures, and lower production costs. Biosynthesis by co-precipitation method using extracts from biological agents is considered the most efficient among other biological methods. Biochemical compound in the extract have a dual role in synthesis, they act as a reducing agent which reduces metal salt to metal ion, and as a capping agent which stabilizes the nanoparticle. Biosynthesis has been shown to result in nanoparticles as good as physical and chemical method. However, several studies report that the synthesized nanoparticles have low stability regardless of the presence of their capping agent, resulting in agglomeration of nanoparticles, which reduces its efficiency. Until now, studies on particle deagglomeration especially during nanoparticle biosynthesis have not been widely carried out. This mini review will explain the phenomenon of agglomeration during biosynthesis. Moreover, deagglomeration treatment using physical and chemical approaches will be examined. Each approach is considered to be able to deagglomerate nanoparticles well, and the combination of the two is projected to be able to provide better results.

1. Introduction
Nanoparticles are defined as materials with particle sizes ranging from of 1 to 100 nm. Consequently, nanoparticles have large surface area, allowing for high number of atoms to be exposed on the surface resulting in higher reactivity than that of bulk form. [1,2]. With this advantage, the nanoparticle has wide applications, such as antimicrobe, catalyst, remediating agent for pollutant and oil waste, sensor, etc. Selection of the appropriate synthesis method is very important as the slightest difference in synthesis routes can have a big influence on the final characteristics [3]. There are various synthesis methods that have been studied, both chemical, physical, and biological. Biological synthesis is a method that has recently been developed as a safe synthesis alternative.

Biosynthesis, also known as green synthesis, utilizes living cells, extracts of biomolecules from living cells, or cell free supernatants to drive nanoparticle formation reactions [3]. Biosynthesis using biomolecular extracts by precipitation method is the most studied due to the ease of the synthesis procedure. Although the synthesis route is different, the characteristics of the biosynthesized
nanoparticles are close to those of the chemical and physical methods. In fact, the organic components attached to the surface of the nanoparticles are able to provide additional functions when the nanoparticles are applied, one of which is to increase the antimicrobial effect [4].

During the synthesis process takes place, the nanoparticles with high reactivity will be susceptible to agglomeration. This resulted in a decrease of nanoparticle toxicity to microbes [4], decrease in pigment degradation photocatalytic activity [5], and poor interfacial/interphase properties and tensile strength of nanocomposite [6], etc. Agglomeration occurs spontaneously and cannot be avoided immediately, so it can becomes a major problem in nanoparticle synthesis [7,8]. Agglomeration does not only occur when the nanoparticles are dispersed in the media, but especially during the synthesis process where the nanoparticles experience strong adhesion between their particles so they are easily agglomerated [1]. For biosynthesis method, it is said that the biological components which are involved in the synthesis process can have a role to prevent agglomeration by acting as a capping agent. Capping agents are amphiphilic compounds capable of covering the surface of the nanoparticles and preventing two or more nanoparticles from approaching each other and join to form large particles [9]. However, several studies stated that these components are still unable to withstand adhesion strength, making agglomeration to be unavoidable [10–12]. So far, there have not been many studies on agglomeration during biosynthesis. Likewise, research of nanoparticles deagglomeration during biosynthesis is limited, especially compared to similar studies performed for chemical synthesis methods. This review will examine how the phenomenon of agglomeration in biosynthesis can occur and also the methods that can be applied to the biosynthesis of nanoparticles to prevent and disperse nanoparticle agglomeration.

2. Nanoparticles Synthesis
Nanoparticles can be produced by reducing the size of the material from a micro or larger scale to a nanoscale called the top-down approach, or the opposite way through the formation of atoms or molecules into nanoparticles, which is referred to as a bottom-up approach. Compared with the top-down, bottom-up approach is more advantageous as it can produce nanoparticles with a size range of 1 to 50 nm with a narrow nanoparticle size distribution [13–15]. Physical and chemical bottom-up methods have been studied for a long time. The physical synthesis requires expensive and complex equipment with the use of high temperatures and pressures. Meanwhile, chemical synthesis frequently involves chemicals that are hazardous to the environment and people [16]. As an alternative, biosynthesis has become a popular bottom-up method currently being developed. Compared to physical and chemical methods, biosynthesis uses a simple set up tools so that it is economical and energy efficient. The reaction for the formation of nanoparticles is fast and the reaction parameters are easy to control [13,17]. Biosynthesis is also often referred to as green synthesis because it avoids the production of synthetic waste, reduces pollution and uses safe materials, and uses renewable feed stocks [18].

