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Synthesis of Cu_2O, Cu_2O/Charcoal, and Cu_2O/Activated Charcoal Composites as Antibacterial Agents

(Sintesis Komposit Cu_2O, Cu_2O/Arang, dan Cu_2O/Arang Aktif sebagai Agen Antibakteri)

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ABSTRAK

Penggunaan antibiotik yang berlebihan untuk pengobatan infeksi bakteri dapat mengakibatkan resistensi sehingga diperlukan bahan antibakteri lain sebagai alternatif. Penelitian ini mengombinasikan arang dan arang aktif dengan oksida logam, yaitu tembaga oksida (Cu_2O) yang bersifat antibakteri terhadap bakteri Gram positif dan Gram negatif, sehingga menghasilkan komposit antibakteri untuk proses pengolahan air. Selain itu, kajian ini juga mengevaluasi pengaruh jenis gula yang berbeda sebagai zat pereduksi terhadap Cu_2O yang dihasilkan, dan mengidentifikasi aktivitas antibakteri Cu_2O dan kompositnya. Sintesis Cu_2O dengan metode raksi seperti-Tollens menggunakan $Cu(NO_3)_2$ sebagai prekursor, kemudian ditambahkan NaOH, NH_4OH, dan gula. Gula yang digunakan adalah sukrosa, gula putih, dan gula merah. Jenis gula sebagai pereduksi memengaruhi ukuran dan morfologi Cu_2O yang dihasilkan. Tambahan Cu_2O pada arang dan arang aktif akan menaikkan sifat antibakteri pada arang dan arang aktif. Tembaga oksida, Cu_2O/arang dan Cu_2O/arang aktif memperlihatkan sifat antibakteri yang tinggi pada *Escherichia coli* (Gram negatif), yaitu secara berturut-turut 5,69 ± 0,02 mm dan 6,23 ± 0,03 mm, karena memiliki lapisan dinding sel yang lebih tipis dibandingkan *Staphylococcus aureus* (Gram positif). Aktivitas antibakteri terbaik ditunjukkan oleh Cu_2O yang disintesis menggunakan gula putih sebagai reduktor dengan zona hambat 8,26 ± 0,19 mm.

Kata kunci: antibakteri, arang, arang aktif, gula, tembaga oksida

ABSTRACT

The excessive use of antibiotics to treat bacterial infections can lead to bacterial resistance, necessitating other antibacterial agents as alternatives. This research combined charcoal and activated charcoal with metal oxide, namely copper oxide (Cu_2O), which has antibacterial properties against Gram-positive and Gram-negative bacteria, thus producing an antibacterial composite for water treatment processes. Furthermore, this study also examined the effect of different types of sugar as reducing agents on the produced Cu_2O and identified the antibacterial activity of Cu_2O and its composites. Synthesis of Cu_2O through the Tollens-like reaction method using $Cu(NO_3)_2$ as a precursor, then adding NaOH, NH_4OH, and sugar. The sugars were sucrose, white sugar, and brown sugar. The type of sugar used as a reducing agent affected the size and morphology of the Cu_2O produced. Adding Cu2O to charcoal and activated charcoal increased antibacterial properties to charcoal and activated charcoal. Copper oxide, Cu_2O/charcoal, and Cu_2O/activated charcoal exhibited high antibacterial properties against *Escherichia coli* (Gram-negative), as of 5.69 ± 0.02 mm and 6.23 ± 0.03 mm, respectively, due to their thinner cell walls compared to *Staphylococcus aureus* (Gram-positive). The Cu_2O synthesized using white sugar as the reducing agent showed the best antibacterial activity, with an 8.26 ± 0.19 mm inhibition zone.

