



UNIVERSITI PUTRA MALAYSIA

***DEVELOPMENT OF DRINKING WATER PURIFIER USING BANANA
WASTE FOR POINT-OF-USE IN RURAL HOUSEHOLD***

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**DEVELOPMENT OF DRINKING WATER PURIFIER USING BANANA
WASTE FOR POINT-OF-USE IN RURAL HOUSEHOLD**

BY

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ABSTRACT

DEVELOPMENT OF DRINKING WATER PURIFIER USING BANANA WASTE FOR POINT-OF-USE IN RURAL HOUSEHOLD

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Introduction: Activated carbon is widely used for water purification due to high specific surface area that provide them excellent adsorbent, high adsorption capacity and high reactivity. Incorporation of silver nanoparticles into activated carbon as an antibacterial water filter treatment is proved to have broad spectrum of bactericidal properties. **Objectives:** The aims of this study was to determine the best ratio of ZnCl₂, SDS and time shaking for silver concentration in filtered water and *Escherichia coli* removal by using activated carbon coated silver nanoparticles. **Methodology:** This study involved four stages starting from material preparation, activated carbon by using chemical activation with ZnCl₂, preparation of silver nanoparticles coating by using chemical reduction method that involved used of suitable reducing agent (NaBH₄) and stabilizer, Sodium Dodecyl Sulphate, and lastly, treatment of artificial water using activated carbon by using conventional batch study. Different shaking time was also investigated during the treatment of the artificial water. **Results and Discussion:** The results shows 100 % of *E.coli* removal using activated carbon. The first 10 minutes of shaking time during treatment of artificial water are sufficient to reduce *E.coli* count from 10⁴ cfu/mL to 0 cfu/mL. SDS ratio starting from 0.5 and ZnCl₂ starting from 0.025 shows the 100% removal of *E.coli*. The highest value of silver concentration in filtered water using activated carbon 0.242 mg/L which at, 0.05 ZnCl₂ ratio, 10 SDS ratio and 10 mins of shaking time. **Conclusion** The minimum removal percentage of *Escherichia coli* was 99.94% and the maximum removal percentage of *Escherichia coli* was 100% by using activated carbon for all ZnCl₂ ratio (0.025,0.05,0.1) with six different SDS ratio (0.5,2,5,10,15,20) and for all different shaking time (10 mins, 20 mins, and 30 mins). The mean for silver concentration was 0.033. The highest value of silver concentration in filtered water using activated carbon was 0.242 mg/L which at, 0.05 ZnCl₂ ratio, 10 SDS ratio and 10 mins of shaking time. Best ratio cannot be determined since that there is no significant difference between ZnCl₂ ratio, SDS ratio, and time shaking for removal percentage of *Escherichia coli* and silver concentrations in filtered water by using activated carbon.

Keywords: Activated carbon, silver nanoparticles, *Escherichia coli* (*E.coli*)

ABSTRAK

PENCIPTAAN PENULENAN AIR MINUMAN MENGGUNAKAN SISA BUANGAN POKOK PISANG UNTUK KEGUNAAN PERUMAHAN LUAR BANDAR

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Pengenalan: Karbon aktif adalah bahan yang digunakan secara meluas untuk pembersihan air kerana mempunyai permukaan yang luas, menjadi penjerap yang sangat baik, dan kapasiti penjerapan yang tinggi. Lapisan nanopartikel perak pada karbon aktif sebagai rawatan air daripada antibakteria telah dibuktikan mempunyai spektrum luas ciri-ciri bakteria. **Objektif:** Tujuan kajian ini adalah untuk menentukan nisbah terbaik $ZnCl_2$ SDS dan masa rawatan terhadap kepekatan perak di dalam air tapisan dan penyingkiran *Escherichia coli* menggunakan karbon aktif bersalut nanopartikel perak. **Metodologi:** Kajian ini melibatkan empat peringkat bermula dari penyediaan bahan, karbon diaktifkan dengan menggunakan pengaktifan kimia dengan $ZnCl_2$, penyediaan lapisan nanopartikel perak dengan menggunakan kaedah pengurangan kimia yang melibatkan penggunaan ejen pengurangan yang sesuai ($NaBH_4$) dan penstabil (SDS), dan akhir sekali, rawatan air tiruan menggunakan karbon yang diaktifkan dengan menggunakan kajian kumpulan konvensional. Masa rawatan yang berbeza juga disiasat semasa kajian dilakukan. **Keputusan dan Perbincangan:** Keputusan dalam kajian ini menunjukkan penyingkiran *E.coli* sebanyak 100% dengan menggunakan karbon yang diaktifkan. Pada 10 minit pertama semasa rawatan menunjukkan masa yang mencukupi untuk mengurangkan jumlah koloni *E.coli* dari 10^4 cfu / mL kepada 0 cfu / mL. Nisbah SDS bermula dari 0.5 dan $ZnCl_2$ bermula dari 0.025 menunjukkan penyingkiran 100% daripada *E.coli*. Nilai kepekatan perak dalam air tapisan paling tinggi adalah 0.242 mg/L pada keadaan 0.05 $ZnCl_2$ ratio, 10 SDS ratio dan 10 minit masa rawatan. **Kesimpulan:** Peratusan penyingkiran minimum *Escherichia coli* adalah 99.94% dan peratusan penyingkiran maksimum *Escherichia coli* adalah 100% dengan menggunakan karbon yang diaktifkan untuk semua nisbah $ZnCl_2$ (0.025,0.05,0.1) dengan enam nisbah SDS yang berbeza (0.5,2,5,10, 15,20) dan masa rawatan berbeza (10 minit, 20 minit dan 30 minit). Nisbah terbaik $ZnCl_2$, SDS dan masa rawatan tidak dapat ditentukan kerana tiada signifikansi antara nisbah $ZnCl_2$, SDS dan masa rawatan terhadap kepekatan perak di dalam air tapisan dan penyingkiran *Escherichia coli* menggunakan karbon aktif bersalut nanopartikel perak.

Kata kunci: Karbon teraktif, nanopartikel perak, *Escherichia coli* (*E.coli*)

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LIST OF ABBREVIATIONS

WHO	World Health Organization
SDS	Sodium Dodecyl Sulphate
DO	Dissolved Oxygen
ZnCl₂	Zinc Chloride



CHAPTER 1

INTRODUCTION

1.1 Background

According to World Health Organization (WHO) (2016) , there are two million diarrhoeal death related to unsafe water, sanitation and hygiene every year and more than one billion people lack access to safe water sources. More than eight out of ten people living in rural area not getting access to safe drinking water (WHO, 2010). Waterborne diseases such as diarrhoea caused by pathogenic microbes that can be directly spread through contaminated water. Eighty-eight percent of diarrhoea cases worldwide result in 1.5 million deaths each year, mostly in young children and occur in developing countries due to lack in improve water and sanitation system (CDC, 2012). In Malaysia, referring from National Water Service Commission (2016) population served for safe water at 2014 for rural area of Sabah and Sarawak are 73.1% and 76.6% respectively, while at 2015 there was improvement such that population served was 76.0% for rural area of Sabah and 78.0% for Sarawak. On the contrary, there was more than 90% population served at others states of Malaysia and urban area mostly got the most population served. Uncontrolled development, industry and agricultural enlargement, ineffective water management can be major reasons for inadequate water supply and low quality of water. (Saimiy et al. 2012). Water that was supplied for drinking was treated and more than 90% water supply is from river but, however, water

supply coverage in rural area of Kelantan, Sabah and Sarawak is lower than Malaysian average of 92.5%. This is due to their location that far away from city with low population density, difficult terrain and poor infrastructure access, which this requires high cost in providing water supply (Eleventh Malaysian Plan, 2016).