Through biosynthesis, nanoparticles are formed by the help of the living organisms activities, including bacteria, yeast, fungi, and algae, and plants [16]. Biological components are reductive, so generally, biosynthesis is carried out for the production of metal or metal oxide-based nanoparticles. This biosynthesis can be carried out intracellularly, extracellularly, or using cell extracts. Intracellular biosynthesis takes advantage of the metabolic system in cells to reduce metal salts by reductase[14], while in extracellular biosynthesis, ionized metal salts adhere to and are reduced by the membrane on the cell surface[19]. The challenge using these biological agents is the need to provide rich grown medium and to be carried in specific environmental conditions during the synthesis process. In addition, the process can take a long time ranging from hours to days [3]. In synthesis using cell extracts, it is the organic compound content that reduces metal ions and stabilizes the formed nanoparticles [20]. The advantages of using cell extracts are that they are able to complete the synthesis in minutes to hours and the synthesis process are simpler than using intra or extracellular [3]. The most common method of biosynthesis using cell extracts is precipitation. In addition, there is synthesis method using microwaves that emerging in recent years because it can be done only in few minutes. However, the microwave method makes it difficult to observe the particle formation reaction at each synthesis time, nor can it be scaled-up for large-scale production [21]. The precipitation method involves more synthesis parameters
that are easily modified so that it is more interesting to study in depth. Therefore, biosynthesis in this paper is focused on the precipitation method.

2.1 Nanoparticle Biosynthesis by Precipitation Method

One of the most studied biological nanoparticle synthesis methods is precipitation. There are several advantages of the precipitation method among other biosynthetic methods, which include ease of preparation, low cost, and high product yield [22]. The principle of the precipitation method is to add the extract of the biological agent to the metal precursor solution gradually until nanoparticles are formed in the form of precipitates. Generally, the extract with precursors is mixed at room temperature for several hours with constant stirring. Synthesis can also be continued by aging at room temperature for 24 hours. The nanoparticle precipitates are then centrifuged and dried with hot air at a temperature of 70-80°C [4,10]. As for metal oxide-based nanoparticles, the dry precipitates are calcined at 350-400°C for 1-3 hours.

In the biosynthesis of nanoparticles, biological extracts act as reducing agents and capping agents. Metal precursors that dissolve in water are ionized into positively charged metal ions, which are further reduced by hydroxyl groups. Zero charged metal atoms undergo nucleation and grow into a larger trench with the attachment of metal ions that have not or have been reduced on the surface of the nanoparticles. Afterwards, the biological component that has a role as a capping agent will cover the nanoparticles through polar interactions with the nanoparticle surface and non-polar interactions with the environment. Capping agent prevents particle overgrowth so that it can keep the size of the nanoparticles small and uniform. Capping agents can also increase the electrostatic and steric repulsive forces of the nanoparticles, where the two repulsive forces can prevent adjacent particles from coming into contact, so that the stability of the nanoparticles in suspension can be maintained properly [9].

3. Agglomeration in Nanoparticle Biosynthesis

As previously mentioned, there is always a chance for the nanoparticles to undergo agglomeration during synthesis. This agglomeration phenomenon is closely related to the size and reactivity of the particles. The very small-sized particle makes the number of atoms that come into contact and react to be high. Therefore, the formed nanoparticles have high reactivity. Nanoparticles will interact with their environment to make them stable, one of which is through van der Waals interactions. The van der Waals force is the interaction of attraction between two or more adjacent nanoparticles through the London dispersive force, Debye inductive force, and Keesom orientational force [23]. Nanoparticles that are reactive and move freely in solution will easily experience collisions, where the collision of particles induces van der Waals force. The nanoparticle clusters will be solidified into agglomerates through chemical bridge between clusters. Next, crystal growth occurs which causes the agglomerates to bind through chemical bonds. Deagglomeration can still be done by shear stress of stirring if the bonds to the agglomerates are still weak [24]. However, as the bonds get stronger, the agglomerates will be more difficult to break even if they are given a larger physical dispersion force. It is very important to pay attention to the phenomenon of agglomeration formation during biosynthesis of nanoparticle.

