COMPARISON BETWEEN BOX-BEHNKEN DESIGN AND CENTRAL COMPOSITE DESIGN FOR OPTIMIZING THE BIOSYNTHESIS OF SILVER PARTICLES USING *Curcuma longa* EXTRACT

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UNIVERSITI SAINS MALAYSIA

2021

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by

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Thesis submitted in fulfilment of the requirements for the degree of Bachelor of Chemical Engineering

JULY 2021

ACKNOWLEDGEMENT

First and foremost, I would like to take this opportunity to express my sincere gratitude to my supervisor, Dr. Masrina Mohd Nadzir for her endless support, guidance, and advice throughout this research project. The writing of this research report has been a great challenge during this pandemic situation, but I am very grateful for all the encouragement given by my supervisor. The completion of this thesis would not have been possible without her guidance and supervision.

I would also like to thank all the staffs and lecturers of School of Chemical Engineering who had spent their time guiding all the students in completing the final year project successfully. All the online lectures and advices was greatly appreciated because the process of completing the final year project thesis was more organized and smoother. Special thanks to my parents, Thinagar Velu and Jeyaletsumy Vengataraman as well as my siblings, Gugan, Sharmila and Janani for their endless support and motivation to complete my studies.

Finally, I would like to express my gratitude to my friends who never fail to provide help and guidance throughout my four years of study including the research process and thesis writing. I would like to thank the School of Chemical Engineering, Universiti Sains Malaysia for providing me the opportunity to carry out the project. Thank you for making this accomplishment possible.

SARANYA THINAGAR
JUNE 2021

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LIST OF ABBREVIATIONS

AgNPs	Silver Nanoparticles
RSM	Response Surface Methodology
BBD	Box Behnken Design
CCD	Central Composite Design
$AgNO_3$	Silver Nitrate
HIV	Human Immunodeficiency Virus
PVP	Polyvinylpyrrolidone
SPR	Surface Plasmon Resonance
DOE	Design of experiment
RSE	Residual Standard Error

LIST OF SYMBOLS

nm	Nanometer
g/L	Gram per liter
°C	Degree Celsius
min	Minutes
mM	Millimolar
%	Percentage
mL	Milliliter
μm	Micrometer
g	Gram

PERBANDINGAN REKA BENTUK BOX-BEHNKEN DAN REKA BENTUK KOMPOSIT PUSAT UNTUK MENGOPTIMUMKAN BIOSINTESIS PARTIKEL PERAK MENGGUNAKAN EKSTRAK *Curcuma longa*

ABSTRAK

Curcuma longa (C. longa) merupakan sejenis tumbuhan saka rizom herba yang tergolong dalam keluarga *Zingiberacae* iaitu keluarga halia. Sebatian paling aktif yang terdapat dalam *C. longa* adalah kurkumin yang mengandungi polifenol yang merupakan komponen penting untuk mengaktifkan proses penurunan semasa sintesis partikel perak. Dalam proses biosintesis, kaedah pengoptimuman penting untuk meningkatkan hasil dan kecekapan dalam penghasilan partikel perak. Tujuan kajian ini adalah untuk menentukan keadaan proses yang optimum, membandingkan reka bentuk Box-Behnken (BBD) dan reka bentuk komposit pusat (CCD) dalam proses pengoptimuman dan menjelaskan interaksi utama antara pelbagai parameter dalam biosintesis partikel perak menggunakan ekstrak *C. longa* yang meliputi: Kepekatan ekstrak *C. longa* (g/L), kepekatan argentum nitrat (mM), suhu (°C) dan masa (minit) dengan menggunakan kaedah gerak balas permukaan (RSM). Model gerak balas permukaan diterapkan oleh BBD dan CCD untuk menentukan keadaan optimum dengan memaksimumkan nilai serapan. Hasil kajian menunjukkan bahawa kedua-dua model BBD and CCD menggambarkan model kuadratik dengan koefisien regresi yang tinggi dengan nilai masing-masing, 0.9889 dan 0.9448. Keadaan optimum yang dicapai untuk model BBD adalah 9.53 g/L, 61.3 °C, 10.7 minit, 1.22 mM dengan keinginan 1.000 manakala 9.57 g/L, 60.5 °C, 10.4 minit, 1.16 mM dengan keinginan 0.914 berdasarkan model CCD. Model BBD yang dihasilkan lebih dipercayai dengan ramalan nilai serapan yang lebih tinggi (1.100) yang sangat dekat dengan nilai sebenar namun ramalan model CCD kurang tepat (1.078) berbanding nilai sebenar. Nilai

tindak balas yang diramalkan untuk model BBD menunjukkan kesepakatan yang sangat baik dengan nilai sebenar dengan baki ralat piawai sebanyak 0.30 % diikuti oleh model CCD dengan baki ralat piawai 2.52%. Oleh itu, didapati bahawa model BBD sangat tepat dan nilai yang berkaitan dengan setiap keadaan optimum cukup dekat dengan nilai yang sebenar. Di antara parameter yang dikaji, kepekatan ekstrak *C. longa* merupakan unsur yang paling signifikan mempengaruhi nilai serapan sedangkan masa inkubasi adalah parameter yang paling tidak signifikan mempengaruhi nilai serapan bagi kedua-duanya model BBD dan CCD.