Department of Statistics Malaysia (2016) stated that, Malaysia's economy rose from 4.7 per cent in 2013 to 6.0 per cent in 2014 and agricultural sector is one of the contributor. Malaysian had been recorded to produce million tonnes of agricultural waste such as from banana, coconut, pineapple, sugarcane, rice, and the highest oil palm fruit. These agricultural left material can be utilized for some application such as Malaysia and Indonesia had used oil palm fiber for building component (Dungani et al. 2016) . Food and Agriculture Organization of the United Nation (2014) stated that agricultural waste produces such as stem, leave and others parts contain sources of cellulose such as banana stem, rice straw and pineapple leaf . In Malaysia, production of banana has increased considerably for the five years from 2005 to 2009 that range from 250000 to 280000 million tonnes per year. In 2009, total production of banana production value estimated to be RM452.4 billion and banana is mainly been exported to Singapore and in small amount to Indonesia, Brunei, Saudi Arabia and Hong Kong. Meanwhile, import of banana mainly from Philippines and Thailand (Hussaain and William, 2011). Figure 1 shows the increment production of banana in Malaysia from 2005 to 2009.

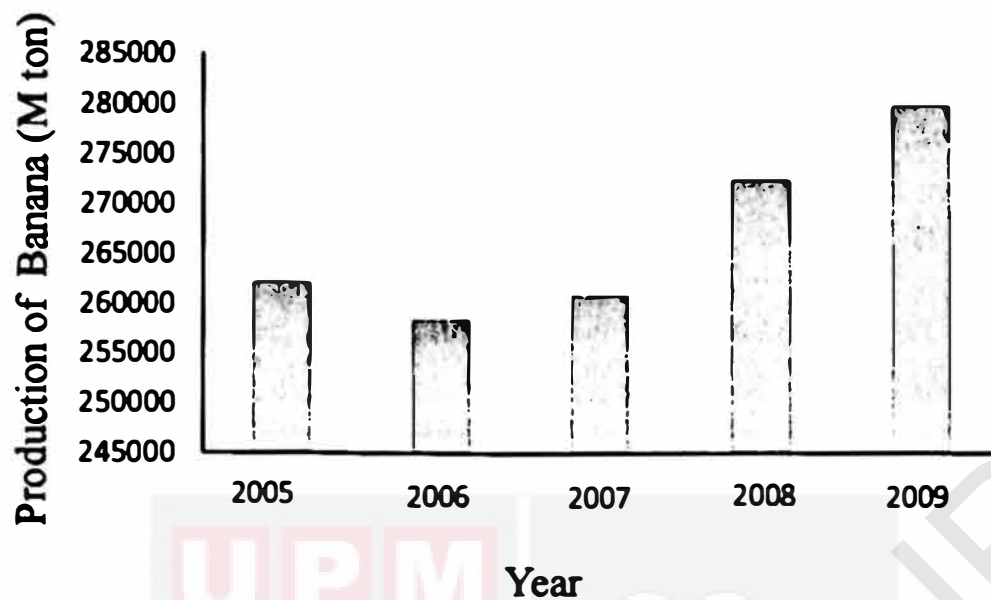


Figure 1.1: Production of Banana in Malaysia from year 2005 to 2009

Source: Department of Agriculture (2011)

Activated carbon is a black solid substance resembling granular or powdered charcoal with a highly developed porous structure that has been proven to be an effective adsorbent for the removal of a wide variety of organic and inorganic pollutants from aqueous or gaseous media (Halim et al., 2000). It is widely used for environmental applications such as gas separation, solvent recovery, and water purification due to its exceptionally high surface area, well-developed internal microporosity, and wide spectrum of surface functional groups. The adsorptive properties of carbon, which enrich nutrient and oxygen concentrations to remove disinfectant compounds. In addition, the presence of a variety of functional groups on the carbon surface, which enhances microbial attachment. Activated carbon is generally produced from coals and lignocellulosic materials and recently the activated carbons was prepared from wastes such as agricultural (Rivera et al., 2001).

Various technologies are being used in drinking water purification, such as nanotechnology. Nanotechnology has been increasingly used in recent years, especially been applied in the water purification in industry, to remove bacteria from drinking water. Water treatment by using nanotechnology is still new and been investigated in several laboratories worldwide, and related to this nanotechnology is using nanoparticle coated with various low-cost material (Sobsey et al.2008). Nanotechnology is discovered to have magnetic interaction, which can remove heavy metals like arsenic in drinking water, and recently nanostructured filter been tested to remove microbial from water. Nanotechnology has a potential to become one of the most important technology in the future, that are capable to detect, identify and filter out chemical and biological agent in the environment with their much higher sensitivity compared to what we have today (Saimy et al. 2013). Recently, nanoparticle that derived from nanotechnology has been widely used and evaluated for water treatment application to remove microorganism by using different material coated (Heidarpour et al. 2011).

1.2 Research Problem

Getting access to safe drinking water is one of the most important issues. Home purification system is one of the types of water technologies available in Malaysia. Department of Statistics (2001) reported that 98% of the urban Malaysian population and 92% of the rural community have access to improved water system. However, in some rural and remote area in Malaysia are still using wells as source of water. Using wells water without any further filtration system have been proved to be more microbial contaminated and they are not regulated by any drinking water standard (Aini et al.,

2007). On the other hand, remote and rural area at villages in Sabah have lower coverage to improved water supply and study shows that for water quality, there are presence of *E.coli* in household water (Zin et al., 2015).

The other problem that Malaysia faced is abundance of agricultural waste has been produce where, approximately 250 to 280 billion tonnes of agricultural waste has been produced yearly (Department of Agriculture, 2009). Banana plantation as the second most widely cultivated fruits in Malaysia generated about 4 tonnes of wastes for every cycle. It only produces fruits once in a lifetime and when the fruit is harvested, normally the banana tree will be cut and leaving the other parts dumped. Consequently, Malaysia face the problem of disposal of portions of banana wastes and other agricultural wastes. The rotten fruits, peel, fruits-bunch-stem, leaves, pseudo-stem and rhizome are among banana wastes generated. The accumulations of agricultural waste need to be manage properly through proper ways of utilization. (Nurhayati et al., 2014).

1.3 Study Justification

Drinking water for hundreds of millions of people is dangerously contaminated or chemically polluted due to inadequate management of urban, industrial and agricultural wastewater (Chan, 2009). According to Centers for Disease Control and Prevention (2014), contamination still occurs after drinking water treatment. With great advance of technology nowadays, there will be more intervention for new water purifier instrument that are cheaper, and affective to remove microorganism (Dungani et al.

2016). Point-of-use water treatment technology has appeared to provide people with safe water quality by home-self water treatment (Malik et al. 2013) .

Output of this study will be used to develop cost effective potable drinking water purifier for point-of-use in rural household by using agricultural waste for water filter material. By using agriculture waste, this study could develop sustainable agriculture practice that will protect environment, public health, and human communities. Utilization of agricultural waste as renewable sources could prevent from environmental pollution and contributed to economy development. Moreover, very limited study has been carried out using banana waste product where, it has been proved to produce the highest percentage of char that act as high adsorption capacity compared to others material (Nurhayati et al., 2014).

