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Study of Synthesis of CuO Nanoparticles with Sol-Gel Method and Its Application as Antibacterial Agent in Portland Cement

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ABSTRACT

In environmental conditions that have a relatively high level of humidity, it can trigger health problems in the people in the residential environment. Humid environmental conditions can cause the interior surface of the building to become an excellent place for the growth of bacteria. This study aims to determine the effectiveness of adding CuO nanoparticles as antibacterial agents in Portland cement with *Escherichia coli* bacteria as a bacterial model. Evaluation of antibacterial properties in Semen-CuO is carried out using the disc diffusion test method. The test was carried out by varying the addition of CuO to Portland cement by 0%, 1%, 3% and 5% (%w/w) and was carried out stirring cuO without sonication and with the help of sonication to obtain a perfect dispersion. Characterization of CuO is carried out using the XRD instrument to obtain information regarding the structure, crystallinity and size of the synthesized CuO. The morphology of the cement-CuO surface was observed using sem instruments. The results showed that the best antibacterial effectiveness was obtained by the addition of CuO as much as 3% into Portland cement which resulted in an inhibitory zone size of 7 mm by stirring using ultrasonics. The presence of an inhibitory zone indicates that the resulting Cement-CuO shows an inhibitory effect on the growth of *Escherichia coli* bacteria.

Keywords: Antibacterial, CuO, Diffusion Discs, *Escherichia coli*, Portland Cement

1. INTRODUCTION

Cement is one of the main basic materials used in construction, so cement is one of the strategic commodities. According to data from the Indonesian Cement Association, domestic cement consumption has increased sharply, especially outside Java. Total domestic cement consumption in January 2022 was around 5.28 million tons, up 7.6% compared to January last year. Meanwhile, cement consumption in 2021 was recorded at 66.21 million tons, an increase of 5.9% from 2020 of 62.51 million tons.

Portland cement is a construction material used as a binder or adhesive. However, in humid environmental conditions it can trigger microbial infections such as bacteria, fungi, and insects on the surface of the cement structure. This is because in humid conditions it can facilitate the attachment of microbes due to the relatively high humidity making the interior surface of the building an excellent place for microbial growth [1], so it is necessary to develop cement-based materials that can function as antimicrobials [2]. This is necessary because most health problems can be caused by the presence of microorganisms such as bacteria, fungi, protozoa, and viruses that can cause health problems for residents in the living environment [3]. In recent developments in science and technology, nanotechnology has received much attention in various fields of science, industry, and research institutions due to its very small size [4]. Nanoparticles have many uses, one of which can be used as an antibacterial.

Nanocomposite is a material with a nano size of 10^{-9}nm which is formed from two or more different materials that have the properties of a mixture of materials [5]. Nanoparticles can be used as antibacterials because in general the size of bacterial cells is around micrometers in size, while the outer cell membrane has a pore size in the nanometer range. Because nanoparticles are smaller than the pores of bacteria, they have a unique ability to penetrate bacterial cell membranes [6]. The use of nanoparticles that have antibacterial properties can be utilized in the composition of concrete and cement. Cement that has been modified to be antibacterial has a promising potential for use in the construction sector [7].

Nanoparticles based on metal oxides have aroused great interest due to their many unique properties associated with nanoparticle size [8]. The antimicrobial activity of metal oxides has attracted attention for use as antimicrobial agents, where the use of these metal oxides has advantages such as: good antimicrobial activity at low nanoparticle concentrations [9], the oxide contains elements that are abundant in nature, very important for humans and can provide high antimicrobial activity in small amounts in the absence of light. In addition, simple metal oxides are widely used in modern technology [1].

Cupric Oxide (CuO) has a monoclinic crystal structure and is a semiconductor material [10]. Conductivity materials that are between conductors and insulators are called semiconductors [11]. CuO nanoparticles show high potential in metal oxide nanoparticles due to their relatively low cost, optical, catalytic and antimicrobial properties [12]. CuO can be used as an antibacterial because it has a relatively low toxicity to human cells, a relatively low cost, has an effective inhibition against various bacteria, the ability to prevent the formation of biofilms and can even remove spores [13]. In addition, CuO nanoparticles produce Reactive Oxygen Species (ROS) which interact with the bacterial cell membrane to enter the cell which results in inhibition of bacterial growth. CuO nanoparticles destroy bacterial cell membranes through the production of ROS [14].