COMPARISON BETWEEN BOX-BEHNKEN DESIGN AND CENTRAL COMPOSITE DESIGN FOR OPTIMIZING THE BIOSYNTHESIS OF SILVER PARTICLES USING *Curcuma longa* EXTRACT

ABSTRACT

Curcuma longa (C. longa) is a rhizomatous herbaceous perennial plant that belongs to the *Zingiberacae*, the ginger family. The most active compound found in the *C. longa* is curcumin that contains polyphenol which is the vital component activating the reduction process during silver nanoparticles (AgNPs) synthesis. In a biosynthesis process, optimization process is important to develop a process that results in higher yield and efficiency. The goal of this study is to determine the optimum process conditions, comparing the Box–Behnken Design (BBD) and Central-Composite Design (CCD) in optimization process and elucidating the main interacting parameters on the biosynthesis of silver particles using *C. longa* extract, which includes: Concentration of *C. longa* extract (g/L), concentration of silver nitrate (mM), temperature (°C), and time (minutes) by using response surface methodologies (RSM). Response surface models were applied by both BBD and CCD for determining optimal condition by maximizing the absorbance value. Results showed that both BBD and CCD models depicted the quadratic models with high regression coefficient of 0.9889 and 0.9448, respectively. The optimum conditions attained for BBD model are 9.53 g/L, 61.3 °C, 10.7 minutes, 1.22 mM with 1.000 desirability whereas 9.57 g/L, 60.5 °C, 10.4 minutes, 1.16 mM with 0.914 desirability based on CCD model. The BBD model generated was more reliable, with a greater prediction of absorbance value (1.100) that was very close to the actual values however the CCD model predictions were less accurate (1.078) to the actual response. The predicted response for BBD model showed excellent agreement with the actual value with residual standard error of 0.30% followed by CCD model with residual standard error of 2.52%.

Consequently, it was discovered that BBD model is highly accurate, and the values associated with each optimal condition are close enough to actual results. Among the variables investigated, the concentration of *C. longa* extract was found to be the most significant element impacting absorbance value whereas the incubation time is least significant parameter influencing the absorbance value for both BBD and CCD model.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Nanoparticles have been used widely across the globe due to the technological advancement in various fields. Nowadays, the nanoparticles have become one of the most prominent research field that deals with various particles with sizes ranging from 1 to 100 nm. The intensity of research involving nanoparticles are very high as it include various fields such as medicine, pharmaceutical, food, cosmetics, electric and electronics, environmental health, energy sector and drug-delivery (Picco *et al.*, 2016). To be more specific, silver is one of the most capitalized nanomaterials with tons of production per year and it is expected to increase tremendously over the years (Ahmed *et al.*, 2016). AgNPs possess novel property such as high temperature tolerance which can hinder the growth of microorganisms compared to other nanoparticles (Khan *et al.*, 2019). Moreover, AgNPs have strong and wide spectrum of bactericidal activities where it plays a profound role in healing and curing wounds due to the anti-inflammatory effects (Hamouda *et al.*, 2019).

There are various routes involved in the synthesis of AgNPs such as biological approach, physical approach, chemical approach and photochemical approach (Haider *et al.*, 2015). Physical and photochemical approach requires very high temperature and power consumptions that involves costly equipment. Chemical approach is the simplest method but it involves the usage of hazardous and toxic substances amid silver synthesis which may harm the environment (Khan *et al.*, 2018). Thus, the present study focuses on the biological approach towards green synthesis of AgNPs. Since plant extracts are used to synthesize silver in this approach, this method emerges as a simple, safe, low cost and feasible alternative to the conventional approach (Maghimaa *et al.*, 2020).

The green synthesis method of AgNPs is receiving great recognition in conjunction with increasing environmental awareness. The main intention of implementing this method is that they are environmentally safe and cost effective (Ziadi *et al.*, 2017). Moreover, the minimal utilization of hazardous chemical substances and low level of waste generated are some of the benefits in a green synthesis method. This method normally focuses in biosynthesis of nanoparticles using the plant extract. Many studies has revealed the exploration of reduction process of metal from various plant extract using different parts of the plant (Reddy *et al.*, 2020). Based on the enormous potentiality of plant as the main source, it would be the best method to synthesize silver compare to the conventional methods. However, it is not easy to acquire high yield of AgNPs by using biological approach. Thus, optimization need to be done to enhance the product yield (Natsuki *et al.*, 2015).

Most of the research papers involve the classical approach by conducting the experiment where one variable is studied at a time while keeping the other variables as fixed conditions. This approach will be time consuming as several sets of experiments need to be carried out and more particularly the interrelation between the variables cannot be analysed (Ahani *et al.*, 2017). Hence, an experimental design like Response surface methodology (RSM) is applied to analyse the interactive effects of multiple factors on the response variables. The main purpose of applying this method is to determine the optimum conditions to produce AgNPs and reduce the number of experimental trials compare to the conventional method. In a synthesizing process, optimizations helps to enhance the product yield by maximizing the efficiency and minimizing the cost (Shahzad *et al.*, 2019).

The present study focuses on optimization of AgNPs synthesis using *Curcuma longa* (*C. longa*) extract by comparing different methods of optimization under response surface methodology. *Curcuma longa* is commonly known as turmeric and it is a rhizomatous herbaceous perennial plant that belongs to the *Zingiberacae*, the ginger family (Kurian *et al.*, 2016). The most active compound found in the *C. longa* is curcumin. Curcumin is a yellow-coloured compound that contains polyphenol which is the vital component activating the reduction process during AgNPs synthesis (Khan *et al.*, 2019). Other than that, plant extract from leaves or rhizome of the *C. longa* is a proven medicinal plant that is traditionally being utilized by various Asian countries for skin disorders, bacterial infections, ulcer and wounds (Rafieian-Kopaei *et al.*, 2014). Apart from that, previous studies have revealed that it can also serve as anti-cancer agent that inhibits the formation and growth of tumour cell by imposing anti-angiogenic effects (Mansouri *et al.*, 2020).

1.2 Problem Statement

Basically, nanoparticles can be prepared by using various methods such as chemical, physical, and biological methods. Chemical and physical methods utilize toxic and hazardous chemicals that eventually effect the environment and human health. Other than that, both these methods require supreme temperature and vacuum conditions to synthesize the nanoparticles which ends up using expensive equipment to withstand the extreme conditions (Natsuki *et al.*, 2015). Thus, it is important to focus on an alternative method that provide cost-effective route and safer environment. Keeping this as our main aspects, the green synthesis under biological method is one step ahead compare to other methods.