1.3 Conceptual Framework

Figure 2 shows the conceptual framework involving existing technologies for drinking water purification that was used as guideline for this study. Water is used for agricultural, industrial and drinking water. In this study, biological contaminant (*Escherichia coli*) will be used as indicator for development of drinking water purifier for point of use water treatment. Various method used for water purification such as reverse osmosis, ultraviolet treatment, distillation and nanotechnology. For this study,

nanotechnology will be studied due to its advantages to have broad spectrum of antibacterial properties.

Water purification was developed for point-of-use water treatment, since recent studies showed that point-of-use treatment of drinking water can help in reducing of waterborne disease. Besides using of ceramic, polymeric membrane, polyurethane for water filter material, this study focusing on agricultural waste that is from banana stem. Effectiveness of different filter material in removal of biological contaminant was to develop cost effective potable drinking water purification for point-of-use in rural household

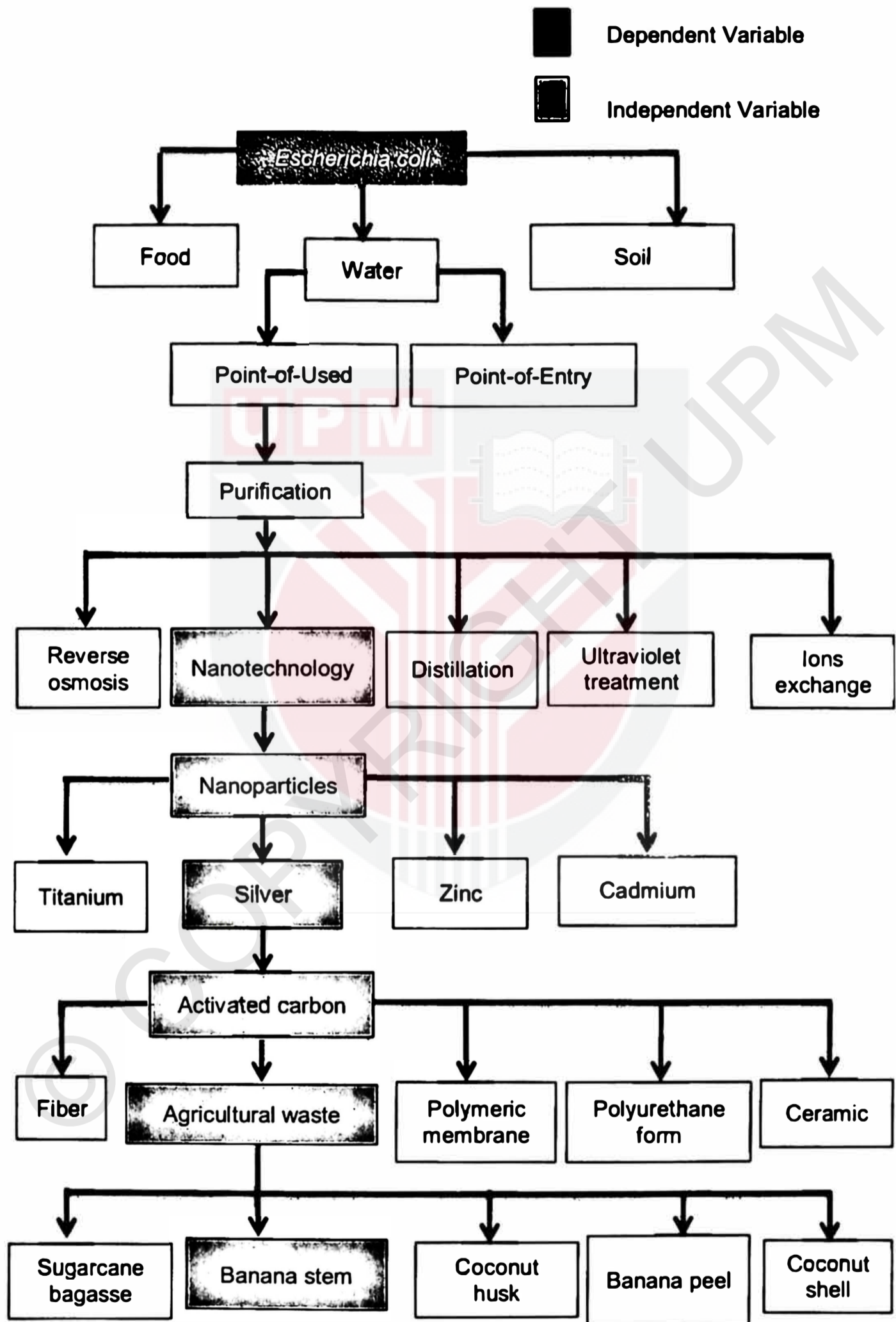


Figure 1.2: Conceptual framework involving technologies for drinking water purification

1.5 Research Objective

1.5.1 General objectives:

To develop drinking water purifier by using banana waste coated with silver nanoparticles for point-of-use in rural household.

1.5.2 Specific objectives:

1. To determine the removal percentage of *Escherichia coli* using activated carbon coated with silver nanoparticle.
2. To determine the silver concentration in filtered water by activated carbon coated with silver nanoparticles.
3. To determine the best ratio of Zinc Chloride ($ZnCl_2$), SDS, and time shaking for silver concentration in filtered water and removal percentage of *Escherichia coli* by using activated carbon coated with silver nanoparticles.

1.6 Hypothesis

There is significant different between Zinc Chloride ratio, SDS ratio, and time shaking for silver concentrations in filtered water and removal percentage of *Escherichia coli* by using activated carbon coated with silver nanoparticles.

1.7 Definition of Variable

1.7.1 Conceptual Definition

1.7.1.2 *Escherichia coli*

Escherichia coli (*E. coli*) are a large group of bacteria found in the environment, foods, and intestines of people and animals. *E. coli* are harmless, and can cause diarrhoea, urinary tract infections, respiratory illness and pneumonia, and other illnesses (CDC, 2016).

1.7.1.3 Activated Carbon

A solid, porous, carbonaceous material prepared by carbonizing and activating organic substances (WHO, 1990).

1.7.2 Operational Definition

1.7.2.2 *Escherichia coli*

Escherichia coli (*E. coli*) is a species of bacteria that can be found in water that indicate human faecal contamination (WHO, 2017).

1.7.2.3 Activated Carbon

Activated carbon is an effective adsorbent for the removal of a wide variety of organic and inorganic pollutants from aqueous or gaseous media (El-Aassar et al., 2013).



CHAPTER 2

LITERATURE REVIEW

2.1 Banana Production

Bananas (Musaceae) are largely produced in tropical and subtropical areas. In Malaysia, the total planted area of banana was 33,495 hectares. Banana plants range in height from 10 feet to 20 feet after one year, and their fruits ripe enough only for 4 to 5 months duration (Aliman, 2010) . By-product from banana plants are great sources of highly valuable raw material for industries used, such as their leaves, stems and others can be used as flavor in food. Banana stem or pseudostem contains 63.9% of cellulose where they are commonly been wasted and left in soil plantation then be used as organic material or mixed with rejected fruit to feed animals. (Abdul Khalil et al. 2006). Recently in Malaysia, banana waste has become one of the renewable energy sources due to their high growth rates (Tock et al. 2009). Figure 2 show description on banana plantation.