The bioactive compounds are believed to act as capping agents that can prevent agglomeration. However, several studies have reported that plant extracts actually induce agglomeration. For instance, biomolecules from Phoenix dactylifera leaf extract produce sphere-shaped Fe3O4 particles that agglomerate into non-uniform flower shapes [12]. The same thing happened in the synthesis of TiO2 synthesized with Phyllanthus niruri leaf extract, where the agglomeration due to the electrostatic forces between the biomolecules on the nanoparticle surface [10]. ZnO synthesized using the pericarp extract of Garcinia mangostana fruit undergoes agglomeration due to the strong polarity and electrostatic attraction between the nanoparticles [11]. In the biosynthesis of nanoparticles using fungi, agglomeration can occur due to the production of enzymes and proteins by the fungi which then accumulate in suspension as happened in extracellular Ag synthesis using Trichoderma longibrachtiu [25]. The same thing happened in the study of Vetchinkina et al [26] who carried out biosynthesis using extracellular from Pleurotus ostreatus, Lentinus edodes, Ganoderma lucidum, and Grifola frondosa.
The resulting Ag nanoparticles are large, irregular in shape, and agglomerated (Figure 1). The potential zeta value of the Ag suspension is -9 to -12 mV, where this figure shows that the repulsion force between the particles is weak. In addition to the influence of capping agents from several types of biological agents, the agglomeration of nanoparticles on biosynthesis is also related to the shape of the particles. Biosynthesis method is advantageous because it is able to control the shape of the nanoparticles into a uniform sphere [27,28]. However, sphere-shaped nanoparticles tend to agglomerate more easily because of the ease of contact between the particles, while nanoparticles with irregular shapes need to adjust their position to suit other particles [29].

Another cause of agglomeration in nanoparticle biosynthesis is the acidity of the biological agent. Most cases of agglomeration occur when biosynthesis is carried out with acidic extracts, where acidic conditions reduce steric repulsion and increase interparticle hydrogen bonding. Biosynthesis of Ag nanoparticles using Mangifera indica peel extract at pH 3 caused the deprotonation of functional group to decrease, resulting in non-uniform and agglomerated nanoparticles [29]. In the synthesis of ZnO using Averrhoa bilimbi fruit extract, the pH which was originally around 3.5-4 decreased to a pH of 1.5-2 due to heating. This extreme acidic condition not only facilitates agglomeration due to decreased capping agent ability, but also slows down ZnO production. In the application of ZnO nanoparticles synthesized with A. bilimbi fruit extract, the formation of nanoparticle agglomerates causes the nanoparticles to settle so that their antimicrobial ability decreases [4]. When the nanoparticle suspension is to be investigated further, filtering using a syringe filter can be performed to eliminate the agglomerates and produce a monodisperse suspension of nanoparticles [30]. However, the effect of filtering was considered insignificant when viewed from the measurement results of particle distribution based on TEM and DLS. For this reason, an additional treatment to prevent agglomeration or to aid the breakdown of the formed nanoparticle agglomerates is needed.

4. Deagglomeration in Nanoparticle Biosynthesis

Various studies have been carried out to disperse the nanoparticle agglomerates formed during synthesis, most of which have been investigated on chemical synthesis. In this section, we will discuss deagglomeration methods that can potentially be applied during the biosynthesis process of nanoparticles, physically and chemically.

4.1 Physical Deagglomeration
Physical deagglomeration aims to break down the agglomerates that are formed. There are various physical deagglomeration that can be done during synthesis and post-synthesis. In this review, the methods discussed are methods that can be applied during the biosynthesis process, namely mechanical stirring and ultrasonication.