In this study, metal oxide nanoparticles were used as antibacterials to be added to Portland cement and CuO nanoparticle synthesis was carried out using the sol-gel method. The advantage of synthesizing nanoparticles with the sol-gel method is that this method is a simple bottom-up synthesis method, has a relatively low cost, and can produce high material purity for nanoparticles [15], high homogeneity of nanoparticle size [16], and low processing temperature [17]. Based on this background, CuO nanoparticles will be synthesized using the sol-gel method as an antibacterial agent to be applied to Portland cement with the *Escherichia coli* bacteria model and antibacterial activity tested using the disc diffusion method in the form of measuring the diameter of the resulting inhibition zone.

2. LITERATURE REVIEW

In the study of making geopolymers with TiO_2 (GP) nanoparticles, the results of the *Escherichia coli* antibacterial test for GP samples were observed by disc diffusion. The resulting inhibition was satisfactory and formed a zone of inhibition around the sample. Moreover, when the GP sample was removed from the nutrient agar, no bacteria were found under the sample [3]. The use of CuO nanoparticles as an antibacterial agent in the manufacture of geopolymers shows that CuO nanoparticles can provide antibacterial effectiveness and increase the compressive strength of both *Bacillus subtillis* bacteria (gram positive) and *Escherichia coli* bacteria (gram negative) [18].

3. EXPERIMENTAL

3.1 Tools and Materials

Tools needed measuring cup, beaker, measuring flask, stirring rod, filter paper, paper puncher, glass funnel, oven, furnace, evaporating cup, drip pipette, watch glass, scales, spatula, mortar and pestle, hot plate stirrer, autoclave, ultrasonic (raw bk-1200), petri dish, erlenmeyer, ose needle, XRD, aquades bottle, oven, SEM, tweezers, autoclave, caliper. The materials used in this study were Portland cement (Semen Padang), $CuSO_4.5H_2O$ 0.1M (Merck), aquades, 0.1 M citric acid (Merck), 1 M NaOH (Merck), *Escherichia coli* ATCC 25922, nutrient agar instant (Merck)

3.2 Procedurs

3.2.1 Synthesis and Characterization of CuO

A total of 250 mL of 0.1M citric acid (Merck) was added to 500 mL of 0.1M $CuSO_4.5H_2O$ (Merck) and stirred with a magnetic stirrer until homogeneous. Then add a few drops of 250 ml of 1M NaOH (Merck) while stirring with a magnetic stirrer until it turns dark blue. Then let stand (aging) for 1 day until many black deposits are formed. Then filtered with filter paper and washed with aquadest. Next, the precipitate was dried at 110^0C until the weight is constant and calcined for 3 hours at 500^0C [18]. Then CuO has been synthesized, characterized using the XRD (X-Ray Diffraction) instrument. Calculations regarding the size of the CuO crystal size that have been synthesized using debye Scherrer's equation:

$$D = K\frac{\lambda}{\beta\cos\theta}$$

Where, D is the size of the crystal, λ is the wavelength of the incoming X-ray beam, β is the full width at half of the maximum intensity of the reflection peak, and K is the Scherrer constant.

3.2.2 Mixing CuO in Portland Cement

For samples containing CuO nanoparticles, 1%, 3% and 5% CuO nanoparticles were added to the water, then stirred with ultrasonic for 15 minutes before adding cement. While stirring without ultrasonic, CuO was stirred manually with a spatula into water until homogeneous. The procedure for mixing water and cement is carried out in a ratio of 1:2 (1 ml of water and 2 g of cement). Then the mixture is stirred to produce a cement paste.

3.2.3 Antibacterial Tests Against *Escherichia coli*

Previously, the manufacture of nutrient media was carried out by weighing as much as 2.8 gr of nutrients to be instantaneous, then added to the aquades up to a volume of 100 mL and stirred and heated until the material was completely dissolved. Then the solution is introduced into the erlenmeyer and tightly closed. Next, a sterile of the necessary medium is carried out using an autoclave at a temperature of 121ºC and a pressure of 15 psi for 15 minutes. After sterilization, before pouring the medium into the petri dish wait until it is lukewarm (± 40°C). Keep the medium tightly at room temperature until it is perfectly compacted and not contaminated.

Disc paper measuring 5 mm is laid or immersed in cement paste samples, positive control, and negative control. Then, the disc paper that has been saturated with the sample along with the control is carefully placed into the media which has previously been spread by *Escherichia coli* bacteria on the nutrient agar media. Then, the sample was incubated at room temperature for two day, then visually observed the results of antibacterial activity formed from the sample in the form of clear areas or resistance areas around the sample and measured by a calipers.

An antibacterial needs to know the activity of its antibacterial power through the response to inhibition of bacterial growth [19].