(Retrieved from <http://www.indiamart.com/harbhole-greenhouse/green-plants.html>)

2.2 Incorporation of Silver Nanoparticle into Water Filter Materials

Various low-cost materials have been used to incorporate with silver nanoparticle that will serve as an antibacterial water filter treatment. These are relatively new solution to access for safe drinking water. Previous review on others low-cost material such as ceramic, polymer, polyurethane, agricultural waste and fiber have been discussed its effectiveness as an antibacterial water filter to remove *Escherichia coli*. *Escherichia coli* removal efficient and silver concentrations in filtered water have been compared among different low-cost materials and refer to WHO and the United State Environmental Protection Agency (USEPA) drinking-water standard. However, use of ceramic for water filter can reduce filter lifetime due to improper handling. While for

agricultural waste , rice husk ash are capable for removing *E.coli*, but, they require higher temperature which more than 500 and 800 C to turn into ash and the higher the temperature used the more better adsorption rate (Malik et al 2013).

2.3 Activated Carbon



Figure 2.2 Activated carbon

Activated carbon have a high specific surface area that provide them excellent adsorbent, high adsorption capacity and high reactivity. Activated carbon can be produced from various agricultural waste such as from banana stem, rice husk ash, coconut ash and etc. Activated carbon made up from banana stem, it consists of 63.9% of cellulose in banana stem, and abundantly found from left over from agricultural. Cellulose is a cost-effective and allows rapid absorption of silver nanoparticles during coating process. By using activated carbon from banana stem, it is easy to be prepared and use lower temperature compared to rice husk ash as activated carbon that involve combustion with high temperature for preparation (Aliman, 2010).

2.4 Silver Nanoparticles

Silver nanoparticles is more preferable since extensive research have been proved that silver has broad spectrum of bactericidal properties, high surface area-to-volume and it is safe to animal cell but highly toxic to bacteria cell (Praveena et al. 2016). Mechanism of silver nanoparticle in microbial removal remain unclear, but, some study demonstrate that silver nanoparticle inhibit microbial growth by attach to cell wall and severely damage the cell's major function. Praveena and Aris (2015) review different low-cost material coated with silver nanoparticles for point-of-used water treatment system, and found that between 92% and 100% of *E.coli* removal during emergency application. In formation of silver nanoparticles, various others method can be used such as polyol method, radiolytic process and the most widely studied are chemical reduction method. This is due to its advantages of yielding nanoparticles without aggregation, high yield and low preparation cost. Reducing agent in the presence of a suitable stabilizer is necessary in chemical reduction method to protect the growth of silver particle through aggregation (Praveena et al. 2016).

2.5 Source of Activated Carbon

Table 1 show that there are other sources from agricultural waste to produce activated carbon and with different purpose and potential in drinking water treatment. Usage of rice husk impregnation with silver nanoparticles has shown to effectively

remove 99.9% of *Escherichia coli* in water treatment. Rice husk ash contains silica that can be used for entrapped silver nanoparticle that useful for antibacterial water filter. However, further studied concerning the removal rate of silver involving the impregnation of silver nanoparticles with rice husk need to be carried out (Praveena and Aris, 2015). Khadijah et al. (2012) studied that sugarcane bagasse activated carbon can be used in removal heavy metal, physical removal contaminant (turbidity, color, etc.) and removal of total coliform of total coliform (*Escherichia coli*) in water treatment. Sugarcane bagasse is an inexpensive material, tested to produce activated carbon through chemical activation and have been proved to remove total coliform in drinking water. Meanwhile, banana peel activated carbon has been studied to be effective in removal of toxic heavy metal, ions, from aqueous solution and industrial waste water (Li et al., 2013). However, limited study regarding banana peel activated carbon in removal of microbial contaminant. Agriculture waste from coconut, which is coconut shell has shown the reduction of physical contaminant (BOD, COD, and turbidity) in water treatment and also more than ninety-nine percent for *Escherichia coli* removal efficiency. Coconut-based agricultural waste has been proved as excellent biosorbents for microbial and inorganic pollutant removal from drinking water (Ratnoji and Singh, 2014).

Table 2.1: Summary of different sources for activated carbon and its purpose as conducted in previous study.

Source for activated carbon	Purpose	<i>E. coli</i> removal capacity (%)	Reference
Rice husk ash	<ul style="list-style-type: none"> • Microbial and heavy metal removal from drinking water. 	99.99	Praveena and Aris (2015)
Banana peel	<ul style="list-style-type: none"> • To remove of toxic heavy metal, ions, from aqueous solution and industrial waste water. 	NA	Li et al. (2016)
Sugarcane bagasse	<ul style="list-style-type: none"> • Removal of heavy metal • Physical removal contaminant (turbidity,color) • Removal of total coliform. 	100	Khadijah et al. (2012)

Coconut shell

- Removal of organic compound >99
- Removal of microbial

Ratnoji and
Singh

(2014)

(Carmalin

Sophia,

Catherine,

&

Bhalambaal,

2013)



UPM



CHAPTER 3

METHODOLOGY

3.1 Study Design

This research is an experimental study design. Experimental study is a method in research that concerned the effect between dependent and independent variable through treatment or intervention (El-Aassar et al., 2013).

3.2 Study Methodology

Methodology of this study involved four stages starting from material preparation, activated carbon preparation, preparation of silver nanoparticles coating, characterization of silver nanoparticles and treatment of artificial water using activated carbon that has been conducted in laboratory scale. Figure 3.3 shows the flowchart of data collection in this experimental study.