4.1.1 Mechanical stirring. Generally, biosynthesis by the precipitation method is accompanied by mechanical stirring. Besides aiming to homogenize the solution, stirring also provides a deagglomeration effect of nanoparticles during synthesis. The effectiveness of stirring for deagglomeration can be increased with the speed of rotation. One study observed the effect of stirring at a speed of 500-900 nm during the synthesis of Fe₃O₄ nanoparticles. Fe₃O₄ crystal size decreased from 24 nm to 22.5 nm by increasing the stirring speed from 500 rpm to 600 rpm. However, TEM observations indicated that the nanoparticles generated at a speed of 600 rpm were agglomerated quite severely [31]. The smaller crystal size is thought to produce a greater magnetic attraction, thereby facilitating agglomeration. On the other hand, the crystal diameter increased to 25 nm when the speed was increased to 900 rpm. At such high speed, the collision between metal ions increases, causing larger crystal growth.

Khan et al. [30] observed the effect of stirring at speeds of 500, 1000, 1500, and 2000 rpm on the growth of ZnO particles and agglomerates. At the highest velocity, ZnO agglomerates are less than that at the lowest velocity. The amount of agglomeration correlated to the lethality of the nanoparticles toward Bacillus subtilis, Eschericia coli, and Candida albicans, where the synthesis at 2000 rpm (lowest agglomeration) had the best inhibition ability of the three microbes. A similar thing was found in a study by Gulrajani et al. [31], where the lowest stirring speed (700 rpm) produced the largest average size of nanoparticles (510 nm), whereas the highest stirring speed (2500 rpm) produced the lowest particle size (370 nm) with the least agglomeration too. The high stirring speed will prevent the growth of particles and induce new particles to produce nanoparticles with small diameter and low agglomeration. In addition, high velocity can also produce a more regular shape of the particles. Based on these studies, deagglomeration in laboratory-scale nanoparticle biosynthesis carried out by the mechanical stirring method will be effective when the stirring speed is at 2000-2500 rpm.

4.1.2 Ultrasonication. Ultrasonication can break down soft-agglomerates effectively and it can even break down hard agglomerates that are difficult to do by stirring or dispersion method with ball milling [32,33]. The principle of ultrasonication is to pass the waves on a liquid medium which can cause rarefaction and compression cycle. Rarefaction occurs when the media pressure is low where air bubbles are formed, which is called cavitation. Furthermore, compression causes stress cavitation due to higher pressure conditions. Rarefaction and compression alternately make the cavitation larger and unstable until it breaks by generating mechanical and thermal energy. This mechanical energy will crush and erode the nanoparticle agglomerates [34,35].

Even though it requires more energy, ultrasonication is able to disperse nanoparticles much more effectively than the stirring method. Kawashima et al. [36] proved that the synthesis of CaCO₃ nanoparticles by stirring, produced aggregates with a size of more than 2 μm, while synthesis process that used ultrasonication did not result in any aggregates. The same thing was reported by Estrada-Monje et al. [37] who observed the effect of the deagglomeration method on the dispersion of TiO₂ nanoparticles in ethylene-vinyl acetate (EVA) solution. STEM observations clearly show that the TiO₂ produced by mechanical stirring forms large agglomerates. Meanwhile, deagglomeration by ultrasonication with 100% amplitude, 80% pulse, for 10 minutes resulted in much smaller agglomerate. In its application, EVA film with TiO₂ which was deagglomerated by ultrasonication was able to inhibit Escherichia coli colonies more than deagglomerated by mechanical stirring.

Parameters that need to be considered when applying ultrasonication as a deagglomeration method include the type of ultrasonicator, time, temperature, pulse, power, and frequency. The probe type ultrasonicator has higher deagglomeration ability than the bath type because it is able to transmit more energy to the nanoparticle system. However, treatment with the sonicator probe can increase the
temperature of the nanoparticle suspension rapidly, which then might induce agglomeration. In addition, sonication time that are too long can also lead to nanoparticle re-agglomeration. The phenomenon of agglomeration in the sonication treatment is related to excess gasification and intense cavitation friction, which changes the charge on the surface of the nanoparticles [32]. Nanoparticle biosynthesis accompanied by sonication deagglomeration can be carried out at room temperature or high temperature up to 90°C, with a time of 10 to 180 minutes. Synthesis time can be shortened by changing other parameters. For example, biosynthesis at 50 W ultrasonication power can be completed in 40 minutes and when the power is increased to 100, 150, and 200 W, the synthesis can be shortened to 20, 16, and 10 minutes, respectively [38].

