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Density of coliform bacteria in well water filtered using a filter coated with silver nanoparticles

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Abstract. *Mufidah LN, Setyono P, susilowati A. 2021. Density of coliform bacteria in well water filtered using a filter coated with silver nanoparticles. Asian J Trop Biotechnol 18: 32-36.* The poor filtration process causes low water quality due to coliform bacteria. Today's nanoparticle technology is growing, one of which is silver nanoparticles known to have antimicrobial properties. Silver nanoparticles can be an alternative for making water filters while also killing bacteria. Therefore, this study was conducted to see the density of coliform bacteria in well water filtered using a filter coated with silver nanoparticles. It is an experimental study with five significant steps: synthesis of silver nanoparticles via reduction, coating filter paper with silver nanoparticles via the shake flask method, water filtration, bacterial density determination via the Most Probable Number (MPN) method, and coliform bacteria viability determination. The main parameter observed in this study was the density of coliform bacteria in well water through a filtering process, namely the MPN value. The synthesis of silver nanoparticles in the form of a gray-green-brown colloidal solution was formed at a wavelength of 437 nm. Silver nanoparticles can stick to the filter paper, which will be used for filtering, indicated by the change in the color of the paper to grayish brown. The MPN value of the control group in all wells was >1,100/100 mL, while the treatment group's wells were 1,2,3, 9.05; 553.65; and 1.5/100 mL. The t-test analysis of the MPN value showed a significant difference between the control and treatment groups with a significance value of <0.05, i.e., 0.04. The coating of silver nanoparticles on the water filter also killed coliform bacteria, which was indicated by the absence of bacteria growth from the filter paper used. The density of coliform bacteria in well water filtered using a filter coated with silver nanoparticles was lower than the control (without silver nanoparticle coating).

Keywords: Coliform, filtering, Most Probable Number, silver nanoparticles, water quality

INTRODUCTION

Water is a source of life because water is vital for all organisms, including humans, animals, and plants. According to Istipsaroh et al. (2016), water is the primary resource with many benefits in life, especially for humans. Humans cannot survive without water because most of the human body consists of water.

Human needs for water always increase from time to time, not only because of the increasing number of people who need water but because of the increasing intensity and variety of water needs. Humans use water to meet their needs, such as washing, bathing, and daily consumption. The rising number of people, the increasing human demand for clean water, and the increasing pollution level (Boretti and Rosa 2019; Hertika et al. 2021). Human activities can lead to water pollution, such as throwing garbage out of place and a lack of awareness of the cleanliness of the surrounding environment (Ferronato and Torretta 2019).

Well water or groundwater comes from the ground or infiltration of rainwater. Well water is widely used by the community, especially in rural areas. However, not all communities have water sources that meet health requirements. Population growth causes the need for clean water or water suitable for consumption. The well water used by the community has reasonable physical, chemical, and biological qualities, which still need to be tested to determine its suitability of the water for daily use (WHO 2006).

People tend to pay less attention to the water quality they consume in the current era, both water content and processing. According to Pradana and Marsono (2013), this is due to the advancement of technology and the increasing busyness of society. Hence, people choose a more practical way to meet their consumption water needs at a lower cost, one of which is filtering techniques.

The poor filtering process can cause water quality not to meet the regulations on drinking water quality. One of the causes is that the coliform bacteria content will be greater due to the filtering system or equipment used. According to Khaq (2016), coliforms are a group of bacteria as an indicator to determine the contamination of pathogenic bacteria in a water source. Examples of a group of bacteria called coliforms are *Escherichia coli, Enterobacter aerogenes, Citrobacter freundii,* and *Shigella* sp., which causes diarrhea.

Nanoparticle technology, called nanotechnology today, is growing. According to Wahyudi et al. (2011), one of the nanotechnology applications in the engineering of metal particles and metal oxides such as silver (Ag), copper (Cu), TiO_2, ZnO, and nanometer-sized MgO, which are applied to various antimicrobial products such as textiles, pulp, and paper, ceramics, and so on. Nanotechnology can be used as an alternative to water filters by manufacturing and applying structures/materials with nanometer dimensions.