The uttermost advantage of green synthesis is the synthesis of nanoparticles can be easily intensified, environment friendly where there is no usage of hazardous chemical, it does not require extreme temperature, pressure and energy to synthesize the nanoparticles (Ahmed *et al.*, 2020). The green synthesis usually involves the bacteria, fungi and plants. Despite that, due to demand of highly aseptic conditions and sustentation, the utilization of microbe mediated synthesis is not feasible for industrial applications (Muthukumar *et al.*, 2020). On that account, the usage of plant extracts should be more favourable mainly due to the greener synthesizing method and simplicity of enhancement.

Besides that, RSM should be implemented to investigate the interactive impact of the parameters as it is vital for biosynthesis of silver. The RSM is advantageous over One-Factor-At-A-Time (OFAT) technique whereby one variable is studied at a time and does not involve any interactions between the variables. This method also provides a statistical model that elucidates the effects of independent factors on the response. Consequently, the required time, cost and materials is reduced as less experimental trials are needed compare to the traditional optimization methods. The optimization of process conditions will help to maximize the product and minimize the expenses (Barabadi *et al.*, 2019).

To date, there is no research being published about the optimization of silver synthesis from *C. longa* extract via response surface methodology. Therefore, this study focuses on the optimization of factor affecting the synthesis of silver particles from *C. longa* extract in terms of concentration of silver nitrate, concentration of *C. longa* extract, temperature and time with the comparison of BBD and CCD to distinguish the better method of optimization for silver synthesis.

1.3 Research Objectives

The main aim of this research is to optimize the operating parameter to synthesize silver particles using RSM and recognize the best optimization method between BBD or CCD for silver particle synthesis which involves plant extract. The objectives of this research are:

i. To optimize the synthesis of silver particles using RSM via BBD and CCD in terms of concentration of silver nitrate, concentration of *C. longa* extract, temperature, and extraction time.
ii. To investigate the interactive effect of concentration of silver nitrate, concentration of *C. longa* extract, temperature, and extraction time on the biosynthesis of silver.
iii. To compare the BBD and CCD in the optimization of silver particles synthesis using plant extract.

1.4 Research Scope

In this study, the data used for the optimization of the silver synthesis was obtained from a research article entitled 'Biofabrication of AgNPs using *C. longa* extract: Effects of extraction and synthesis conditions, characteristics, and its antibacterial activity' (Trung *et al.*, 2020). The selection of process factors and their levels were determined, followed by optimization of extraction parameters via RSM using BBD and CCD model. The parameters studied for the optimization process were limited to the concentration of *C. longa* extract (7.5-12.5 g/L), temperature (50-70 °C), incubation time (5-15 min) and concentration of $AgNO_3$ (1.00-1.50 mM). The model equation was obtained for both BBD and CCD models. Statistical analysis was carried out to compare and test the adequacy of BBD and CCD models obtained from the optimization process. The model graphs of 3D and 2D contour plots were analyzed to investigate the interactive effect of concentration of silver nitrate, concentration of *C. longa*, temperature, and extraction time on the biosynthesis of silver. Finally, the accuracy and precision of two optimization methods which are BBD and CCD was verified by evaluating the residual standard error to determine the best model suitable for optimization of biosynthesis of silver particles using plant extract.

1.5 Organization of Thesis

The contents for each chapter in this study are stated below.

Chapter 1: An overview of this study and the importance of synthesis of silver particles by green synthesis. This chapter also includes the problem statement, research objectives, research scope and the organization of thesis.

Chapter 2: Review of the literature related to this study. A comprehension into silver particles in general, the methods available for silver synthesis and the factors influencing the synthesis of silver particles. Finally, the statistical tools used to perform and evaluate the optimization studies between BBD and CCD was discussed.

Chapter 3: This chapter provides clear description on the methodology of this research which includes the optimization of the process parameters, statistical analysis based on ANOVA, response surface plots for synthesis of silver particles and the verification of the models.

Chapter 4: This chapter presents the results and discussion of the model fitting of Box-Behnken Design and Central Composite Design followed by statistical optimization by applying RSM along with the response surface analysis using 3D and 2D contour plot. Then, the interactive effects of the process parameters were discussed followed by the optimization of the conditions for silver synthesis. The verification of both BBD and CCD model was evaluated by comparing the residual standard error. Last part, the sustainability aspect related to this thesis was considered and explained in detail.

Chapter 5: This chapter concludes the research and recommendation for future improvement to this research project are proposed.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Silver Particles

AgNPs compose of peculiar chemical and physical properties that makes them applicable in various fields hence many researchers have shown their interest to deal with AgNPs (Ndikau *et al.*, 2017). The total production of AgNPs was estimated to be 500 tonnes per year in 2009 whereas this number was expected to hike up to 900 tonnes per year by 2025 (Islam *et al.*, 2021). The major application of AgNPs is medical products as it represents 30% of overall application followed by 25% in coatings and paints, 15% in cosmetics and personal care products, 15% in textiles and 15% for other applications (Islam *et al.*, 2021). **Figure 2.1** shows the applications of AgNPs in various fields.

