SYNTHESIS, CHARACTERIZATION AND EFFECT OF SILVER NANOPARTICLES ON *Citrobacter* sp. A1 AND *Enterococcus* sp. C1 IN A SIMULATED ENVIRONMENT

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Bioscience)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

JUNE 2017

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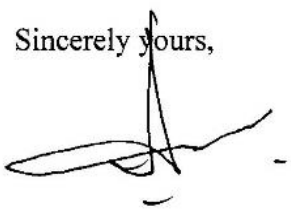

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Specially dedicated to my beloved parents,

'Thank you for always being there; your endless love, faith, and encouragement never fail to strengthen me'

To my beloved sister,

'Thank you for the endless support during my ups and downs'

ACKNOWLEDGEMENTS

First and foremost, I would like to express my endless thanks and gratefulness to my project supervisor, Prof. Dr. Noor Aini Abdul Rashid for her high-level inspiration, extensive editing, never ending support and guidance. My heartfelt appreciation to my late supervisor, Prof. Dr. Alias Mohd. Yusof. His motivation and knowledge had enabled me to develop experience in speciation and environmental chemistry. To my three co-supervisors; Assoc. Prof. Dr. Jafariah Jaafar, Dr. Mohd Firdaus Abdul Wahab and Dr. Chan Giek Far, my gratitude is for their useful suggestions and opinions. All the patience, criticism and ideas from my supervisor and co-supervisors throughout this study are greatly appreciated. Without their continuous support, the research objectives and thesis writing would not be accomplished.

A million thanks goes to all my labmates and seniors; Dr. Zarita, Liyana, Azura, Zarini, Nazanin, Safwan and Syima for giving me their support, encouragement and for just being a part of the Nanomaterial Lab. My sincere appreciation also goes to all the lab assistants of Faculty of Bioscience and Medical Engineering, UTM, for their assistance. Special thanks to the Faculty of Bioscience and Medical Engineering for the research facilities. I would like acknowledge Dr. Chua Lee Suan (Institute of Bioproduct Development, UTM) and her staff for the analyses using the ICP-MS. Also much thanks to staff from University Industry Research Laboratory, UTM, especially, Madam Norzubaidha, Miss Ain and Miss Aini for their invaluable technical and instrumental support. Thanks to Mr. Mohammed and Mr. Amin from Ibnu Sina Institute, UTM for their help on characterizing silver nanoparticles by TEM and SEM analysis. To Madam Irmazian and her staff from Institute of Bioscience, UPM, my appreciation for aiding me with the preparation of the bacterial cells for TEM analysis. My appreciation also goes to Ministry of Higher Education for MyPhD scholarship for given financial support and Ministry of Science, Technology and Innovation through Techno Fund Grant, National Nanotechnology Directorate (Vot 4H009) for funding this research.

Last but not least, I would like to extend my deepest gratitude and appreciation to my family and my close friends for their encouragement and moral support. Thank you very much for everything.