The antimicrobial properties of colloidal silver can kill all pathogenic microorganisms, and there have been no reports of microbes that are resistant to silver (Ariyanta et al., 2014). The antimicrobial properties of silver can be used as an alternative for making water filters while also killing bacteria by coating the filter.

So far, many studies have been carried out on the application of silver nanoparticles. Some examples of silver nanoparticle applications that have been carried out include silver nanoparticles as a catalyst, a real-time optical sensor, and an antimicrobial agent (Haryono et al. 2008). As an antimicrobial agent, silver nanoparticles are used for lining wound dressings (Ariyanta et al., 2014). In addition, silver nanoparticles can be inoperable on other materials such as water and air filters and textile fibers. Therefore, this study was conducted to test a water filter coated with silver nanoparticles against the density of coliform bacteria.

This study aimed to determine the density of coliform bacteria in well water filtered using a filter coated with silver nanoparticles.

MATERIALS AND METHODS

Research time and place

This research was carried out from October 2018 to April 2019 at the Biology Laboratory and Integrated Mathematics and Natural Sciences Laboratory of Faculty Mathematics and Natural Sciences, then the Central Laboratory of Universitas Sebelas Maret, Surakarta, Central Java, Indonesia.

Ingredient

The materials used in this research include silver nanoparticles synthesis materials, silver nanoparticle activity test materials, silver nanoparticle coating materials on filter paper, water filtering materials, coliform bacteria test materials, and bacterial viability test materials. The materials used to synthesize silver nanoparticles include $AgNO_3$ 1.0 mm, sodium citrate 1%, and aquadest. The test materials for silver nanoparticle activity include sample water, silver nanoparticles, and NA medium. The silver nanoparticle coating material includes filter paper no. 1, aquadest, and silver nanoparticles on filter paper. Water filtering materials include sample water and filter paper infused with nanoparticles. Distilled water, lactose broth single media, lactose broth double media, BGLB media, aluminum foil, plastic wrap, and label paper are used in the coliform bacteria test. The materials used to determine bacterial viability are NA medium and filter paper used to filter water.

Procedures

Silver nanoparticle synthesis

Synthesis of silver nanoparticles was made by heating 150 mL of $AgNO_3$ with a concentration of 1.0 mm to boiling in an Erlenmeyer flask. 1% sodium citrate was added to this solution in 15 mL of distilled water dropwise. The mixture was stirred using a magnetic stirrer until it turned pale yellow during the heating process. Pale yellow indicates that silver nanoparticles have been formed (Mailu et al., 2010).

Silver nanoparticle characterization

According to Ariyanta et al. (2014) research, the silver nanoparticles formed were then characterized. Characterization was carried out using UV-vis Spectro, which determines whether or not silver nanoparticles are formed. The appearance of the absorbance peak at a wavelength of ± 410 nm to determine that silver nanoparticles have been formed.

Silver nanoparticle activity test

The silver nanoparticles that have been created are then combined with sample water in a ratio of 1:2, 1:3, or 1:5, depending on the amounts of silver nanoparticles present in the sample water. When silver nanoparticles are mixed with sample water, the results are diluted by a factor of 10^{-2} and 10^{-3}, and each dilution is planted on NA media for two repetitions.

Silver nanoparticle coating on filter paper

The coating of silver nanoparticles on filter paper was carried out by Duran et al. (2007). The filter paper used is filter paper no. 1. Filter paper soaked in silver nanoparticles, then incubated using a shaker incubator for 36 hours and allowed to stand for 5 minutes. The filter paper coated with silver nanoparticles was dried again in an oven at a temperature of 70ºC for 5 minutes.