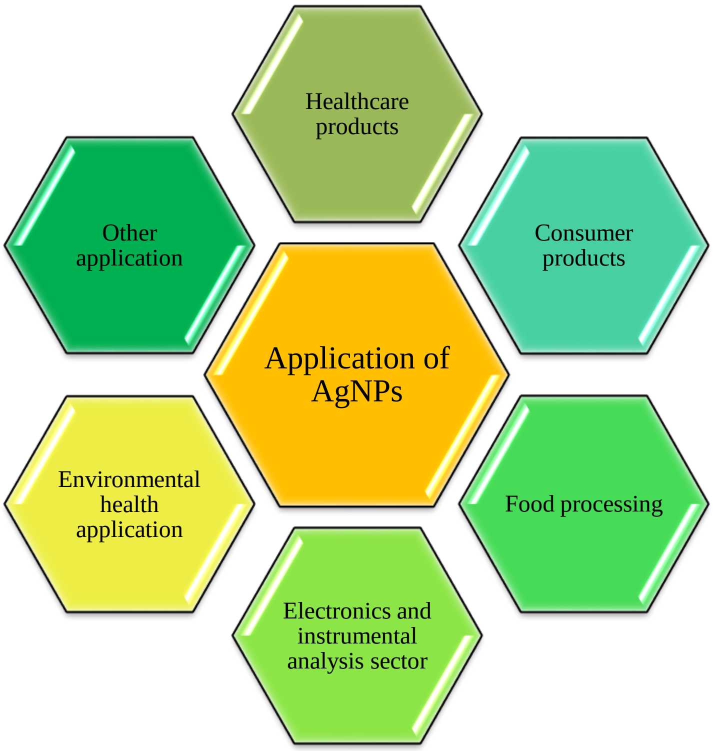

Figure 2.1 Applications of AgNPs (Islam *et al.*, 2021)

AgNPs are being used extensively in various healthcare products because of its unique antimicrobial properties that are able to fight with bacteria, viruses and fungi (AbuDalo *et al.*, 2019). Wound care products, antimicrobial bandages, breathing tubes and catheters are the examples of healthcare products made up of AgNPs. Other than that, silver compounds were utilized as a treatment for wounds to prevent any antimicrobial activity in the affected area before antibiotics was discovered. To add onto that, AgNPs could be utilized as antitumor agents where it can treat human cancer cells especially lung cancers and also proven to possess inhibitory effect on human immunodeficiency virus (HIV) cells (Jennings *et al.*, 2020).

Moreover, AgNPs can be found being used in many consumer products such as cosmetics, personal care products, soaps and pastes due to the antimicrobial effect although the concentration of AgNPs is very low (Talapko *et al.*, 2020). Besides that, AgNPs are suitable to be used in electronics and instrumental products as it is stable and possess very low electrical resistivity. For instance, silver-containing transistors, optical fibres, photonic and anti-reflective materials are common examples (Islam *et al.*, 2021).

In food processing industry, AgNPs are used in packaging containers and wrapping consumables to prevent growth of bacteria, consequently lengthen the shelf-life of the food products (Bajpai *et al.*, 2018). On the other hand, low concentration of AgNPs are being utilized in few food supplements that are safe for humans and able to kill the microorganisms. Although AgNPs have many advantages, the high concentration of silver is hazardous to living organism and aquatic life. Thus, ingestion of silver will result in serious health risks for humans (Islam *et al.*, 2021).

2.2 Overview of Curcuma longa

Curcuma longa is a rhizomatous herbaceous perennial plant with short stem and large oblong leaves. Its rhizomes are in ovate shape and are often branched. These plants require 20 to 30 °C of temperature and consistent amount of rainfall throughout the year to grow healthily. These plants are known for their medicinal purposes and being use in the entire world (Sharifi-Rad *et al.*, 2020). The plant belongs to the ginger family, *Zingiberaceae* which is native to India. The inner side of the rhizomes are orange in color whereas the outer layer is yellowish brown.

The rhizomes can be blended into yellow powder form when the rhizomes are dried. They are slightly bitter and possess certain odor which are slightly pungent. The largest producer of *C. longa* in the world is India where they dominate 90% of the total yield worldwide (Baliga *et al.*, 2018). Other than that, *C. longa* is also being used as a spice, dental health improvement, perfumes, natural coloring agent and food additives which are approved by the responsible authorities (Lim *et al.*, 2016).

2.3 Potential Application of *Curcuma longa*

C. longa is an herb with bright yellow pigment that has been used in various application all over the world. *C. longa* is also revealed to have diverse biological effects such as antimicrobial, antioxidant, anti-inflammatory, antitumoral and hypolipidemic properties (Ahmad *et al.*, 2020). Curcumin diferuloylmethane is known as the active compound found in *C. longa* which was first discovered in 1910 (Vaughn *et al.*, 2016). Moreover, curcumin appears as an effective compound to deal with the pathological pain that comes along with chronic diseases. Oral cavity related conditions such as tooth pain, cavity sealant and treatment for malignant lesion could utilize the *C. longa* for pain relief (Normando *et al.*, 2019). Curcumin is proven to reduce hyperlipidaemia and the resistance to insulin consequently influence high-density lipoprotein functions.

On top of that, fatty liver among patients can be minimized in the presence of curcumin compound but only applicable for non-alcoholic fatty-liver disease (Tranchida *et al.*, 2017). Furthermore, curcumin act as an anticancer agent especially for those who were diagnosed with head and neck squamous cell carcinoma. Gastric, colon and breast cancer also can be suppressed by using curcumin (Mansouri *et al.*, 2020). Other than this, liver cancer induced by tobacco smoke can also be prevented. Another most recognized function of curcumin is that it acts as an antimetastatic agent and has cytotoxic effects on tumour cells (Bachmeier *et al.*, 2018).

In the course of chemotherapy, curcumin act as a chemosensitizer and minimizes the side effects from chemotherapy drugs by reducing the toxicity. Curcumin also shows protective effects towards damaged cell caused by ultraviolet rays. Additionally, *C. longa* has immense effects on skin health and able to treat psoriasis as the curcumin compound has antibacterial, antiviral, and antifungal effects. Also, curcumin has advantageous effects on Alzheimer’s disease, able to treat major depressive disorder and plays an important role in reducing the indication of premenstrual syndrome (Chen *et al.*, 2018).