ABSTRACT

Silver nanoparticles (AgNPs) are one of the most widely used commercial nanomaterials due to their antimicrobial properties which could pose as environmental hazard. However, lack of study on the fate and effect of AgNPs released into the environment has prompted this research. Thus, the purpose of this study was to investigate the fate of AgNPs in the environment and also to assess the possible adverse effect of AgNPs on model environmental isolates; *Citrobacter* sp. A1 (designated A1) and *Enterococcus* sp. C1 (designated C1). AgNPs capped with polyvinylpyrrolidone (PVP) were synthesized using ultrasound-assisted chemical reduction. It shows that low pH value, high ionic strength and the content of the media influenced the stability of PVP-capped AgNPs. A1 and C1 were contacted with varying concentrations of PVP-capped AgNPs (10, 100, 1000 mg L^{-1}) in nutrient-rich media or 0.1 M phosphate buffer (PB). No significant effect of PVP-capped AgNPs on both A1 and C1 in nutrient-rich media was observed. However, at 1000 mg L^{-1} PVP-capped AgNPs in 0.1 M PB, A1 retained viability for 6 h while C1 only 3 h. A1 appeared to be more resistant to AgNPs than C1. It is possible that silver ions (Ag^+) were released from PVP-capped AgNPs and contributes to the antibacterial effect. The antibacterial mechanism of PVP-capped AgNPs was evaluated. Ag^+ or Ag^+-adsorbed AgNPs may attack the cell membrane and internalized, leading to bacterial cell death. The effect of the final rinse from a Sharp washer with Ag^+ ions releasing function was determined. Although only Ag^+ ions were released by the washer, the presence of a mix of AgNPs, Ag ions and other forms of Ag may suggest Ag^+ ion transformation. The final rinse water was found to be toxic towards A1 and C1. Additionally, the distribution, transformation and effect of AgNPs under simulated environment containing stream water and sediment was studied. Bacterial consortium of A1 and C1 was introduced into the simulated environment with AgNPs spiked over a period of 50 days. The results revealed that most of the Ag, either PVP-capped AgNPs or Ag^+ spiked into the simulated environment migrated from the aqueous phase into the sediment possibly due to aggregation and formation of Ag complexes. The viability of A1 and C1 from the water and sediment in the simulated environment remained unchanged, even at high concentration of AgNPs, at 6500 mg L^{-1} introduced due to the formation of Ag-organic matter complexes. However, A1 and C1 lost viability when spiked with Ag^+ ions exceeding 0.35 mg L^{-1} which indicates that Ag^+ ions were more toxic than AgNPs. It can be speculated that the fate of Ag (either AgNPs or Ag^+) is closely tied to the chemistry of the environment into which they are released while the toxic effect of Ag (either AgNPs or Ag^+) depends on their fate and the types of bacterial strains present.

ABSTRAK

Nanopartikel argentum (AgNPs) adalah salah satu bahan nano komersial paling banyak digunakan kerana ciri antimikrobnya yang kuat tetapi boleh mengundang bahaya kepada alam sekitar. Walau bagaimanapun, kekurangan kajian mengenai nasib dan kesan AgNPs selepas dibebaskan ke dalam alam sekitar telah mendorong kajian ini. Tujuan kajian ini adalah untuk mengkaji nasib AgNPs pada alam sekitar dan juga untuk menilai kemungkinan kesan buruk AgNPs pada pencilan model persekitaran; *Citrobacter* sp. A1 (A1) dan *Enterococcus* sp. C1 (C1). AgNPs ditukup dengan polivinilpirolidon (PVP) yang telah disintesis dengan menggunakan penurunan kimia berbantukan ultrabunyi. Ia menunjukkan bahawa nilai pH yang rendah, kekuatan ionik yang tinggi dan kandungan media dapat mempengaruhi kestabilan AgNPs ditukup PVP. Bakteria A1 dan C1 didedahkan dengan kepekatan AgNPs ditukup PVP yang berbeza-beza (10, 100, 1000 mg L^{-1}) dalam media yang kaya dengan nutrien atau 0.1 M penimbal fosfat (PB). Tiada kesan AgNPs ditukup PVP yang ketara diperhatikan pada kedua-dua bakteria A1 dan C1 di dalam media yang kaya dengan nutrien. Walau bagaimanapun, pada 1000 mg L^{-1} AgNPs ditukup PVP dalam 0.1 M PB, bakteria A1 mengekalkan kebolehhidupan selama 6 jam manakala bakteria C1 hanya 3 jam. Bakteria A1 didapati lebih tahan kepada AgNPs berbanding bakteria C1. Ada kemungkinan bahawa ion-ion argentum (Ag^+) yang dibebaskan daripada AgNPs ditukup PVP memberi kesan antibakteria. Seterusnya, mekanisma antibakteria AgNPs ditukup PVP dinilai. Ag^+ atau Ag^+ terjerap AgNPs dapat menyerang sel membran membawa kepada kematian sel bakteria. Kesan bilasan terakhir dari mesin basuh Sharp dengan fungsi pelepasan ion-ion Ag^+ ditentukan. Walaupun hanya ion-ion Ag^+ dikeluarkan oleh mesin basuh, kehadiran campuran AgNPs, ion-ion Ag dan lain-lain bentuk Ag mencadangkan transformasi ion Ag^+. Air bilasan terakhir didapati toksik terhadap bakteria A1 dan C1. Selain itu, pengagihan, transformasi dan kesan AgNPs di bawah simulasi persekitaran yang mengandungi aliran air dan sedimen juga dikaji. Konsortium bakteria A1 dan C1 yang diperkenalkan ke dalam simulasi persekitaran dengan AgNPs melonjak dalam tempoh 50 hari. Keputusan menunjukkan sebahagian besar Ag; sama ada AgNPs ditukup PVP atau Ag^+, melonjak ke dalam simulasi persekitaran bertukar daripada fasa akues kepada sedimen mungkin melalui pengumpulan dan pembentukan kompleks Ag. Kebolehhidupan bakteria A1 dan C1 daripada air dan sedimen di dalam simulasi persekitaran kekal tidak berubah, walaupun pada kepekatan AgNPs yang tinggi (6500 mg L^{-1}) diperkenalkan kerana pembentukan kompleks Ag-bahan organik. Walau bagaimanapun, bakteria A1 dan C1 hilang kebolehhidupannya apabila dilonjakkan dengan ion-ion Ag^+ melebihi 0.35 mg L^{-1}. Ini menunjukkan bahawa ion-ion Ag^+ lebih toksik daripada AgNPs. Ia boleh dispekulasikan bahawa nasib Ag (AgNPs ataupun Ag^+) berkait rapat dengan kimia alam sekitar, iaitu lokasi dibebaskan manakala kesan toksik Ag (AgNPs ataupun Ag^+) bergantung kepada spesies Ag sedia ada dan jenis strain bakteria yang hadir.