Water sampling (well water)

The wells used were three sampling locations around the river flow, Banjarsari District, Surakarta. The sample container used was washed and sterilized by covering the mouth of the bottle using aluminum foil and coated with plastic wrap, and then sterilized using a sterilization autoclave. The sampling device is lowered into the well. Then the sampling device is removed after filling the sample. The water is transferred from the sampling device into the sample container preheated with a Bunsen burner. After being filled, it is heated again with a Bunsen burner and closed again (SNI 6989.58:2008).

Water filter

Filter paper that has been coated with silver nanoparticles is then applied to filter water samples. The filter paper is placed or affixed to the funnel. Then the funnel is inserted into the culture bottle as a container. The water sample was then poured into a funnel that had been pasted with nanoparticle filter paper at a speed of 10 mL/min. The filtered water was then tested for bacteria using the Most Probable Number (MPN) method.

Coliform bacteria density and viability test

Coliform bacteria test was carried out on filtered water samples and filter paper used for filtering water samples. Coliform bacteria in filtered water samples were counted using the MPN method. In contrast, on filter paper used for filtering water, samples were grown on NA media and seen whether coliform bacteria were alive or not.

Most Probable Number (MPN) test. The test for the MPN consists of two stages: the presumptive stage and the confirmed stage. 10 mL, 1 mL, and 0.1 mL samples were inoculated on Lactose Broth media in three series of tubes for each volume, then incubated at 37ºC for 24 hours. Positive tubes were identified by the presence of gas and a change in the purity of the Durham tube. The verified test is next performed on the positive tube from the presumptive test. A sample of 1 ose needle inoculated with Lactose Broth media was transferred to BGLB media and incubated at 37ºC for 24 hours. The number of positive tubes (cloudy and gaseous) was counted, and the number of coliform bacteria per 100 mL of sample was calculated using the MPN table.

Bacterial viability test. The viability test was carried out by planting the filter paper directly on NA media and incubating it for 24 hours at 37ºC. Planting results were observed to see the viability of bacteria.

Data analysis

The data obtained is the number of coliform densities in water filtered using silver nanoparticles and controls (without silver nanoparticles). The data were then compared and analyzed using the T-test method.

RESULTS AND DISCUSSION

Silver nanoparticle synthesis

The principle of synthesizing silver nanoparticles uses a reduction reaction with silver nitrate ($AgNO_3$) precursor and sodium citrate as a reducing agent. The reduction reaction occurs when the dropwise addition of a reducing agent and a stabilizer, namely sodium citrate solution, is added to a boiling silver nitrate solution. The colloidal solution synthesized by silver nanoparticles is shown in Figure 1.

The colloidal solution formed from the synthesis of silver nanoparticles is gray-green brown. The color indicates that silver nanoparticles have been formed, as stated by Zielinska et al. (2009), that the color of the colloidal silver nanoparticle solution is red to grayish-green depending on the ratio of the reducing agent. Apart from looking at the appearance of the color, it is necessary to carry out characterization to ensure further that silver nanoparticles have been formed.

Silver nanoparticle characterization

The purpose of characterizing colloidal silver nanoparticle solution is to ensure that silver nanoparticles form successfully in the solution. Characterization utilizing a UV-Vis spectrophotometer in which UV light with a wavelength of 300 to 600 nm is fired onto a colloidal solution of silver nanoparticles. Silver nanoparticles formed successfully will exhibit wavelengths between 400 and 500 nm, as these are the wavelengths of silver nanoparticles (Bagyalakshmi and Haritha 2017). The findings of the UV-Vis spectrophotometer's characterization are displayed in Figure 2.

The UV-Vis spectrophotometer determined the colloidal silver nanoparticle solution had a wavelength of 437 nm and an absorbance of 1.49403. These findings are consistent with previous research, which indicates that the produced silver nanoparticles have a wavelength of 418-419 nm (Ariyanta et al. 2014), a wavelength of 420 nm (Jain and Pradeep 2004), and a wavelength of 411.4-432.7 nm (Wahyudi et al., 2011). Characterization with a UV-Vis spectrophotometer revealed that silver nanoparticles were generated throughout the manufacturing procedure.