2.4 Synthesis of Silver Particles

Generally, there are two methodologies which are associated in the synthesis of nanoparticles, either "top to bottom" approach or a "bottom to top" approach. These two synthesis approaches of silver particles involve the chemical, physical, and biological methods. The top to bottom approach involves breaking down of appropriate bulk material into small particles via size reduction involving many techniques. This approach usually involves the physical method where evaporation condensation is used to synthesize metal nanoparticles using tube furnace at atmospheric pressure. The major disadvantages of utilizing this equipment are excessive energy consumption, use up large space, extreme power consumption, requires plenty of time to reach thermal stability and high cost. Moreover, the nanoparticles formed via these methods are usually imperfect in the surface structure and the physical properties are highly dependent on the surface structure (Ahmed *et al.*, 2016).

In contradictory, for bottom to top approach, biological and chemical methods are used in silver synthesis by self-assemble of atom to new nuclei that grow into nanoscale particles. In this approach, AgNPs are commonly synthesized by using chemical reduction. Chemical methods usually use toxic chemicals which will produce non-eco-friendly by-products. Thus, biological method via green route is more preferred as it does not involve toxic chemicals. This biosynthesis of nanoparticles involves biological bodies such as plant extract and microorganisms. The major advantages of biosynthesis via green route are environment friendly, low-cost production and can be easily scaled up (Natsuki *et al.*, 2015).

2.4.1 Physical Method

Physical method is known to produce large quantities AgNPs in a single process (Natsuki *et al.*, 2015). There are few techniques for synthesis of AgNPs via physical method such as vapor condensation, laser ablation, irradiation, and lithography. Evaporation and condensation are the process involved in vapor condensation which are conducted by using tube furnace at atmospheric pressure. Carrier gas will be obtained by vaporizing the source material within a boat centred at the furnace. The targeted materials are vaporized by utilizing heat source and hastily condensed which will result in the synthesis of AgNPs (Yusuf *et al.*, 2019). However, a tube furnace is known to consume vast amount of power and longer heating time in order to achieve stable operating conditions (Natsuki *et al.*, 2015).

Laser ablation is another method to synthesis AgNPs. This technique does not involve the usage of chemical substances however emerge as an efficient method to synthesize AgNPs. By applying this method, the particle size of the colloids can be controlled by varying the number of laser pulses. The laser ablation onto metallic bulk silver salts in liquid state can result in AgNPs (Yusuf *et al.*, 2019). The method is more advantageous than conventional methods because the preparation of metal colloids does not involve chemical substances hence no toxic materials are generated from this method (Natsuki *et al.*, 2015).

Additionally, AgNPs can be synthesized by using irradiation method where a silver is targeted with a laser beam in pure water. A small size nanoparticles and tapered size distribution are produced from this technique by using high-power laser and laser beam with small spot sizes. Moreover, nanosphere lithography method is used to produce large-sized nanoparticles and well-ordered 2D nanoparticles arrays which is simple and inexpensive method (Güzel *et al.*, 2018).

Having said that, the synthesis of AgNPs using physical methods have abundant stumbling block because of their large size which occupies large space, utilizes large amount of energy subsequently increasing the surrounding temperature and a lot of time is needed to attain the thermal stability. Moreover, this method is very expensive due to high energy consumption (Singh *et al.*, 2019).

2.4.2 Chemical Method

Besides physical approach, chemical reduction is one of the popular methods in AgNPs synthesis due to its simplicity and convenience. In order to obtain small sized and spherical shaped nanoparticles, the growth of the nanoparticles need to be controlled. The benefits of utilizing chemical approach are the AgNPs can pe synthesized at low cost and high yield can be obtained. Metal precursors, reducing agents and stabilizing agents are three main components generally needed in chemical synthesis of AgNPs. Two stages involved in the formation of colloidal solutions from the reduction of silver salts are nucleation and subsequent growth (Haider *et al.*, 2015). These stages play the main role in determining the size and shape of the synthesised AgNPs.

Moreover, all nuclei are desired to form at the same period in order to acquire monodispersed AgNPs of consistent size distribution. Thus, all the nuclei will have same subsequent growth and expected to have similar size approximately. These initial two stages mentioned before can be controlled by modifying the reaction variables such as pH, precursors, reaction temperature, reducing agents and stabilizing agent. The examples of reducing agents are glucose and sodium borohydride whereas sodium oleate and Polyvinylpyrrolidone(PVP) are the examples of stabilizing agents (Haider *et al.*, 2015; Natsuki *et al.*, 2015). The usage of protective agents are crucial to stabilize and protect the nanoparticles that can cohere onto nanoparticle surfaces and also to avoid agglomeration during the production of AgNPs (Abbasi *et al.*, 2016).

Other than that, the existence of surfactants such as acids, amines, alcohol and thiols also help in protecting and stabilizing the particles from agglomeration or any other interactions that may affects the changes in the surface properties. To add onto that, polymeric compounds possess the same functions as surfactants to protect and stabilize the AgNPs. For instance, poly(vinyl alcohol), poly(vinylpyrrolidone), poly(ethylene glycol), poly(methacrylic acid) and polymethylmethacrylate are the examples of polymeric compounds (Abbasi *et al.*, 2016). However, there are few drawbacks associated with chemical methods such as usage of toxic chemicals which acts as reducing agents or surfactants which will aggravate and create harmful environmental effects (Abdellah *et al.*, 2018).

2.4.3 Biological Method

Biological method is an alternative method of synthesizing AgNPs by using biological routes which involves microorganisms and plants. Nowadays this method is more preferred by researchers compared to chemical and physical methods due to several reasons. One of the reasons are the usage of chemical stabilizer and reducing agents in the chemical methods result in production of contaminated AgNPs (Singh *et al.*, 2019). Besides that, the synthesis of AgNPs using physical methods have many disadvantages such as utilization of large amount of energy subsequently increasing the surrounding temperature and a lot of time is needed to attain the thermal stability. Moreover, this method is very expensive due to high energy consumption (Singh *et al.*, 2019). Thus, an inexpensive, safe, valid, and green approach are considered in the synthesis of AgNPs with desired size and shape. For instance, most green method does not involve the usage of expensive chemicals, has less energy consumption and the product formed are environmentally friendly (Khan *et al.*, 2017). Hence, all these added benefits prove that biological method is more advantageous compared to physical and chemical methods.