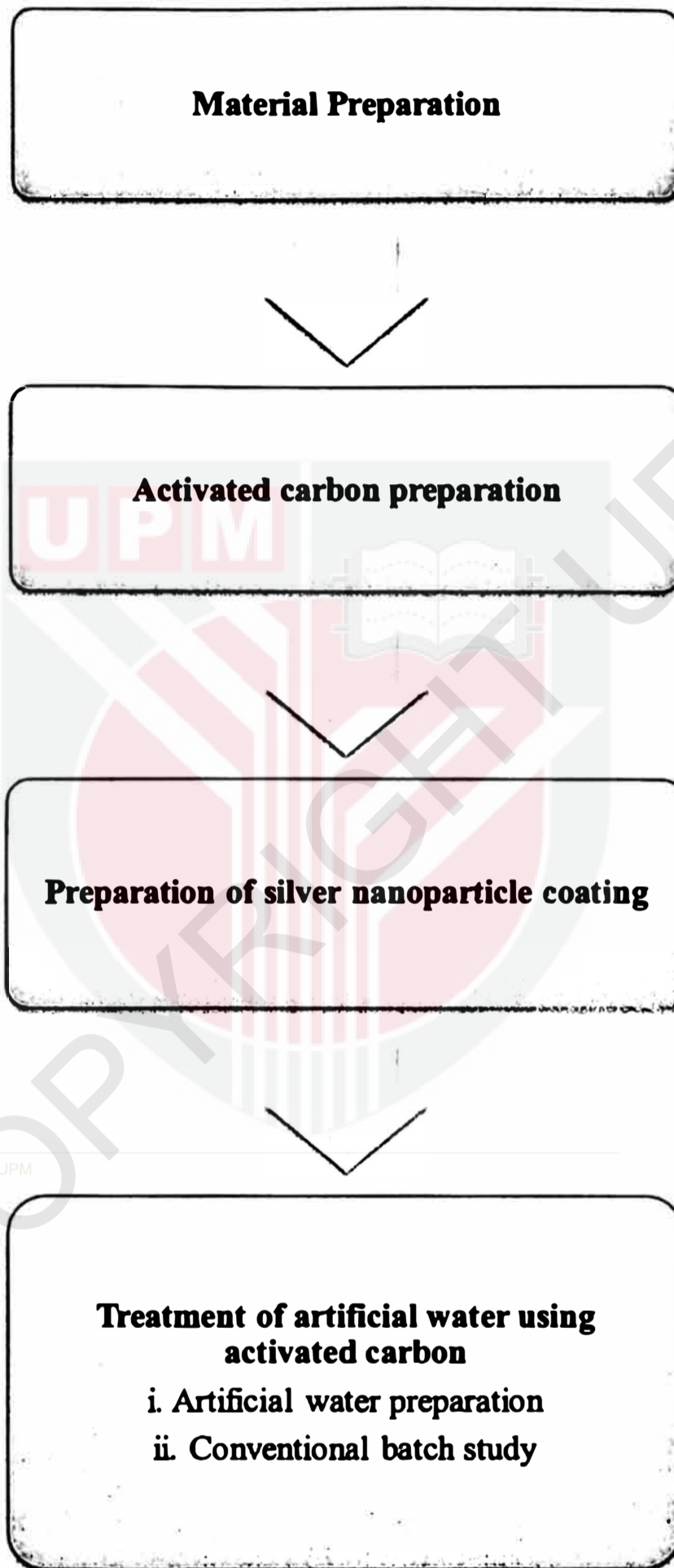


Figure 3.1 Data collection flowchart

3.2.1 Materials preparations

Banana stem were obtained and were cut into pieces (2 cm) and immersed in a mixture of 1% potassium metabisulfite for 24 hours to inhibit oxidation. Banana stem pieces were arranged into aluminum tray and dried in a drying oven at 90°C- 110°C for 24 hours and then milled using knife mill. The dried banana stem was washed with ethanol to remove lipid fractions and dried in a drying oven at 60 °C for 24 hours. The samples were sieved through a 200-mesh sieve to obtain homogenous and afforded microparticles. Material preparation was based on methods applied by Tibolla et al. (2014).

3.2.2 Activated carbon preparation

The preparation of activated carbon was done using chemical activation with zinc chloride ($ZnCl_2$). According to Ucar et al. (2009) and Salman & Hameed (2010) chemical activation method with Zinc Chloride could produce high surface area of activated carbon. Homogenized banana stem were weighted based on the ratio selected which were 0.025, 0.05, and 0.1 and then added to 40% $ZnCl_2$ (0.006 M) solution that was prepared by dissolving 400g $ZnCl_2$ into 500 ml ultrapure water. Dried banana stem was mixed using shaker for 24 hours at 250rpm. Next, the mixture was dried at 90-110°C for 24 hours. Carbonization was done in a reactor at temperatures of 450°C for four hours under nitrogen gas flow.

3.2.3 Preparation of silver nanoparticles coating

Silver nanoparticles coating on the best activated carbon were done by using chemical reduction method which involving a reducing agent with a suitable stabilizer. Chemical reduction method is chosen because it can yield high silver nanoparticles without aggregation with low preparation cost. Silver nitrate (AgNO_3 , 99.5%) was used as precursor to prepare silver nanoparticles, while sodium borohydride (NaBH_4 , 98%) and sodium dodecyl sulphate (SDS, 98%) was used as a reducing agent and stabilizer. The stabilizer is necessary to protect the growth of silver nanoparticles through aggregation. Molar ratio of 0.5, 2, 5, 10, 15, and 20 (SDS: AgNO_3) were selected using 0.001M of AgNO_3 solution were prepared by dissolving the 0.1699g of AgNO_3 in 1000 mL of ultrapure water. 20 gram of activated carbon were immersed and stirred using magnetic stirrer at 220rpm in solution of 40 ml of AgNO_3 for 30 minutes. The solution then let to settle down before the upper layer of solution was removed using pipette. After that the solution was rinsed with ethanol to remove any unabsorbed silver nitrate, then was filtered through filtration method. Next, NaBH_4 and SDS were stirred together in 50 mL of ultrapure water using magnetic stirrer for 30 minutes. Silver nanoparticles was produced by pouring the activated carbon NaBH_4 and SDS solution slowly for 15 minutes to obtain stable silver nanoparticles. The activated carbons coated with silver nanoparticles were placed in oven at 80°C for 2.5 hours for drying (Song et al., 2009).

3.2.4 Treatment of artificial water on the selected activated carbon

3.2.4.1 Artificial water preparation

Artificial water was prepared in this experiment to represent household water contaminated by *Escherichia coli*. Bacteria were culture in LB agar plate for overnight before being sub-culture in a broth form. Bacteria in a broth form then was shaken and incubated for overnight. The next day, bacteria were then diluted with autoclave broth and measure to initial absorption of approximately 0.5 by UV Spectrophotometer at 600nm represented 10^7 cfu/ml (Molecular Biology Handbook, 2017). Next, saline water was prepared by dissolving 9g of sodium chloride (NaCl) together in 1L of ultrapure water and autoclave for 20 minutes at 121 °C. Artificial water was prepared by added 1ml of bacteria suspension and mix into 1L of saline water to represent 10^4 cfu/ml. Biological (*Escherichia coli*) were analysed according to procedures described by American Public Health Association (1998).

3.2.4.2 Conventional batch studies

A series of conventional batch studies were carried out by using 2g of the best activated carbon with highest surface area with 200 mL of artificial water. The activated carbon solution was then shaken using orbital shaker for different shaking time which were 10 minutes, 20 minutes and 30, minutes. Then, the solution was settled down for 6 hours. Next, 100 ml of treated water was pipette then filtered through 0.45µm Whatman filter paper and analysed for physical (pH, dissolved oxygen and turbidity), biological (*Escherichia coli*) and chemical characteristics (heavy metal) according to procedures

described by American Public Health Association (APHA). 0.45 μ m Whatman filter paper then was incubating by using Lauryl Sulphate broth for 14 hours at 44°C. Two factors (silver nanoparticle coated on activated carbon and concentration of zinc chloride) were analysed in terms adsorption process. Quantification of silver in filtered water were performed using Induced Coupled Plasma Mass Spectrometry (ICPMS). Conventional batch studies was done to determine the best activated carbon coated with silver nanoparticles.



3.2.5 Data analysis

The collected data was analysed using the statistical computer software (Statistical Package Service and Solution-SPSS). The type of analysis used for this study is based on the objectives of this study.

Objectives	Statistical Test
To determine the removal percentage of <i>Escherichia coli</i> using activated carbon coated silver nanoparticle.	Descriptive Analysis
To determine the silver in filtered water by activated carbon coated silver nanoparticle.	Descriptive Analysis
To determine the best ratios of Zinc Chloride, SDS and time shaking for silver concentration in filtered water and <i>Escherichia coli</i> removal by using activated carbon coated silver nanoparticle.	General Linear Model (Multivariate)

3.3 Quality Assurance and Quality Control

Apparatus used during this experiment were first rinsed with tap water and brushed to remove any impurities before soaked in acid wash for overnight. Then the apparatus were rinse again using ultrapure water and allowed to dry. Glassware and culture media were also autoclaved at temperature of 121°C for 20 minutes for (Laboratory Safety Guide, 2004). Culture media and *Escherichia coli* broth were place in the chiller at 4°C to prevent from contamination and prevent from growth of colony.