Table 1 Bacterial Inhibition Power Categories According to Davis-Stout

Inhibitory Power of Bacteria	Category
≥ 20 mm	Very strong
10-20 mm	Strong
5-10 mm	Keep
≤ 5 mm	Weak

3.2.4 Cement-CuO Characterization

Cement-CuO paste, which has been made before, is allowed to stand until the paste hardens and forms an acian. Then the Semen-CuO is characterized using SEM (Scanning Electron Microscopy).

4. RESULTS AND DISCUSSION

4.1 Synthesis and Characterization of CuO

The resulting Copper (II) Oxide (CuO) nanoparticle powder is a black solid. In this study, the CuO nanoparticle synthesis process was carried out using the sol-gel method, where the sol-gel method is a nanoparticle synthesis method consisting of two different phases, namely solution and gelation. The sol-gel method was chosen because this method only requires simple reagents and uses a relatively low temperature to be able to produce nanoparticles with high purity and good size when compared to other synthesis methods [20].

The CuO synthesis process was carried out by adding 500 ml of 0.1 M $CuSO_4.5H_2O$ which is a metal salt and 250 ml of citric acid which functions as a chelating agent. In the synthesis process, mixing $CuSO_4.5H_2O$ with citric acid, the resulting solution is stirred to form a sol. The addition of base is done to modify the pH so that it can increase the binding of cations to citrate, where in this study NaOH was used as a base [21]. The addition of NaOH is intended to form CuO compounds in the synthesis process [22], according to the following reaction:

$$2Cu^{2+} + SO_4^{2-} + 2NaOH \rightarrow Cu(OH)_{2(s)} + Na_2SO_4 + 5H_2O$$

$$Cu(OH)_{2(s)} \rightarrow CuO_{(s)} + H_2O$$

$$CuO_{(s)} \xrightarrow{calcination} CuO_{(s)}$$

Mixing this solution is done by stirring accompanied by heating which causes a condensation process to turn the sol into a gel. There is a blue precipitate of copper (II) hydroxide which when heated can turn into copper (II) oxide which is black in color due to dehydration. The resulting solution, then allowed to stand (aging) for 1 day to get the gel tissue back to settle. Then, the precipitate is filtered and oven dried to remove possible residual ions and moisture. Then, the sample was calcined at 500^0C for 3 hours to form oxides and organic compounds will decompose during the increase in temperature.

Figure 1. CuO nanoparticles

The synthesized CuO nanoparticles were then characterized using XRD (X-Ray Diffraction) instruments. Analysis using XRD (X-Ray Diffraction) aims to obtain information about the size, structure and crystallinity of the synthesized CuO nanoparticles. In this study, the XRD pattern was obtained as shown in the following figure:

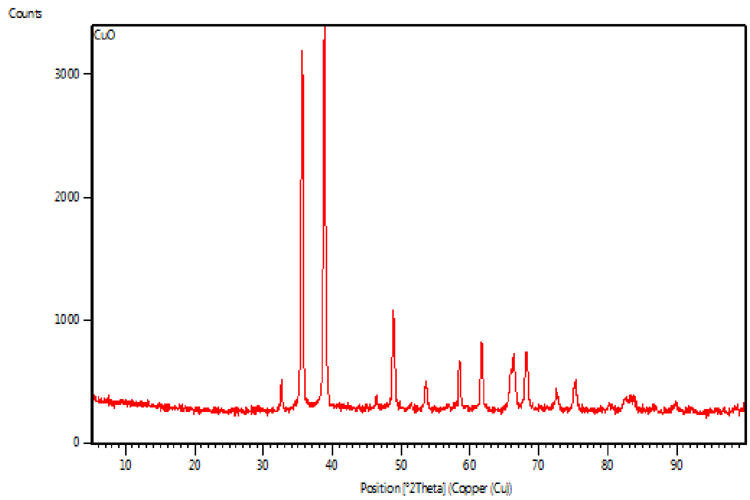

Figure 2. XRD Pattern of CuO

From the results of the XRD characterization of the samples, the spectrum of the XRD characterization results was matched with the Inorganic Crystal Structure Database (ICSD) data and 19 specific peaks were obtained at the diffraction angle (2θ) and according to each hkl value. The diffraction peaks are well defined and show the peaks of CuO nanoparticles and the orientation of the crystal planes according to the ICSD database number 01-070-6830 which indicates a monoclinic structure. The sharp peaks produced from the graph indicate that the CuO nanoparticles produced have high crystallinity properties. The sharpness of the XRD spectral graph is related to its crystallinity quality. The sharpness of the peak also affects the width of the curve or what is commonly referred to as the full width at half maximum FWHM (full width half maximum). The FWHM value is related to the size of the crystal diameter, where the larger the FWHM value, the smaller the diameter of the resulting crystal [23]. Based on the results of calculations using the Debye-Scherrer formula, the crystal size of CuO is 18.60 nm.