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LIST OF ABBREVIATIONS

8-oxoG	-	8-oxoguanine
A1	-	*Citrobacter sp.* A1
AgNPs	-	Silver Nanoparticle
ASTM	-	American Society for Testing and Materials
ATP	-	Adenosine triphosphate
BER	-	Base excision repair
BSA	-	Bovine serum albumin
C1	-	*Enterococcus sp*. C1
CFU	-	Colony forming unit
CPE	-	Cloud Point Extraction-Based Separation
CTAB	-	Cetyltrimethylammonium bromide
CTAC	-	Cetyltrimethylammonium chloride
DMF	-	*N*, *N*-dimethyl formamide
DNA	-	Deoxyribonucleic acid
DO	-	Dissolved oxygen
EDX	-	Energy dispersive X-ray
EPS	-	Exopolysaccharide
FBME	-	Faculty Biosciences and Medical Engineering
FCC	-	Face-centered cubic
FTIR	-	Fourier transform infrared spectroscopy
FWHM	-	Full width at half maximum
GSH	-	Glutathione
GSSG	-	Gluthathione disulfide
HIV	-	Human immunodeficiency virus
ICP-MS	-	Inductively coupled plasma mass spectrometer
ICP-OES	-	Inductively couple plasma-optical emission spectroscopy
ISE	-	Ion selective electrode

JCPDS	-	Joint Committee on Powder Diffraction Standards
LC50	-	Lethal concentration 50%
LPS	-	Lipopolysaccharide
MIC	-	Minimum Inhibition Concentration
MW	-	Molecular weight
NA	-	Nutrient agar
$NaBH_4$	-	Sodium borohydride
NADH	-	Nicotinamide adenine dinucleotide
NB	-	Nutrient broth
NOM	-	Natural organic matter
NPs	-	Nanoparticle
OD	-	Optical density
OECD	-	Organization for Economic Co-operation and Development
PB	-	Phosphate buffer
PEG	-	Polyethylene glycols
PGA	-	Poly-beta-1,6-N-acetyl-D-glucosamine
PNEC	-	Predicted no observed effect concentration
PU	-	Polyurethanes
PVA	-	Polyvinylalcohols
PVP	-	Polyvinylpyrrolidone
RND	-	Resistance-nodulation-cell division
ROS	-	Reactive oxidative species
SDS	-	Sodium dodecyl sulfate
SDS	-	Sodium Dodecyl Sulphate
SEM	-	Scanning electron microscopy
SP-ICP-MS	-	Single particle-inductively couple plasma-mass spectroscopy
SPR	-	Surface plasmon resonance
TAE	-	Tris-acetate-EDTA
TCS	-	Two component system
TEM	-	Transmission electron microscope
TOC	-	Total organic carbon
U.S.	-	United States
USEPA	-	United States Environmental Protection Agency
UTM	-	University Teknologi Malaysia