Silver nanoparticle activity

The silver nanoparticles that have been formed are tested for their antibacterial activity by mixing a colloidal solution of silver nanoparticles and sample water with various comparisons to then calculate the number of bacteria that grow with the total plate count. The results of the antibacterial activity test with a total plate count are presented in Table 1.

In the control group, seeding a solution of colloidal silver nanoparticles and sample water at a dilution of 10-2 revealed that bacteria grew in wells 1 and 2 but were too few to count (TFTC), but well 3 had 25 x 102 cfu/mL. Bacterial growth on the growing media was not seen in the treatment group; it means that when silver nanoparticles come into direct contact with a material, they behave as an effective antibacterial.

Figure 1. Solution of colloidal silver nanoparticles

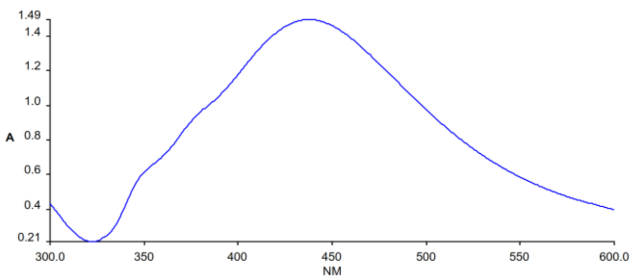

Figure 2. Graph of wavelength and absorbance of colloidal silver nanoparticle solution

Silver nanoparticle coating on filter paper

The filter paper gets coated by immersing the filter paper in a colloidal solution containing silver nanoparticles. The results of putting silver nanoparticles on filter paper are shown in Figure 3. After 36 hours of soaking in a colloidal silver nanoparticle solution, the filter paper produced a color change. Initially white, the filter paper gradually took on a grey-brown hue. This color change indicates the presence of silver nanoparticles on the filter paper. These findings corroborate Jain and Pradeep's research (2004) on silver nanoparticle coatings on polyurethane.

Most Probable Number (MPN) filtrate

The MPN method determines the density of coliform bacteria in the filtrate. The MPN test consists of two stages: estimation and affirmation. The presumptive test is used to predict the presence of coliform bacteria. In contrast, the confirmation test is used to confirm the presence of coliform bacteria based on the outcome of the presumptive test. The comparison of MPN test reactions during the estimation and confirmation stages is shown in Figure 4. A color change in the media and the formation of bubbles in the Durham tube (Figure 4 A1, A2, B1) indicate the presence of coliform bacteria in the sample water; on the other hand, if no color change occurs in the media and no bubbles form in the Durham tube (Figure 4 A3, A4, B2), there are no coliform bacteria in the sample water. The MPN value of filtered water from three wells with two replications is reported in Table 2.

According to the MPN value, there is a decrease in the density of coliform bacteria in the sample water filtered with a filter coated with silver nanoparticles compared to water filtered with a filter not coated with silver nanoparticles. MPN levels for water samples in all control groups exceeded 1100/100 mL, but MPN values in the silver nanoparticle coating treatment were 9.05; 553.65; and 1.5/100 mL, respectively. The t-test analysis revealed a significant difference between the control and treatment groups, with a significance value of 0.05 or 0.04. This demonstrates that adding silver nanoparticles to the filter reduces the density of coliform bacteria in the filtering process.