4.2 Chemical Deagglomeration
The key to nanoparticle agglomeration is the high reactivity on the surface which causes the attraction between the particles to increase. Therefore, it is necessary to control the repulsive force on the surface of the nanoparticles which can be done by chemical deagglomeration. This method involves adding a chemical that can modify the electrostatic and steric stability of the nanoparticles to maintain the stability of the nanoparticles in suspension.

4.2.1 Electrostatic Stabilization. Electrostatic stabilization aims to increase the repulsive force by the charged layer on the surface of the nanoparticles called the Electric Double Layer (EDL). EDL consists of unreduced metal atoms and ions from biological extracts that are attracted to the surface of the nanoparticles. It is this collection of charged ions that produces an electrostatic repulsion so that when the nanoparticles are close together and the EDL of each particle overlaps, the ions experience an osmotic repulsive interaction which then cause them to drifts away from one another [39]. Increasing the electrostatic repulsive force can be achieved by adjusting the pH during biosynthesis. Nanoparticle agglomeration occurs when the pH of the synthesis approaches its isoelectric pH, which is pH where the electric potential value is 0. At this pH, the attraction between the particles reaches a maximum, so it is easy to form hard agglomerates [40]. pH also affects the characteristics of the extract. Changes in pH will change the ability of biomolecules to chelate and reduce metal ions, which in turn changes the shape and yield of the resulting nanoparticles [20]. Biological extracts, especially plants, tend to be acidic. The level of acidity can affect the ability of phytochemical compounds to bind and reduce metal ions. In addition, the more acidic the extract pH, the slower reduction and nucleation processes of nanoparticles will be, so that agglomerates and aggregates are easier to form [41].

In addition to pH modification, electrostatic stability control can also be done by adding a surfactant type dispersing agent that can be ionized in the water. Espitia et al. [42] tried to add Na2P2O7 dispersing agent to increase the stability of ZnO nanoparticles. Na2P2O7 ionizes to form Na+ and P2O7^2- ions, likewise, some of the Zn atoms from the nanoparticles are also ionized in the water. Positive Zn atoms will be attracted to the surface of the ZnO nanoparticles to form a sterl layer. Pyrophosphate which is negatively charged will cover the outer side to form a diffuse layer, and the counter ion from pyrophosphate, namely natrium, will inhabit the outer side. The higher the pyrophosphate anion that covers ZnO, the greater the electrical potential, so the greater its electrostatic repulsive force also. The level of electrostatic stability can be calculated by the zeta potential, where good stability that prevents agglomeration can be achieved when the value is more than positive or negative 25 mV [43]. Moreover, addition of dispersing agents that have the potential to be used to increase the electrostatic stabilization of nanoparticles can also be done. Some examples of dispersing agents that can be used in the biosynthesis of metal nanoparticles are sodium dodecyl sulphate, cetyltrimethyl ammonium bromide, tertiary butyl ammonium, sulphonium, phosphonium, and imidazolium moieties [9,44]. However, the interaction of these chemicals with the biological extract has yet to be determined.

4.2.2 Steric Stabilization. To maintain their stability, nanoparticles can attract complex components such as polymers and alcohols to form an additional protective layer apart from EDL, that is steric layer [39,44]. The repulsion force caused by sterl layer maintains the position of the nanoparticles with a
minimum distance so there is no interaction that led to agglomeration. The components involved in the formation of this layer are capping agents, and in biosynthesis, capping agents can be polyphenols, citric acid, vitamins, biodegradable polymers, silica, and etc. [9]. This capping agent provides steric stabilization on the nanoparticle suspension to prevent agglomeration. The role of steric stabilization may be weak if the effectiveness and concentration of the biochemicals that act as capping agents are low, or if the biochemicals are damaged by drying and calcination as they cannot withstand high temperatures [28].