2.4.3.1 Synthesis using Bacteria

Microbial synthesis of nanomaterials involves the usage of prokaryotes and eukaryotes. Microorganisms are known to be associated with many biological activities and the metals or non-metals are commonly related to these activities (Kushwaha *et al.*, 2015). In general, bacteria are the most lavishly found organism in our atmosphere. Bacteria is the main course in the production of inorganic materials either via intracellular or extracellular synthesis. Thus, bacteria emerge as one of the crucial organisms in the synthesis of noble metals such as AgNPs as shown in **Figure 2.2** (Rafique *et al.*, 2017).

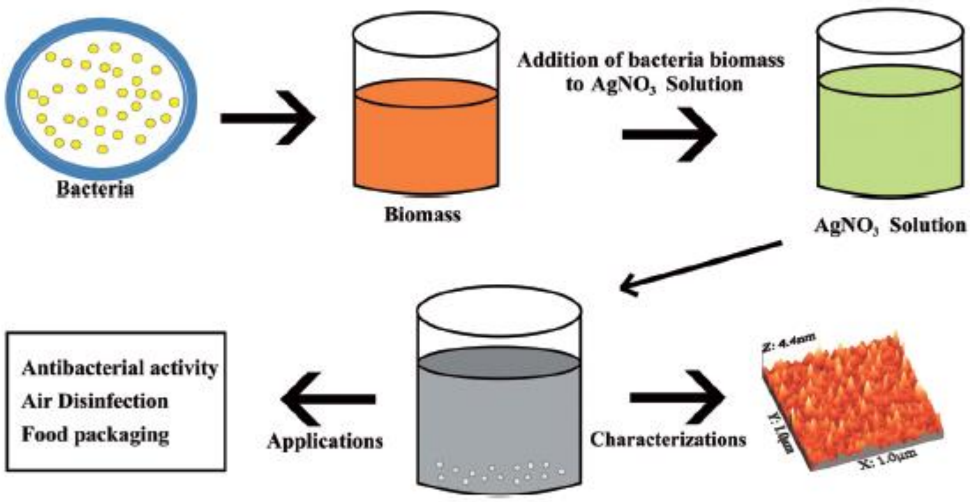

Figure 2.2 Schematic diagram for synthesis of AgNPs using bacteria (Rafique *et al.*, 2017)

Bacteria turns up as an appropriate, flexible, and suitable choice for large scale green synthesis of AgNPs. This method is also known to be advantageous because the biologically synthesized nanoparticles have various applications especially in the medical fields. The vital reason for the recent hike up in the evolution of silver based products is the astonishing antimicrobial activity reported by most of the researchers (Kushwaha *et al.*, 2015).

Table 2.1 shows the bacteria used in the green synthesis of AgNPs. However, there are some drawbacks of utilizing bacteria in silver particles synthesis such as slow production rate and the size and shape of silver particles are restricted in contrast with the conventional routes. Moreover, it is difficult to obtain uniform distribution of the particle size, similar shape and structure, chemical constituent, and arrangements (Abdelghany *et al.*, 2018).

Table 2.1 Green synthesis of AgNPs using bacteria

Bacteria	Shape of AgNPs	Size (nm)	References
Aeromonas sp.	Spherical	8-16	(Singh *et al.*, 2017)
Bacillus sp.	Spherical	42-92	(Das *et al.*, 2015)
Agrococccus sp.	Spherical	5-80	(Suman *et al.*, 2014)
Ochrobactrum sp.	Oval	38-85	(Thomas *et al.*, 2015)
Exiguobacterium mexicanum sp.	Spherical	5-40	(Padman *et al.*, 2014)
Euphorbia hirta L.	Spherical, oval, cubic, hexagonal	10-60	(Syed *et al.*, 2016)
Novosphingobium sp.	Spherical	8-25	(Du *et al.*, 2017)
Kinneretia sp.	Spherical	15-20	(Singh *et al.*, 2017)
Bacillus methylotrophicus sp.	Spherical	10-30	(Wang, C. *et al.*, 2016)
Rhodococcus spp.	Spherical	5-50	(Otari *et al.*, 2015)

2.4.3.2 Synthesis using Fungi

Fungi is considered as one of the appropriate choices for synthesis of AgNPs as they are capable of secreting large number of enzymes and can adapt easily to other mediums to reduce the silver nitrate solutions (Abdelghany *et al.*, 2018). For instance, *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Trichoderma* sp., and *Cladosporium* sp. are normally utilized in these processes. **Table 2.2** shows the synthesis of AgNPs by using various fungi. Other added advantages of silver particles synthesis using fungi are good bioactivity, low toxicity and enhance the control of pathogen. Thus, this method emerge as one of the popular method to synthesize silver particles (Guilger-Casagrande *et al.*, 2019).

Table 2.2 Green synthesis of AgNPs using fungi

Fungus	Precursor	Size (nm)	References
Penicillium oxalicum	$AgNO_3$	10-40	(Rose *et al.*, 2019)
Fusarium oxysporum	Nitrate reductase	24	(Hamedi *et al.*, 2017)
Sclerotinia sclerotiorum	$AgNO_3$	10-15	(Saxena *et al.*, 2016)
Arthroderma fulvum	$AgNO_3$	15.5±2.5	(Wang, L. *et al.*, 2016)
Trichoderma longibrachiatum	$AgNO_3$	5-30	(Elamawi *et al.*, 2018)
Duddingtonia flagrans	$AgNO_3$	30-409	(Costa *et al.*, 2017)
Aspergillus fumigatus	$AgNO_3$	322.8	(Shahzad *et al.*, 2019)
Aspergillus oryzae	$AgNO_3$	7-27	(Phanjom *et al.*, 2017)

One of the main drawbacks of using fungi is it require nitrogen in large amounts and nitrogen is considered as the limiting factor for the growth of fungi although they are capable of utilizing amino acids, ammonium and nitrate as a source of nitrogen (Zomorodian *et al.*, 2016). **Figure 2.3** shows the schematic diagram of AgNPs synthesis using fungi.