For quality control, the samples were stirred and shake at the same speed which was 220rpm to avoid these factor to affecting the result of analysis. The samples were also analysed in duplicate to increase the accuracy and precision (American Public Health Association, 1998).

CHAPTER 4

RESULTS AND DISCUSION

4.1 Descriptive statistic of filtered water using activated carbon

Table 4.1 shows result of in-situ parameters, *E.coli* removal and silver concentration of water after filtration by using activated carbon with different $ZnCl_2$ (ratio), SDS (ratio) and shaking time.

Table 4.1: Descriptive analysis of water after filtration using activated carbon

ZnCl₂ (ratio)	SDS (ratio)	Time (min)	Turbidity (NTU)	pH	Dissolved Oxygen (mg/L)	Silver Conc. (mg/L)	<i>E.coli</i> removal (mean)	
0.025	0.5	10	0.37	7.02	6.93	0.0548	99.99	
		20	0.66	5.57	7.93	0.0427		
		30	0.41	5.48	7.21	0.0395		
	2	10	0.54	6.94	6.95	0.0136		
		20	0.66	5.79	7.97	0.0521		
		30	0.65	5.47	8.15	0.0453		
	5	10	2.24	6.96	7.41	N/D		
		20	1.02	5.51	7.64	0.0260		
		30	2.52	6.98	8.13	0.0599		
	10	10	5.63	5.19	5.35	0.0589		
		20	0.85	5.63	7.42	0.0263		
		30	1.30	6.94	7.85	0.0210		
	15	10	2.79	5.26	7.98	0.0797		
		20	0.63	6.99	8.40	0.0559		
		30	0.97	7.35	8.23	0.0657		
	20	10	9.99	5.07	7.93	0.1437		
		20	4.31	5.18	8.34	0.1357		
		30	8.46	6.95	8.29	0.021		
	0.05	0.5	10	0.23	6.97	7.11	0.0155	
			20	0.53	5.92	7.43	N/D	
			30	0.48	5.59	7.29	N/D	
		2	10	0.54	6.94	6.95	0.0040	
			20	0.38	6.00	7.07	0.0237	
			30	0.52	5.21	7.91	0.0849	
5		10	1.16	6.95	7.97	0.0141		
		20	0.45	5.76	7.44	0.0101		
		30	2.74	6.95	8.17	0.0347		
10		10	4.77	5.22	7.00	0.2417		
		20	0.42	5.3	7.42	0.0251		
		30	1.36	7.00	7.90	0.0284		

ZnCl₂ (ratio)	SDS (ratio)	Time (min)	Turbidity (NTU)	pH	Dissolved Oxygen (mg/L)	Silver Conc. (mg/L)	<i>E.coli</i> removal (mean)
	15	10	2.01	0.28	7.43	0.1118	
		20	0.5	6.95	8.51	0.0615	99.99
		30	2.77	7.29	8.16	0.0439	
	20	10	9.99	5.13	7.91	0.0307	
		20	5.70	5.29	8.16	0.0294	
		30	5.99	6.97	6.2	0.0318	
0.1	0.5	10	0.32	7.02	6.75	N/D	
		20	0.40	6.12	7.12	0.0003	
		30	0.34	6.03	6.88	N/D	
	2	10	0.45	6.96	6.33	N/D	
		20	0.35	6.04	8.46	N/D	
		30	0.36	5.09	7.9	N/D	
	5	10	1.48	6.94	7.66	N/D	
		20	0.39	5.84	6.54	N/D	
		30	3.47	6.94	8.12	0.0454	
	10	10	7.17	5.30	7.70	0.0173	
		20	0.58	5.90	7.02	0.0003	
		30	0.40	7.02	8.09	N/D	
	15	10	3.45	5.23	7.49	0.0130	
		20	0.57	6.94	8.54	0.0155	
		30	5.00	7.28	8.09	0.0114	
	20	10	9.56	5.06	7.93	0.0029	
		20	6.25	5.39	7.94	N/D	
		30	2.64	6.97	6.42	N/D	

Based on the Table 4.1, by using 0.025. ZnCl_2 (ratio), the highest value of turbidity were recorded at SDS ratio 20 (9.99 NTU), SDS ratio 20 (4.31 NTU), and (8.46 NTU) after 10 minutes, 20 minutes and 30 minutes shaking time, respectively. Meanwhile, the lowest turbidity value were recorded at SDS ratio 0.5 (0.37 NTU), SDS ratio 0.5 and 2 (0.66 NTU), and (0.41 NTU) after 10 minutes, 20 minutes and 30 minutes shaking time, respectively. For pH value, the highest pH was sample of SDS ratio 0.5 (7.02), SDS ratio 20 (5.18) and SDS ratio 2 (5.47) for 10 minutes, 20 minutes and 30 minutes, respectively. Meanwhile, the lowest value of pH were SDS ratio 20 (5.07), SDS ratio 20 (5.18) and SDS ratio 2 (5.47) after 10 minutes, 20 minutes and 30 minutes shaking time, respectively. For dissolved oxygen, the highest value were at sample SDS ratio 15 (7.98) , SDS ratio 15 (6.99) and SDS ratio 20 (8.29) for 10 minutes, 20 minutes and 30 minutes, respectively. Meanwhile, the lowest dissolved oxygen value were recorded at SDS ratio 10 (5.35), SDS ratio 20 (5.18), SDS ratio 0.5 (7.21) after 10 minutes, 20 minutes and 30 minutes shaking time, respectively.

Next, by using 0.05 ZnCl_2 (ratio), the highest value of turbidity were recorded at SDS ratio 20 (9.99 NTU), SDS ratio 20 (5.70 NTU), and SDS ratio 20 (5.99 NTU) after 10 minutes, 20 minutes and 30 minutes shaking time, respectively. Meanwhile, the lowest turbidity value were recorded at SDS ratio 0.5 (0.23 NTU), SDS ratio 15 (0.50NTU), and SDS ratio 0.5 (0.48 NTU) after 10 minutes, 20 minutes and 30 minutes shaking time, respectively. For pH value, the highest pH was sample of SDS ratio 0.5 (6.97), SDS ratio 15 (6.95) and SDS ratio 15 (7.29) for 10 minutes, 20 minutes and 30 minutes, respectively. Meanwhile, the lowest value of pH were SDS ratio 15 (0.28), SDS ratio 20 (5.29) and SDS ratio 2 (5.21) after 10 minutes, 20 minutes and 30

minutes shaking time, respectively. For dissolved oxygen, the highest value were at sample SDS ratio 5 (7.97), SDS ratio 15 (8.51) and SDS ratio 5 (8.17) for 10 minutes, 20 minutes and 30 minutes, respectively. Meanwhile, the lowest dissolved oxygen value were recorded at SDS ratio 2 (6.95), SDS ratio 2 (7.07), SDS ratio 20 (6.20) after 10 minutes, 20 minutes and 30 minutes shaking time, respectively.