UV-Vis	-	UV-Visible
XRD	-	X-ray diffraction
XRF	-	X-ray fluorescence

LIST OF SYMBOLS

nm	-	Nanometer
min	-	Minute
ppb	-	Part per billions
g	-	Gram
μm	-	Micrometer
M	-	Molar
mM	-	Mili molar
h	-	Hour
L	-	Litre
ppm	-	Part per millions
% w/v	-	Weight per volume percent
°C	-	Celcius
kPa	-	Kilopascal
% v/v	-	Volume per volume percent
rpm	-	Revolutions per minute
θ	-	Theta
Å	-	Angstrom
λ	-	Lambda
α	-	Alpha
β	-	Beta
kV	-	Kilo volt
kW	-	Kilowatts
K	-	Kelvin
atm	-	Atmosphere
s	-	Second
keV	-	Kilo electronvolt
GHz	-	Gigahertz

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nanotechnology has been growing and progressing drastically in both scientific and industrial sectors since the 20th century. The rapid development of the nanotechnology industry has even brought nanomaterials/nanoparticles (NPs) into many aspects of daily life (Li, 2011). NPs refers to particles with at least one of the dimensions ranged between 1 to 100 nanometers in term of size (ASTM, 2006). Among the NPs, silver nanoparticles (AgNPs) are the most common and widely used. According to Fabrega *et al.* (2011), there are existing 259 AgNPs-containing products out of 1015 NPs-containing commercial products in the market.

AgNPs have gained extensive uses in several applications such as pharmaceutical, cosmetic, electronics, bio-sensing, optical devices, environmental remediation, catalysis and material sciences (Badawy *et al.*, 2011). It can also be found in many commercial products ranging from paint, cosmetics, medical and textiles to sportswear and electrical appliances such as washing machines and air-conditioners. This is mainly due to their strong antimicrobial properties to prevent infection and growth of microorganisms (Maynard *et al.*, 2006; Guzmán *et al.*, 2006; Ansari *et al.*, 2011). In a study by Rues (2011), AgNPs was shown to possess stronger antimicrobial activity than CuO NPs, Cu_2O NPs, CuNPs and ZnO NPs. Other than having strong antimicrobial properties, low toxicity to human cells at low concentrations, high thermal stability and low volatility of AgNPs also contribute to the employment of AgNPs in various applications (Durán *et al.*, 2007).

The broad spectrum antimicrobial properties of AgNPs lead to *Aspergillus niger* (fungus), *Staphylococus* sp. and *Bacillus* sp. (Gram-positive bacteria) being susceptible to AgNPs (Jaidev and Narasimha, 2010). However, the frequent use of AgNPs may increase microbial resistance (Gong *et al.*, 2007). Although AgNPs give a vast range of benefits, a certain portion of the AgNPs will inevitably enter the environment and lead to pollution (Hiriart-Baer *et al.*, 2006; Wiesner *et al.*, 2006; Brar *et al.*, 2010). This may affect the microbial genes that are essential for various metabolic processes including organic matter degradation, element transformation and nutrient recycling to support and continue all forms of life (Bradford *et al.*, 2009; El-Rafie *et al.*, 2014). Therefore, more studies are needed to understand the impact of AgNPs on microorganisms in natural or built environmental systems.

When the AgNPs are released into the environment, AgNPs will generally stay as individual particles in suspension and/or transform physiochemically in terms of aggregation and chemical speciation (Luoma, 2008; Yin *et al.*, 2015b). This will be strongly decided by the environment in which they are exposed to and this includes different chemical, physical and biological conditions (Khaksar *et al.*, 2015). A study carried out by Liu and Hurt (2010) showed that AgNPs are not persistent in aquatic systems containing dissolved oxygen. This indicates that AgNPs can dissolve into Ag ions (Ag^+) and they will lose the properties of the primary particle. Liu and Hurt (2010) also demonstrated that Ag^+ are released faster with temperature in the range 0-37°C and slower with increasing pH or the addition of humic or fulvic acids. Ag^+ release is slower is due to the aggregation that induced by high pH or addition of humic or fulvic acids. Aggregation of NPs can also be induced by the bacterially-produced proteins (Aruguete and Hochella, 2010). With the occurrences of the transformation process, it is suggested that AgNPs and Ag^+ can coexist in the environment (Akaighe *et al.*, 2011). Furthermore, Ag^+ can adsorb to AgNPs (Kennedy *et al.*, 2010; Liu and Hurt, 2010) and high particle concentrations provide more binding surfaces for Ag^+ (Kennedy *et al.*, 2010).