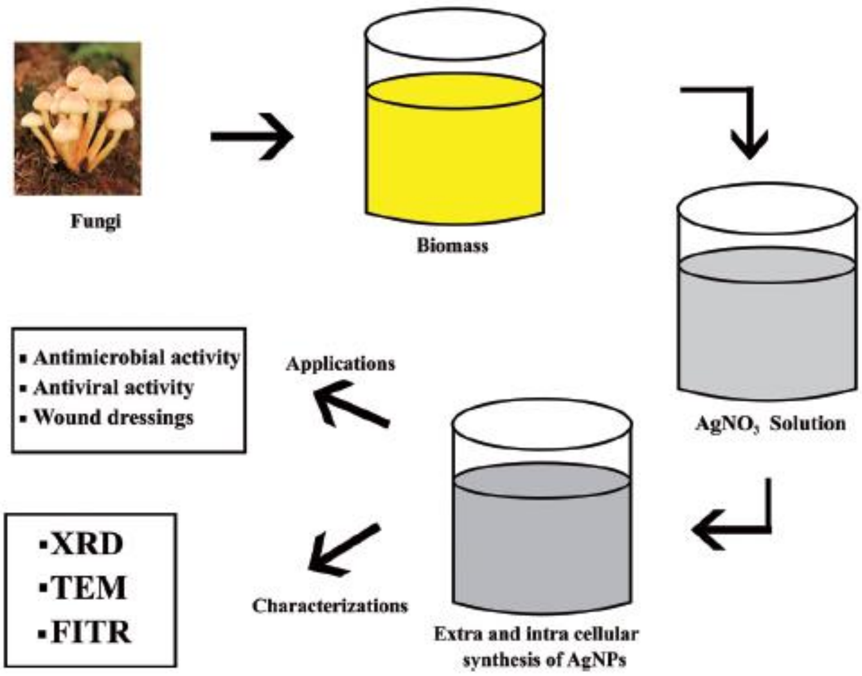

Figure 2.3 Schematic diagram of silver particles synthesis using fungi (Rafique *et al.*, 2017)

AgNPs are formed on the surface of mycelia for the medium that involves fungi. Due to the electrostatic force between the enzymes and the ions, the silver particles will be adsorbed on the surface of fungal cells. The enzymes present in cell walls reduces the silver particles to silver nuclei. As compared to bacteria, fungi are more preferred due to its straightforward downstream processing, biomass treatment and higher secretion of proteins which eventually increases the yield of silver particles obtain through this route (Rafique *et al.*, 2017). Optimization of the silver particles synthesis can be implemented by manipulating certain variables such as pH, silver nitrate concentration, amount of biomass, incubation temperature and cultivation time.

2.4.3.3 Synthesis using Plant Extract

The utilization of plant extracts for synthesis of silver particles is more advantageous than other conventional methods because there is no risk of bacterial contamination, simplicity, and less energy requirement. Besides that, the AgNP synthesis by using plant extracts reduces the metal ions due to the existence of functional molecules such as amides, ketones, phenols, carboxylic acid and many more. The content of specialised metabolites is known as the reducing agents in the synthesis of silver particles using plant extract (Masum *et al.*, 2019).

In general, the synthesis of AgNPs utilizing plants involves the dried biomass of the specific plant, metallic salts, and precursor. There are three mains steps that are followed for the synthesis of AgNPs via biological route. Firstly, the choice of solvent medium is determined followed by the determination of reducing agent that are environmental friendly and lastly, the capping agent is decided where it helps to stabilize the synthesized nanoparticles (Parveen *et al.*, 2016). Apparently, biological agents play the role as reducer or stabilizer in the production of silver particles (Aritonang *et al.*, 2019). **Figure 2.4** shows the schematic diagram for synthesis of AgNPs by using plant extracts.

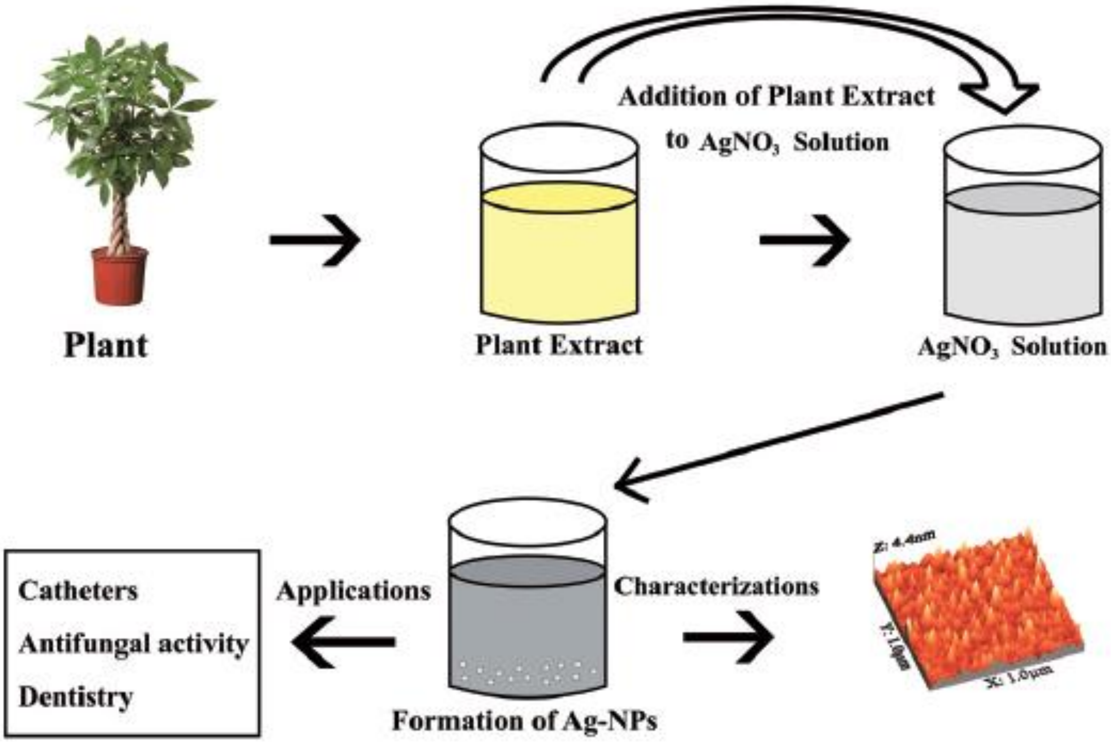

Figure 2.4 Schematic diagram for synthesis of AgNPs by using plant extracts (Rafique *et al.*, 2017)