Steric stabilization can be increased by the addition of a complex dispersing agent. Thus, the protective layer on the nanoparticles will be thicker so that the steric repulsive force is able to prevent agglomeration and maintain the stability of the nanoparticles during synthesis and also after drying [44]. Polymer materials such as poly(ethylene glycol), poly(vinyl alcohol), and poly (vinyl pyrrolidine) are commonly used to increase steric repulsive force [9]. It should be noted that in order to produce maximum steric stability, the determination of the type and concentration of the dispersing agent must be accurate. Qiang et al. [45] tried four types of dispersing agents to stabilize CuO nanoparticles. It is said that each type of dispersing agent has a different effect, even if it is added with the same concentration. This is related to the absorption of dispersing agent on the surface of the particles that change the characteristics and reactivity of the nanoparticles [46]. For CuO dispersed in water with a concentration of 2% wt, sodium polyacrylate with a concentration of 0.4 to 0.8% (w/w) under conditions of pH 10 gave the best stability. At a concentration that is too low, the amount of dispersant is not yet equivalent to CuO resulting in sedimentation of the nanoparticles. On the other hand, a too much dispersing agent causes flocculation which is also undesirable. The use of dispersing agent with polymer types is considered to be able to maintain the stability of the nanoparticle solution for a long time, as evidenced by the Ag nanoparticles resulting from biosynthesis with PVA addition that remains stable for 3 months with room temperature storage [47]. In its potential use in biosynthesis method, a dispersing agent should be added to the metal precursor solution before mixing it with the biological extract.

4.3 Combined Deagglomeration Treatments
Some researchers combine two deagglomeration methods simultaneously to maximize the dispersal of the nanoparticles. It has been mentioned that ultrasonication is known to be able to break down agglomerations well but does not eliminate the potential for re-agglomeration after the sonication process is complete. To maintain the deagglomeration effect longer, a component that can increases its repulsive or steric stability can be added [32,33]. Pradhan et al. [46] conducted a research to determine optimum dispersing method for Mn and Cu nanoparticles. They proved that sonication for 15 minutes with the addition of a dispersing agent of Bovine Serum Albumin (BSA) solution in 0.05 vol% resulted in the higher percentage of the Mn and Cn nanoparticles measured at <20 nm than the treatment without the addition of BSA. Another study by Kawashima et al. [36] showing that CaCO₃ nanoparticles dispersed by the probe ultrasonicator settles and are not affected by large variations in sonication intensity and hydrophobic properties of CaCO₃. However, when combined with a polycarboxylate-based superplasticizer dispersing agent, the stability of the deagglomerated nanoparticles is maintained. The increase in sonication time and amplitude also has a positive effect on CaCO₃ dispersion in solution, where the best stability is produced at 3 hours and an amplitude of 40%. Cunniffe et al. [48] also states that the synthesis of high concentrations of nanoparticles accompanied by sonication deagglomeration produces micron-sized particles, while additional deagglomeration using a dispersing agent can reduce the particle size to the nanoscale. In their research, sonication for 4 minutes produced nanoparticles with a size of less than 100 nm. This combination of deagglomeration methods can be referred to for application in nanoparticle biosynthesis.

Conclision
This review has discussed the biosynthesis of nanoparticles and the accompanying agglomeration issues, along with the methods that can solve these problems. The environmental friendliness and easy procedures make biosynthesis a widely explored method of producing nanoparticles. However,
nanoparticles resulting from biosynthesis method is prone to agglomeration. Therefore, some potential deagglomeration is proposed in this study. Physical deagglomeration can be done by mechanical stirring at a speed of 2000-2500 rpm or by ultrasonication using a bath type ultrasonicator. Chemical deagglomeration can be carried out by increasing electrostatic stability through pH modification and addition of ionic surfactants or increasing steric stability by adding a polymer type dispersing agent. The combination of these methods also has the opportunity to improve the deagglomeration and stability of the nanoparticles. The discussion in this review is limited to the principle of deagglomeration, the discussion in this review is limited to the principle of deagglomeration, the discussion in this review is limited to the principle of deagglomeration, the discussion in this review is limited to the principle of deagglomeration, the discussion in this review is limited to the principle of deagglomeration, the discussion in this review is limited to the principle of deagglomeration, the discussion in this review is limited to the principle of deagglomeration, the discussion in this review is limited to the principle of deagglomeration, the discussion in this review is limited to the principle of deagglomeration, the discussion in this review is limited to the principle of deagglomeration, the discussion in this review is limited to the principle of deagglomeration.

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