Other than the influence of the surrounding environment, the characteristics of AgNPs themselves, such as capping agent and particle size, as well as manufacturer's preparation also play a role in the transformation (Wiener *et al.*, 2006; Badawy *et al.*,

2010; Tolaymat *et al.*, 2010; Stebounova *et al.*, 2011; Yin *et al.*, 2015b). All these transformation processes of AgNPs have great impacts on toxicity in the ecosystem (Levard *et al.*, 2012). For example, aggregation of AgNPs reduced their toxicity towards bacteria (Kvítek *et al.*, 2008). In another study, long chain alkyl carboxylates-capped AgNPs lost their stability in solution after treatment at pH 2 for 30 mins. The antibacterial activity towards *E. coli* O157:H7 of this acid-treated long chain alkyl carboxylates-capped AgNPs decreased (Qu *et al.*, 2010). However, the oxidative dissolution of AgNPs to Ag^{+} increased their toxicity to *E. coli* (Xiu *et al.*, 2012).

From the above mentioned reports, there are indication that different types of AgNPs either as NPs alone or as complexes can found be in different environments leading to different fate and toxicity level. Hence, one of the principal aims of this study was to investigate the fate of the prepared AgNPs in the environment and also to assess the possible adverse effect of AgNPs on selected microorganisms.

1.2 Problem Statement

In recent years, nanotechnology has developed rapidly with demand due to the benefits brought from its production and application. However, the safety of the nanotechnology product is the question asked increasingly by many quarters, which may have impacts on not only the environment but also human health if higher level. Owing to their strong antimicrobial properties, AgNPs has become the most common commercial product among nanomaterials (Beer *et al.*, 2012; Marambio-Jones and Hoek, 2010). The amount of AgNPs released into the environment increases because of the production, use and disposal of AgNPs-containing products. But lack of knowledge regarding the fate of AgNPs released into the environment has prompted this work. Over time, the accumulation of AgNPs in the environment increases. This has become a worrying factor that could present serious negative consequences in the natural system which might cause possible destruction to the environment and to useful microorganisms. Hence, the fate and effect of AgNPs released into the environment is an environmental issue which requires urgent action.

Generally, AgNPs can be synthesized using various techniques resulting in different characteristics, including sizes and shapes, for use in several applications. The characteristics may influence their fate, transport and toxicity in the environment (Flory, 2012). Based on Badawy (2010), the great majority of AgNPs synthesis processes are not environmental friendly (76%). This is due to the non-environmental friendly synthesis methods are expected to provide more control over the reaction process and produce AgNPs with novel characteristics. However, the drawback of green synthesis is that it often produces large particle sizes (Badawy, 2010). Tolaymat *et al.* (2010) reported that most of the fabricated AgNPs that are spherical in shape with an average diameter of less than 20 nm and are considered the most reactive. They also inferred that these AgNPs (less than 20 nm) are seldom fabricated using green synthesis methods. Among the synthesis methods of AgNPs, chemical reduction by organic and inorganic reducing agent (such as sodium citrate, sodium borohydride) is the most common approach. The method for chemical synthesis of AgNPs is still being developed and the synthesized AgNPs may experience instability problems like aggregation of NPs and poor size distribution (Iravani *et al.*, 2014). These problems could be minimized by the use of capping agent (Ju-Nam and Lead, 2008; Badawy, 2010; Mahltig *et al.*, 2013). Polyvinylpyrrolidone (PVP) is a capping agent that commonly used in the formation of AgNPs (Shin *et al.*, 2004a). AgNPs stabilized by PVP is also widely found in consumer products (Arnaout, 2012). This study is focused on the fate and effect of AgNPs that are released into the environment. Thus, the AgNPs used in this study should have similar properties as that manufactured and incorporated into AgNP products. Then, synthesis of PVP-capped AgNPs with an average diameter of less than 20 nm is targeted to ensure their performance and mode of action are similar to manufactured ones.