By using 0.1 ZnCl₂ (ratio), the highest value of turbidity were recorded at SDS ratio 20 (9.56 NTU), SDS ratio 20 (6.25 NTU), and SDS ratio 15 (5.00 NTU) after 10 minutes, 20 minutes and 30 minutes shaking time, respectively. Meanwhile, the lowest turbidity value were recorded at SDS ratio 0.5 (0.32 NTU), SDS ratio 0.5 (0.40 NTU), and SDS ratio 0.5 (0.34 NTU) after 10 minutes, 20 minutes and 30 minutes shaking time, respectively. For pH value, the highest pH was sample of SDS ratio 0.5 (7.02), SDS ratio 15 (6.94) and SDS ratio 15 (7.28) for 10 minutes, 20 minutes and 30 minutes, respectively. Meanwhile, the lowest value of pH were SDS ratio 20 (5.06), SDS ratio 20 (5.39) and SDS ratio 2 (5.09) after 10 minutes, 20 minutes and 30 minutes shaking time, respectively. For dissolved oxygen, the highest value were at sample SDS ratio 10 (7.70), SDS ratio 15 (8.54) and SDS ratio 5 (8.12) for 10 minutes, 20 minutes and 30 minutes, respectively. Meanwhile, the lowest dissolved oxygen value were recorded at SDS ratio 2 (6.33), SDS ratio 5 (6.54), SDS ratio 20 (6.42) after 10 minutes, 20 minutes and 30 minutes shaking time, respectively.

Result shows that the mean of silver in filtered water was 0.033, meanwhile, highest value 0.242 mg/L which at, 0.05 ZnCl₂ ratio, 10 SDS ratio and 10 minutes of

shaking time. The highest value was exceeding Malaysian Drinking Water Quality Standard and United State Environmental Protection Agency which are 0.05 mg/L and 0.1 mg/L, respectively. The silver in filtered water, was an indication for silver leached from activated carbon (Heidarpour et al., 2011). The retention of silver nanoparticles on the surface of activated carbon was dependable to the method of silver nanoparticles coating (Reidy et al., 2013). Studied by Song et al (2006), state that inadequate amount of SDS as stabilizer will result silver nanoparticle to aggregate thus increase the silver concentration in filtered water. However, some value for silver in filtered water was not detected by ICPMS because the reading was below than detection limit.

The *E.coli* removal after treatment using activated carbon shows mean of 99.99. This indicate that all ratio of Zinc Chloride, SDS and time shaking used was sufficient to remove *E.coli*. Ratio of $ZnCl_2$ was sufficient to produce high surface area of activated carbon while, adequate amount of SDS function to absorb on the surface of silver nanoparticles thus protect the silver nanoparticles from aggregation. The first 10 minutes of shaking time was sufficient and optimum time for adsorption of the *E.coli* by prepared activated carbon, where complete removal of *E.coli* has occurred.

4.2 Removal percentage of *Escherichia coli*

Table 4.2 shows the minimum removal percentage of *Escherichia coli* was 99.94% and the maximum removal percentage of *Escherichia coli* was 100% for all $ZnCl_2$ ratio (0.025,0.05,0.1) with six different SDS ratio (0.5,2,5,10,15,20) and for all different shaking time (10 mins, 20 mins, and 30 mins).



Table 4.2 Removal percentage of *Escherichia coli*

ZnCl ₂ (ratio)	SDS (ratio)	<i>E coli</i> removal (%)		
		10 mins	20 mins	30 mins
0.025	0.5	100	100	100
	2	100	100	100
	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100
0.05	0.5	100	100	100
	2	100	100	100
	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	99.94
0.1	0.5	100	100	100
	2	100	100	100
	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100

Based from the results in Table 4.2, by using activated carbon at different ratio of Zinc Chloride ($ZnCl_2$) in removing *E.coli* in 100mL of artificial water shows 100% removal of *E.coli* from 10^4 cfu/mL to 0 cfu/mL. Chemical activation by using $ZnCl_2$ as activating agent under nitrogen atmosphere has reported to produce activated carbon with high surface area (Ucar et al., 2009). The amount of $ZnCl_2$ used as the precursor during impregnation has an effect on the surface area of activated carbon produce. Previous study shows that impregnation ratio of $ZnCl_2$ within 0.5 to 2.0 appears to form activated carbon with well enough surface area as compared with commercially produce activated carbon. High surface area of activated carbon has been proved to be an effective adsorbent for the removal of bacteria from water (El-Aassar et al., 2013). In this batch study, it shows that the first 0.025 ratio of $ZnCl_2$ was sufficient to produce high surface area of activated carbon thus capable of removal the *E.coli* count to zero.

From this conventional batch study, activated carbon coated with silver nanoparticles has been used to study the removal of *E.coli* from drinking water. During the silver nanoparticle coating by chemical reduction method, different SDS ratio has been used to obtain stable silver nanoparticles. Sodium Dodecyl Sulphate (SDS) is important as stabilizer to prevent the silver from aggregation. Adequate amount of SDS function to absorb on the surface of silver nanoparticles thus protect the silver nanoparticles from aggregation. Based from previous study, increasing SDS ratio shows well –dispersed state of silver particles has been observed (Song et al., 2009). Results from this study revealed that, starting from SDS ratio 0.5 varying until 20 shows to 100% remove of *E.coli*. This shows that SDS ratio used were able to protect the silver

nanoparticle form on the activated carbon. Based on some literature review they proposed that the bacterial inactivation with silver when there was an interaction of silver with bacteria protein. The interaction cause the structural change in bacterial cell membrane thus inhibit the growth of bacteria (Heidarpour et al., 2011).

Besides, another factors affecting the removal percentage of *E.coli* was the shaking time which was the time of exposure of the artificial water to the prepared activated carbon coated silver nanoparticles. Results from this study shows that by increasing the shaking time, the activated carbon could 100% reduce the *E.coli* count. The first 10 minutes of shaking time was sufficient and optimum time for adsorption of the *E.coli* by prepared activated carbon, where complete inhibition of *E.coli* has occurred. Based from the literature review, the longer the microbial sample water stayed contact with the activated carbon, it could reduce the bacterial load for over 99% of its original amount (Kenneth & Emmanuel, 2015). As the treatment time increases , the rate of removal efficiency of *E.coli* also increase (Yoon et al., 2008).

4.3 Best Activated Carbon

Table 4.3 shows result to determine the best ratio of Zinc Chloride ($ZnCl_2$), SDS, and time shaking for *Escherichia coli* removal and silver concentration in filtered water by using activated carbon.

Table 4.3 Best activated carbon

Silver Conc.	<i>E.coli</i> removal	P value	P value	P value	R^2
Mean (95% CI)	Mean (95% CI)	($ZnCl_2$ ratio)	(SDS ratio)	(time)	
0.033 (-0.008,0.075)	99.99 (99.99,99.99)	0.157	0.428	0.359	0.995

***General Linear Model (Multivariate)**

Based on the above result, no significant different shows between $ZnCl_2$ ratio, SDS ratio and time shaking for silver concentration and removal percentage of *Escherichia coli* in filtered water. Thus, null hypothesis is failed to be rejected. However previous study shows that the result of *Escherichia coli* removal and silver concentration were highly affected by the $ZnCl_2$ ratio, SDS ratio and time.

The ratio of $ZnCl_2$ has effect on the surface area of activated carbon produce, where high surface proved to be an effective adsorbent for the removal of bacteria. (El-Aassar et al., 2013). Stabilizer plays an important roles to ensure the stability of silver nanoparticle in aqueous solutions. Previous study shows that the smallest output count of silver concentration in filtered water indicate the stability of silver and the ability of the stabilizer used to retain the silver on the surface of activated carbon. The mechanisms of

silver nanoparticles as antibacterial agents remain unclear. However, previous study revealed that silver nanoparticles inhibit microbial growth by attaching to the cell membrane and severely damage the cell's major functions (Heidarpour et al., 2011; Praveena, et al., 2016). Previous study shows the effect of contact time, where the longer the microbial sample water stayed contact with the activated carbon, this will increase the reduction of bacteria (Kenneth & Emmanuel, 2015).