Table 1. Bacterial growth in well water after added colloidal silver nanoparticles

Well	Control (without nanoparticles silver)	Treatment (addition of silver nanoparticles)		
		1:2	1:3	1:5
1	TFTC (5x10^2 cfu/mL)	Not growing	Not growing	Not growing
2	TFTC (8x10^2 cfu/mL)	Not growing	Not growing	Not growing
3	25x10^2 cfu/mL	Not growing	Not growing	Not growing

Note: 1:2; 1:3; 1:5 = ratio of colloidal silver nanoparticle solution and sample water

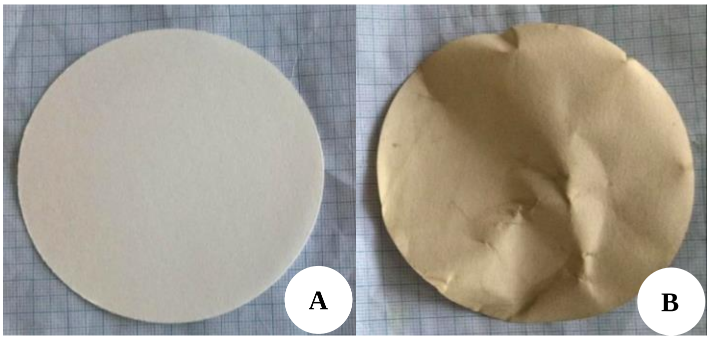

Figure 3. Filter paper: A. No silver nanoparticle coating, B. After silver nanoparticle coating

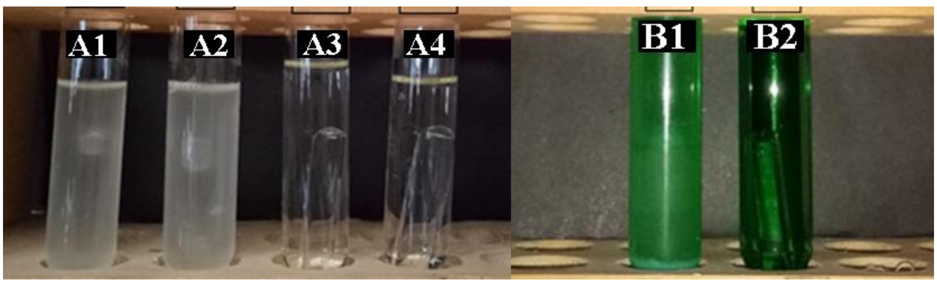

Figure 4. Reaction to MPN Test: A. Prediction test; B. Affirmation test

Table 2. MPN value of well water after filtration

Category	Well	Coliform Test (MPN/100 ml)
Control	1	>1100
	2	>1100
	3	>1100
Treatment	1	9.05
	2	553.65
	3	1.5

Table 3. Observation of bacterial viability on filter paper

Category	Well	Bacterial viability	
		Growing	Not growing
Control	1	√	
	2	√	
	3	√	
Treatment	1		√
	2		√
	3		√

Bacterial viability on filter paper

Bacterial viability testing on filter paper is used to establish whether silver nanoparticles can actively kill bacteria or work mechanically to decrease the pores in the filter paper. Table 3 summarizes the results of observations of bacterial viability on filter paper.

The results of the bacterial viability test on the filter paper used for filtering are shown in Table 3. When uncoated filter paper was planted on NA medium after filtering, bacteria grew (control group). In contrast, when coated filter paper was planted on NA media, bacteria did not grow (treatment group). These findings suggest that covering filter paper with silver nanoparticles acts as a filter and kills bacteria throughout the water filtering process.

Ariyanta et al. (2014) also reported on the capacity of silver nanoparticles to kill germs. According to his research, the wound dressing cloth coated with silver nanoparticles eliminated the bacteria *E. coli, Bacillus subtilis*, and *Staphylococcus aureus* in vitro using the shake flask method followed by a total plate count to determine the bacteria's quantity.

According to Franci et al. (2015), silver nanoparticles can physically interact with the surface of bacterial cells. It damages the cell membrane, causing structural changes that make the bacterium more permeable. It is well established that when silver nanoparticles accumulate on the cell membrane of *E. coli* bacteria, they generate a gap that penetrates the bilayer, increasing permeability and ultimately cell death.

In conclusion, the density of coliform bacteria was significantly lower in well water filtered through a filter coated with silver nanoparticles than in control water (without silver nanoparticle coating).

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