4.2 Cement-CuO Characterization

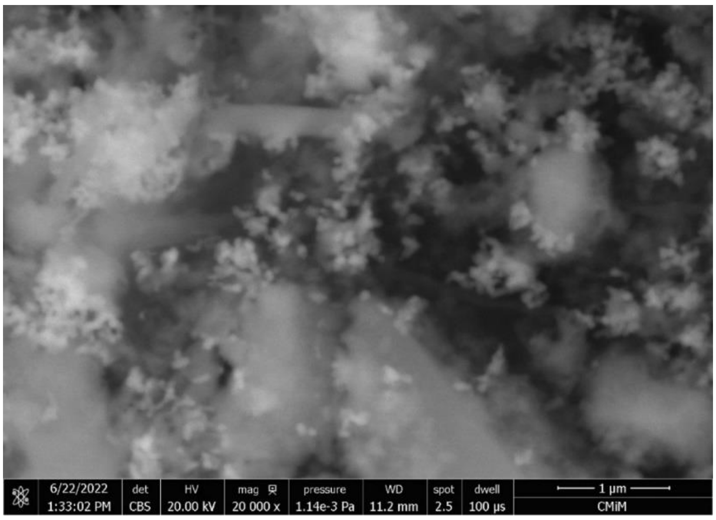

Figure 3 SEM Images of Cement-CuO

In the image above, the surface morphology of Cement-CuO can be observed through the results obtained from the SEM instrument. At 20,000x magnification, the CuO cement sample shows the presence of hydration products such as hydrated calcium silicate gel (C-S-H gel) and portlandite. The C-S-H gel which is the main product of hydration of Portland cement is in the form of a gel that can crystallize, and portlandite or calcium hydroxide which tend to form large crystals with a hexagonal prism morphology and can also be observed CuO nanoparticles that form spherical particles.

4.3 Antibacterial Test Against *Escherichia coli*

4.3.1 Stirring CuO without using ultrasonic

Table 1 Average diameter of inhibition zone and growth inhibition response with CuO stirring without ultrasonic

Treatment	*Average Diameter of the Inhibitory Zone (mm)*	*Growth Barrier Response*
0%	*1*	Weak
1%	*2,33*	Weak
3%	*3,33*	Weak
5%	*4,67*	Weak
CuO 100%	*18,5*	Strong
Aquadest	-	*Not response*

Based on data from the results of research that has been done, Portland cement without the addition of CuO and Portland cement added with CuO produced various diameters of the inhibition zone. Antibacterial activity testing was carried out by observing the inhibition zone that appeared around the paper disc placed in a petri dish. Inhibition zones appeared in all petri dishes, except for distilled water which acted as a negative control. Aquades is a negative control which results in the growth of *Escherichia coli* bacteria growing in nutrient agar media.

CuO with a concentration of 100% has antibacterial properties. Based on the grouping of antibacterial activity through the response to bacterial growth inhibition according to Davis and Stout, 100% CuO has a relatively strong inhibitory activity. The ability of CuO as an antibacterial agent is indicated by the presence of Cu^{2+} which is released indicating its high affinity for bacteria. This is because Cu^{2+} particles attract amine and carboxyl groups on the surface of bacterial cells so that on the surface of bacterial cells there is disruption of the cell membrane which results in cell damage. When the surface of the bacterial cell wall is damaged and penetrated, the metabolites will leak and the function of other cells in the bacteria will stop, preventing bacteria from reproducing [24]. Based on the theory of HSAB (Hard Soft Acid Base), Cu^{2+} ions which are included in borderline metal ions (border areas) have stronger complex bonds with N atoms and S atoms which are included in weak bases. Complex bonds between Cu^{2+} and S or N atoms in enzymes and proteins cause structural changes in enzymes and proteins and result in the death of bacteria [18]. In addition, Cu^{2+} ions can produce Reactive

Oxygen Species (ROS) or free radicals such as •OH (hydroxyl), •O_2 (superoxide), H_2O_2 (hydrogen peroxide) which can inhibit the growth of bacterial cells by oxidizing the double bonds in phospholipids so that they can damage proteins. which plays a role in adhesion and biofilm formation [25].