Keywords: activated charcoal, antibacterial, charcoal, copper oxide, sugar

INTRODUCTION

Inorganic materials have become important antibacterial agents used in the biological field. Metal oxide materials show broad-spectrum antibacterial activity on Gram-positive and Gram-negative bacteria (Hong *et al.* 2018). Copper oxide (Cu_2O) is an excellent material for antibacterial applications due to its high antibacterial activity, effectiveness, and good storage stability (Hemmati *et al.* 2019). Copper oxide has shown multitoxicity across many bacteria species (Xiong *et al.* 2015). Copper oxide particles have been reported to exhibit lower toxicity than silver oxide (Bezza *et al.* 2020). Copper oxide has been widely used in the antibacterial field as an antiseptic and germicide in daily life (Kiaune & Singhasemanon 2011), antibacterial for medical treatment tools (Allaker & Memarzadeh 2014), and in removing pathogens from the aquatic environment (Fan *et al.* 2012). Synthesis of Cu_2O by chemical reduction (Tollens-like reaction)

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requires a reducing agent. Typical reducing agents are sodium borohydride, hydrazine or borane compounds, and sugar (Gawande *et al.* 2016). Sugar is a low-cost and environmentally friendly reducing agent to produce Cu_2O.

Copper oxide, with its antibacterial properties, can be combined with other materials to form antibacterial composites. Charcoal and activated charcoal have been widely used in water treatment to remove organic and inorganic pollutants because of their high surface area, amount, and adsorption rate. However, when charcoal and activated charcoal are used to purify water, bacteria will adhere due to the excellent biocompatibility properties of charcoal and activated charcoal. It causes bacteria to breed on charcoal and activated charcoal during the water purification process so that charcoal and activated charcoal become polluted (Zhao *et al.* 2013). This problem can be overcome by combining charcoal and activated charcoal with copper oxide, which has antibacterial properties.

Combining charcoal and activated charcoal with copper oxide will produce a composite. *Composite* is a material formed from a combination of two or more materials that have different mechanical properties from their constituents. Composite formation aims to improve certain mechanical properties and/or specific properties to obtain materials that have new properties from their constituents. Copper oxide can be used as a composite with charcoal. Therefore, this research combines charcoal and activated charcoal with metal oxide, namely copper oxide (Cu_2O), which has antibacterial properties against Gram-positive and Gram-negative bacteria, thus producing an antibacterial composite for water treatment processes. Furthermore, this study also examines the effect of different types of sugar as reducing agents on the produced Cu_2O and identifies the antibacterial activity of Cu_2O and its composites.

MATERIALS AND METHODS

The experiment used analytical balance (Precissa XT 220 A), micropipette (Nichipet EX), hotplate (Torrey Pines Scientific), oven (Memmert), UV-Vis spectrophotometer (Jenway 7300), and Fourier-transform infrared (FTIR) (Perkin Elmer Spectrum One). The materials included Cu(NO3)2.3H2O (Merck), commercial charcoal, commercial activated charcoal (Setiaguna), NaOH, NH4OH (Merck), ethanol (Merck), distilled water, Mueller Hinton agar (Oxoid), sucrose, white sugar (Gulaku), and brown sugar.

Cu_2O Synthesis through Tollens-like reaction

The $Cu(NO_3)_2.3H_2O$ 10 mM precursor consisted of 2 mL, prepared in 3 test tubes. Each test tube was added with 1 mL of 0.4 M NaOH and 1 mL of 10 mM NH_4OH. The next step was adding a reducing agent of 37% (b/v) sugar, as much as 1 mL. The sugars used were sucrose, white sugar, and brown sugar. The test tube containing the precursor and reagents was heated using a hotplate at 80°C for 10 minutes. In the next step, the sample is washed with ethanol and then distilled water until the pH is neutral. The supernatant was separated from the precipitate and then dried using an oven at 105°C for 1 hour. Cu_2O synthesized using sucrose as a reducing agent was labeled as Cu2O (1), Cu_2O which uses white sugar as a reducing agent was named Cu_2O (2), and Cu_2O which is synthesized using brown sugar as a reducing agent was labeled as Cu_2O (3).