Most research has been done with laboratory bacterial strains or clinical pathogens (Chudasama *et al.*, 2010; Lara *et al.*, 2010; Le *et al.*, 2012; Kora and Rastogi, 2013), and there is a lack of research involving bacterial species found in real environment (Gao, 2011). Two environmental isolates, *Citrobacter* sp. A1 (designated A1) and *Enterococcus* sp. C1 (designated C1) from an oxidation pond at Universiti Teknologi Malaysia (Johor, Malaysia) were selected to be used in this study. *Citrobacter* sp. A1 is a Gram-negative coccobacillus from the *Enterobacteriaceae*

family. It has a good potential in heavy metal reduction, nitrate reduction, sulfate assimilation, quorum sensing, and biofilm formation, making it important in the biodegradation of various xenobiotics and bioremediation of heavy metals (Chan *et al.*, 2012a). *Enterococcus* sp. C1 is a Gram-positive facultative anaerobic diplococcus, classified as lactic acid bacteria. The presence of regulatory systems and phage elements in this strain improved their ability and potential in bioremediation (Chan *et al.*, 2012b). These bacterial strains have been extensively researched on the biodegradation of several azo dyes, of which Amaranth was most studied (Chan *et al.*, 2012c). However, the tolerance of A1 and C1 against AgNPs is still unknown. Different bacterial strains possess different capabilities of defense against AgNPs. Hence, evaluating the response of A1 and C1 in the presence of AgNPs appears to be another set goal.

The release of AgNPs are not only from the AgNPs-containing products, but it could also happen to conventional Ag-containing products (Geranio *et al.*, 2009). Previous studies also found that various Ag forms (coarse Ag, AgNPs, Ag^+ and Ag complexes) exist in the released water from the AgNPs- or Ag related-containing products (Lorenz *et al.*, 2012; Mitrano *et al.*, 2014; Mitrano *et al.*, 2015). This phenomenon can be explained by the transformation process. Nowadays, several electronic companies produced washers which release Ag as antibacteria during washing. The incorporation of Ag into the washer was firstly initiated by Samsung followed by Sharp, LG and several other electronic companies. It is believed that AgNPs and other Ag species will be release from the discharge of these washers. The release of these Ag species will end up on the clothings or in the wash water which will be disposed into the environment (Farkas *et al.*, 2011). The release of these Ag species into the environment is of special concern, since silver is a strong antibacteria and toxic to aquatic organisms. Few data are currently available on the levels of Ag species released from washer with incorporated Ag function and their effect on bacteria (Jung *et al.*, 2008; Farkas *et al.*, 2011). Therefore, an attempt to characterize the discharge of varying Ag species released from a commercial washer with incorporated Ag function and subsequently investigating their effect on environmental isolates, A1 and C1.

The aquatic environment is where the released Ag species from Ag/AgNPs-containing products will likely to end up. The accumulation of AgNPs in aquatic environment is a major concern in this study. AgNPs are not persistent and transformed when expose to the different environment (Luoma, 2008; Liu and Hurt, 2010; Yin *et al.*, 2015b). The transformation affect their fate, bioavailability and toxicity in the ecosystem. In addition, different manufacturer synthesis method or type of AgNPs causes them to behave differently (Badawy *et al.*, 2012; Lin *et al.*, 2012). Therefore, the need to set up a simulated aquatic environment for PVP-capped AgNPs exposure is an imperative task in order to provide better understanding of their fate and effect on bacteria. Previous study reported that AgNPs and Ag^+ can coexist in the environment and AgNPs may be transformed to Ag^+ or vice versa (Akaighe *et al.*, 2011). Hence, the fate of Ag^+ and their effect on bacteria in simulated environment are important aspects of this research.

1.3 Objectives of Study

The objectives of the study were as listed below:

- To synthesize highly stable and non-aggregated form of AgNPs.
- To investigate the effect of synthesized PVP-capped AgNPs on *Citrobacter* sp. A1 and *Enterococcus* sp. C1.
- To explore the effect of Ag released from a washer with incorporated silver function on *Citrobacter* sp. A1 and *Enterococcus* sp. C1.
- To elucidate the fate and effect of PVP-capped AgNPs and Ag^+ in the simulated environment.

1.4 Scope of Study

In this study, there are four scopes to be completed. The first scope involved stable and well dispersed of PVP-capped AgNPs preparation by ultrasound-assisted chemical reduction and characterization using UV-Visible (UV-Vis) spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), zeta potential analyzer, transmission electron microscope (TEM) and energy dispersive X-ray (EDX) spectroscopy. The effect of pH and different biological media on the prepared PVP-capped AgNPs were also investigated.