Various plant extracts are being used for the AgNPs synthesis due to its advantageous nature such as environmentally friendly, fast synthesis rate, economical and non-toxic. The plant extracts can be acquired from various section of the plant such as leaves, root, bark, stem, fruits and peels (Castillo-Henríquez *et al.*, 2020). Plants have the potentials to take part in the synthesis of AgNPs are shown in **Table 2.3**. Moreover, plant extracts act as a reducing agent to reduce the silver nitrate solution to produce silver ions. This mechanism is normally observed through a UV-Visible spectrophotometer that demonstrate the absorbance value at specific wavelength (Roy *et al.*, 2017).

Table 2.3 Green synthesis of AgNPs using plant extract

Plant extract	Shape of AgNPs	Size (nm)	References
Gomphrena globasa leaf	Spherical, hexagonal, triangular	15-25	(Tamilarasi *et al.*, 2020)
Citurs limetta peel	Spherical	18	(Dutta *et al.*, 2020)
Pedalium murex leaf	Spherical	20-50	(Anandalakshmi *et al.*, 2016)
Azadirachta indica leaf	Spherical, irregular shapes	34	(Ahmed *et al.*, 2016)
Parkia speciosa leaf	Spherical	31-35	(Ravichandran *et al.*, 2019)
Luffa Acutangla leaf	Spherical	15-41	(Taruna *et al.*, 2016)
Musa paradisiaca peel	Spherical	23.7	(Ibrahim, 2015)

2.5 Factor Affecting the Synthesis of Silver Particles

There are several experimental parameters that are affecting the efficiency of silver particles synthesis such as concentration of plant extract, concentration of silver nitrate solution, temperature, pH, incubation time and ratio of plant extract to silver nitrate solution.

2.5.1 Concentration of Plant Extract

The concentration of silver nitrate solution plays a vital role in the biosynthesis of silver particles. Many researchers have published the influence of concentration of plant extracts on the synthesis of AgNPs. Ahmed (2016) had studied the influence of plant extract concentration on the silver synthesis by using *Azadirachta indica* aqueous leaf extract to obtain the best yield. It is reported that when the concentration of leaf extract in silver nitrate solution was increased from 9 % (v/v) to 29 % (v/v), the intensity of the absorbance value also increases together with longer wavelength.

In another finding, Zulfiqar *et al.* (2019) reported the same hypothesis where when the concentration of *Fagonia cretica* extract in silver nitrate solution was increased from 17 % (v/v) to 29 % (v/v), there was a sharp peak absorbed in the UV-Vis spectrum proving that there is formation of AgNPs. Whereas at low concentration of the plant extract, there was no observable peak in the UV-Vis spectrum may be due to the insufficient reducing agent to form the silver particles.

However, when the concentration of the plant extract in silver nitrate solution was further increased up to 44 % (v/v), the absorption peak started to reduce which result in poor yield of the silver particles. The results obtained by Zulfiqar *et al.* (2019) correspond with the result that is reported by Trung *et al.* (2020) when using *C. longa* stem extract. It is reported that when the concentration of *C. longa* extract was increased up to 12.5 g/L, the intensity of the absorption peak started to decrease and resulting in accumulation of silver particles. Thus, the most optimum concentration of plant extract needs to be precise to avoid the possibility of insufficient or oversupplied reducing agent where both will result in poor yield of silver particles.

2.5.2 Concentration of Silver Nitrate Solution

The concentration of silver nitrate solution plays a major role in the biosynthesis of silver particles. Several researchers have demonstrated the impact of concentration of silver nitrate solution on the biosynthesis of silver particles. Jalani *et al.* (2018) had studied the effect of concentration of silver nitrate solution ($AgNO_3$) onto the synthesis of AgNPs by using fresh *Citrus grandis* fruit peel extract to obtain the most optimum value. It is observed that when the concentration of $AgNO_3$ solution was increased from 1 mM to 5 mM, the Surface Plasmon Resonance (SPR) band switch to a longer wavelength. This phenomenon prove that large AgNPs are formed at high concentration of $AgNO_3$ solution.

The maximum absorbance value was achieved at 2 mM of $AgNO_3$ solution however the value of absorbance decreases as the concentration was elevated to 5mM. This occurrence is due to the availability of functional groups in the peel extract that is limited to react with the silver ions to form AgNPs (Vanaja *et al.*, 2013). The results obtained by Jalani *et al.* (2018) is corresponding with the result that is reported by using *Piper chaba* stem extracts (Mahiuddin *et al.*, 2020), *Coleus aromaticus* (*C. aromaticus)* leaf extract (Vanaja *et al.*, 2013) and *C. longa* extract (Trung *et al.*, 2020). Thus, lower concentration of $AgNO_3$ solution such as 1 mM is the optimum concentration for nanoparticles synthesis.

2.5.3 Temperature

The most crucial parameter that affect the synthesis of silver particles is temperature. In many instances, the temperature required to synthesis silver particles by using green synthesis method is at room temperature or less than 100 °C. Vanaja *et al.* (2014) reported that the productivity of silver particles increased when