In the cement sample without the addition of CuO, it was seen that there was a cloudy inhibition zone around the paper disc of only 1 mm, which according to Davis-Stout, the sample had a weak inhibitory response to bacterial growth. Meanwhile, the addition of 1%, 3%, and 5% CuO resulted in an increase in the diameter of the inhibition zone along with the addition of CuO as an antibacterial. This happened because the higher the concentration of CuO added, the greater the amount of compounds that functioned as antibacterials released, thus facilitating the penetration of these compounds into cells. However, the antibacterial activity produced also has a relatively weak inhibitory activity.

4.3.2 Ultrasonic stirring of CuO

Table 1 Average diameter of inhibition zone and growth inhibition response with CuO stirring with ultrasonic

Treatment	Average Diameter of the Inhibitory Zone (mm)	Growth Barrier Response
1%	5,5	Keep
3%	7	Keep
5%	6,33	Keep

Based on data from the results of research that has been carried out, both the addition of CuO of 1%, 3% and 5% resulted in a cloudy inhibition zone with moderate inhibitory activity. Where, in cement the addition of 3% CuO resulted in a larger diameter of the inhibition zone than the addition of 1% CuO, but there was a decrease in the diameter of the inhibition zone with the addition of 5% CuO when compared to the variation of the addition of 3% CuO. The addition of antibacterial concentration may not be directly proportional to the diameter of the inhibition zone formed. This can occur due to the differences produced by the inhibitory power which is influenced by differences in the sensitivity of the organism, the mechanism and the process of antibacterial action.

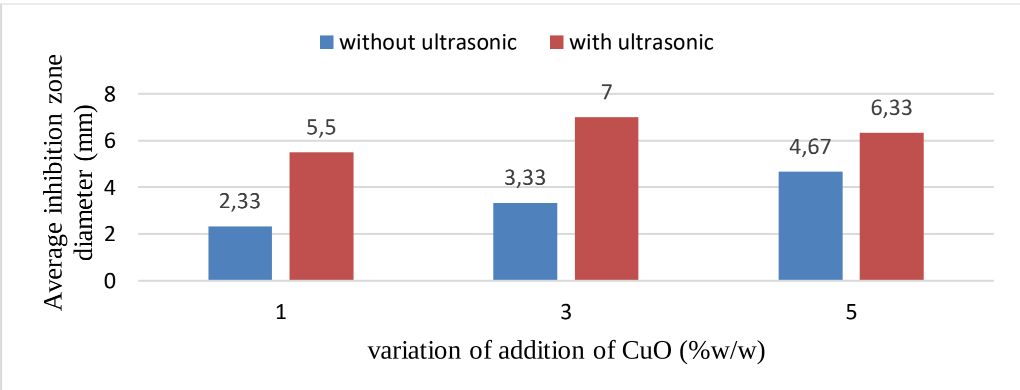

Figure 4. Bar chart comparison of the average diameter of the inhibition zone

Based on the diagram, it can be seen that there is an effect of stirring nanoparticles on the quality of the antibacterial activity produced. Where, the treatment of stirring CuO nanoparticles with ultrasonic assistance before being added to cement had a better response to inhibition of bacterial growth or antibacterial activity compared to stirring CuO into cement manually or without ultrasonic assistance. To disperse nanomaterials, a dispersion medium in the form of water is needed in the manufacture of composite cement. However, since the mixing of water in the manufacture of cement pastes or mortars requires the ratio of water to cement practiced in civil engineering to be lower than or equal to 0.5 or 1:2, this causes the amount of water available for dispersion to be limited. Nanoparticles when mixed with water show agglomeration tendencies due to Van der Waals forces. The use of mechanical methods using ultrasonic assistance allows for effective dispersion of nanoparticles in Portland cement so that later maximum results can be obtained in the process of applying CuO to Portland cement to be tested for antibacterial activity against *Escherichia coli* bacteria. The ideal dispersion can be described as a state in which the nanoparticles are completely separated from each other and no clusters or agglomerates are present. Ultrasonication can be used to prevent agglomeration of nanoparticles by using ultrasonic wave energy and cavitation in water.

5. CONCLUSION

The addition of CuO nanoparticles can provide antibacterial effectiveness in the form of inhibiting the growth of *Escherichia coli* bacteria, but in each variation the addition of CuO does not produce a significant difference. Antibacterial cement with ultrasonic agitation of CuO has better antibacterial activity than manual or non-ultrasonic agitation of CuO. The best variation to produce antibacterial cement is the addition of 3% CuO with ultrasonic stirring.

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