The second scope of the study involved the determination of the effect of PVP-capped AgNPs against A1 and C1. The antibacterial activities at varying concentrations of PVP-capped AgNPs against A1 and C1 were studied over a time frame. The antibacterial mechanism of PVP-capped AgNPs was investigated through viability study, scanning electron microscopy (SEM), TEM and agarose gel electrophoresis. Bacterial defense mechanism against PVP-capped AgNPs was also speculated by draft genome sequence analyze. For comparison purpose, the antibacterial activities of commercial AgNPs and Ag^+ against A1 and C1 was studied.

Subsequently, a commercial washer by Sharp with Ag^+ releasing function was purchased. The manufacturer claimed that the washer releases Ag^+ during final rinse. Thus, the final rinse water under varying wash procedures was collected. For the third scope, speciation of Ag (total Ag, AgNPs and Ag^+) in the final rinse water were determined by Cloud Point Extraction-Based Separation (CPE) and ultrafiltration followed by microwave-assisted acid digestion and quantification using inductively coupled plasma mass spectrometer (ICP-MS). AgNPs in Ag-containing final rinse water were also characterized using TEM-EDX. Then, the effect of Ag-containing final rinse on A1 and C1 was studied through viability study, SEM, TEM and agarose gel electrophoresis.

The fourth scope of work encompassed the elucidation of the fate of PVP-capped AgNPs and Ag^+ followed by the investigation of their effect in the simulated environment. Simulated environment containing stream water and sediment (with or

without bacterial consortium A1 and C1) were set up. PVP-capped AgNPs or Ag^+ was added progressively over a period of 50 days. Then, speciation of different Ag species as well as the viability of A1 and C1 in water and sediment portion were evaluated.

1.5 Significance of Study

AgNPs has been significantly noted for its antibacterial potential. However, the behavior of AgNPs will vary due to the different in AgNPs preparation (Badawy *et al.*, 2012; Lin *et al.*, 2012). This will subsequently affect their antibacterial properties (Qu *et al*., 2010). This is the first report on the antibacterial activity of PVP-capped AgNPs synthesized via ultrasound-assisted chemical reduction used against *Citrobacter* and *Enterococcus* spp. In a previous study, PVP-capped AgNPs synthesized by this method was determined based on the electrical and rheological properties (Goharshadi and Azizi-Toupkanloo, 2013). Hence, further study of antibacterial properties was investigated in this research. These two bacterial strains A1 and C1 have shown to posess high tolerance towards toxic and recalcitrant compounds like azo dyes and heavy metals. Nevertheless, the tolerance level towards AgNPs has yet to be determined.

Most researchers are only focusing on the benefits brought about by AgNPs and have not considered their effects on the environment and to human health. Since there is an increase in the use of AgNPs containing products in the world, the importance of studying the fate and effect of AgNPs after being discharged into the aquatic environment is necessary. It was mentioned earlier that there is a lack of knowledge regarding this issue, especially in Malaysia. Yet, it is noteworthy and significant to study the effect of silver released from a commercial washer with incorporated silver function (as one of the anthropogenic activities contributing to the accumulation of silver in natural water/wastewater system) on A1 and C1. Similarly, clarification on the transport and transformation process of Ag and their effect on bacteria in simulated environment is also important. The findings obtained should provide further insight on the fate of the released Ag into the ecosystem and the possible outcomes upon exposure of the bacteria to Ag in the environment. This study

could also serve to increase awareness of public, industries and the government on the use of AgNPs- or Ag-related products because of the direct/indirect influence on ecosystem.

1.6 Thesis Outline

This thesis begins with Chapter 1 describing the background of study, problem statement, objectives, scopes and significance of this study. Chapter 2 reviewed the literatures related to the application of AgNPs, AgNPs synthesis, AgNPs on their release, fate and toxic effect on the environment and bacteria. Chapter 3 briefly outlined the research design and methodology. Meanwhile, chapter 4, 5, 6 and 7 concerned with the data processing and discussion according to the four objectives respectively. The conclusions and recommendation for future studies are as stated in Chapter 8.

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