Since the result shows no significance was found, this indicate that 40% Zinc Chloride used in this experiment was not the best percentage to find the best activated carbon. $ZnCl_2$ plays an important role in increasing the surface area of the activated carbon. The increment of surface area may be achieved via the creation of new micropores as indicated by the rise in micropores surface area and volume. Surface area of activated carbon (0 % $ZnCl_2$) is much lower compared to that of chemically activated carbon. The micro-pore surface area increased with increasing percentage of zinc impregnation solution (Abdullah et al., 2001).

4.4 Study limitation

This study has some limitations which have to be highlighted. Various factors are need to be considered during this batch study as it could affecting the results. Coating method for silver nanoparticles also affecting the result of this study. Preparation of silver nanoparticles can be done by using chemical, physical and biological method. However, the most popular method being used is chemical approaches including chemical reduction method by using various reducing agent. Different method will result in different stability and aggregation of silver nanoparticle size of silver form and silver distribution (Iravani et al., 2014). For physical approaches, metal nanoparticles are generally synthesized by evaporation–condensation, which could be carried out using a tube furnace at atmospheric pressure. Nanoparticles of various materials, such as Ag, Au, PbS and fullerene, have previously been produced using the evaporation/condensation technique. Meanwhile, for chemical approaches, common used of reducing agent are borohydride, citrate, ascorbate and elemental hydrogen was used to reduce silver ions (Ag^+) in aqueous solution thus produced colloidal silver with particle diameters of several nanometres (Kholoud et al., 2010).

Others limitation in this study is the percentage of Zinc Chloride use during chemical activation process to produce activated carbon. The amount of ZnCl_2 used as the activating agent will affect the surface area of activated carbon produce. Based from previous study, a further increase in percentage of ZnCl_2 used result to decrease surface area of activated carbon, which may be cause by the incomplete carbonization of banana stem. These results indicate that ZnCl_2 plays an important role in increasing the surface area of the activated carbon. This factor become limitation in this study since that only

40 % of $ZnCl_2$ was used to prepare the activated carbon (Abdullah et al., 2001). Thus, this study cannot show the minimum and maximum percentage of $ZnCl_2$ in order to produce high surface area of activated carbon.



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CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the minimum removal percentage of *Escherichia coli* was 99.94% and the maximum removal percentage of *Escherichia coli* was 100% by using activated carbon for all ZnCl ratio (0.025, 0.05, and 0.1) with six different SDS. The mean for silver concentration was 0.033. The highest value of silver concentration in filtered water using activated carbon was 0.242 mg/L which at, 0.05 ZnCl₂ ratio, 10 SDS ratio and 10 mins of shaking time. Best ratio cannot be determined since that there is no significant difference between Zinc Chloride ratio, SDS ratio, and time shaking for removal percentage of *Escherichia coli* and silver concentrations in filtered water by using activated carbon coated with silver nanoparticles.

5.2 Recommendation

Further study for removal of *Escherichia coli* using activated carbon coated with silver nanoparticles should consider several methods for silver nanoparticle coating and also different percentage of activating agent to compare the effectiveness. Not only that, since this experiment are involve with use of chemical, instead of quantification of silver, quantification of Zinc Chloride also need to take into consideration. Further study also need to include control for activated carbon without coated with silver nanoparticles to compare the effectiveness in *E.coli* removal. Lastly, more parameter in drinking water other than *E.coli* such as *Vibrio cholera*, *Salmonella sp.* etc. recommended to be tested to represent for worldwide situation.

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Appendix A: Table of calculation

ZnCl (ratio)	Shaking time(min)	AgNO ₃ /SDS (ratio)	Duplicate	Number of colony	Bacteria removal effectiveness calculation (%)	Average	
0.025	10	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
			D1	0	$\frac{10^4 - 0}{10^4} \times 100$		
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
	5	2	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
				D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
	10	5	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
				D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
	15	10	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
				D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
20	15	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
			D1	0	$\frac{10^4 - 0}{10^4} \times 100$		
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
20	20	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
			D1	0	$\frac{10^4 - 0}{10^4} \times 100$		
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
	5	2	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
				D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
5	5	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		

ZnCl (ratio)	Shaking time(min)	AgNO ₃ /SDS (ratio)	Duplicate	Number of colony	Bacteria removal effectiveness calculation (%)	Average
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		10	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
		15	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
		20	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
		30	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
		0.5	D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
		2	D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
		5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
		10	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
					$\frac{10^4 - 0}{10^4} \times 100$	

ZnCl (ratio)	Shaking time(min)	AgNO ₃ /SDS (ratio)	Duplicate	Number of colony	Bacteria removal effectiveness calculation (%)	Average	
0.05	10	15	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
		20	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
		0.5	2	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		2	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
		5	5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		10	10	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		15	15	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
				D2	0	$\frac{10^4 - 0}{10^4} \times 100$	

ZnCl (ratio)	Shaking time(min)	AgNO ₃ /SDS (ratio)	Duplicate	Number of colony	Bacteria removal effectiveness calculation (%)	Average
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		20	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
	20	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
		2	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
		5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
	10		D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100
		15	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	100

ZnCl (ratio)	Shaking time(min)	AgNO ₃ /SDS (ratio)	Duplicate	Number of colony	Bacteria removal effectiveness calculation (%)	Average
		20	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
	30	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		2	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		10	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		15	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		20	D1	6	$\frac{10^4 - 6}{10^4} \times 100$	99.94

ZnCl (ratio)	Shaking time(min)	AgNO ₃ /SDS (ratio)	Duplicate	Number of colony	Bacteria removal effectiveness calculation (%)	Average	
0.025	10	0.5	D2	6	$\frac{10^4 - 6}{10^4} \times 100$	100	
			D1	0	$\frac{10^4 - 0}{10^4} \times 100$		
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
			2	D1	0		$\frac{10^4 - 0}{10^4} \times 100$
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
			5	D1	0		$\frac{10^4 - 0}{10^4} \times 100$
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
			10	D1	0		$\frac{10^4 - 0}{10^4} \times 100$
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
			15	D1	0		$\frac{10^4 - 0}{10^4} \times 100$
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$		
			20	D1	0		$\frac{10^4 - 0}{10^4} \times 100$
D2	0	$\frac{10^4 - 0}{10^4} \times 100$					

ZnCl (ratio)	Shaking time(min)	AgNO ₃ /SDS (ratio)	Duplicate	Number of colony	Bacteria removal effectiveness calculation (%)	Average
	20	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		2	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
	5		D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
	10		D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
	15		D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
		20	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100
			D2	0	$\frac{10^4 - 0}{10^4} \times 100$	
	30	0.5	D1	0	$\frac{10^4 - 0}{10^4} \times 100$	100

ZnCl (ratio)	Shaking time(min)	AgNO ₃ /SDS (ratio)	Duplicate	Number of colony	Bacteria removal effectiveness calculation (%)	Average
			D2	0		
	2		D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0		100
	5		D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0		100
	10		D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0		100
	15		D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0		100
	20		D1	0	$\frac{10^4 - 0}{10^4} \times 100$	
			D2	0		100
					$\frac{10^4 - 0}{10^4} \times 100$	

Appendix B: Ethic's Approval Letter



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