Synthesis of Cu_2O/charcoal and Cu_2O/activated Charcoal Composites

Charcoal and activated charcoal were pulverized first to 100 mesh size. A physical mixing method was used to synthesize copper oxide/charcoal composite. The initial steps of the synthesis were the same as the synthesis of copper oxide through the Tollens reaction until heating at a temperature of 80°C for 10 minutes. After that, 10 mg of the commercial activated charcoal was added to each test tube and shaken until homogeneous. In the next step, the sample was washed with ethanol and then distilled water until the pH was neutral. The supernatant was separated from the precipitate and dried using an oven at 105°C for 1 hour.

Specific Surface Area Calculation (Okeola *et al.* 2012)

Calculating the specific surface area (S) used methylene blue adsorption with the isotherm adsorption model, which was the Langmuir model. The value of Xm is the amount of solute adsorbed on the monolayer per gram of adsorbent (mol/g), Am is the surface area occupied by the adsorbent either in the form of molecules or aggregates on the surface (m2), NA is Avogadro's number, and Mw is the molecular weight of adsorbate. The calculation of the specific surface area follows the following equation:

$$S = \frac{Xm \times Am \times NA}{Mw}$$

The Adsorption Capacity of Methylene Blue

The adsorption capacity of methylene blue is the maximum amount of dye that can be adsorbed by 1 g of adsorbent. This analysis began with preparing a 2000 ppm methylene blue stock solution. The methylene blue stock solution was diluted to eight concentrations (50, 125, 250, 500, 750, 1000, 1500, and 2000 ppm). A total of 20 mg of charcoal and activated charcoal were added to 5 mL of methylene blue solution of various concentrations in different containers, then shaken every hour. Adsorption was carried out for 24 hours. The filtrate concentration was determined using a visible light spectrophotometer at λ maximum. The adsorption data was calculated for its capacity and analyzed using the Freundlich and Langmuir equations.

Freundlich equation:

$$\text{Log}\frac{X_m}{m}=\text{Log } k+\frac{1}{n}\text{Log Ce}$$

where Ce is the ion concentration after adsorption (mg/L), *X*m/m is the mass of ions adsorbed per adsorbent mass (mg/g adsorbent), k is the maximum adsorption capacity (mg/g), and n is the Freundlich constant.

Langmuir equation:

$$\frac{Ce}{Xm/m}=\frac{1}{a\cdot b}+\frac{1}{a}Ce$$

where Ce is the ion concentration after adsorption (mg/L), *X*m/m is the mass of ions adsorbed per adsorbent mass (mg/g adsorbent), a is the maximum adsorption capacity (mg/g), and b is the Langmuir constant.

Antibacterial Test on Disc Diffusion

Inoculating the pure bacteria of *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) were prepared with a 103 CFU/mL concentration. A total of 20 mL of liquid Mueller Hinton agar (MHA) media was poured into a petri dish and then allowed to stand until agar was formed. A total of 1 mL of bacteria culture was spread evenly on the surface of the media. Paper discs with a diameter of 5 mm were dripped with 20 L of sterile distilled water, and 0.5 mg of copper oxide samples were added. The paper disc containing the sample was placed on the surface of the media at a certain distance, and the procedure was repeated three times. The plates were incubated at 37°C for 24 hours. The diameter of the inhibition zone for each sample was measured. The sterile distilled water blank was used as a comparison.

Analysis of scanning electron microscope

Samples were prepared and coated with gold using a Hitachi MC1000 instrument and then analyzed using a JSM-IT300LA SEM (EDS Detector JED-2300 Series) at 1000× and 5000× magnification.

Data analysis

The antibacterial data were the mean and standard deviation of the triple replications. The data was analyzed using one way ANOVA (analysis of variance) and continued with the Tukey test to identify differences in the test data. The difference was considered statistically significant if $p<0.05$.

RESULT AND DISCUSSION

Cu_2O Synthesis through Tollens-like Reaction

Synthesis of Cu_2O used a chemical reduction method by modifying the Tollens method. Tollens reagent, consisting of a solution of $AgNO_3$ in this study, was replaced by $Cu(NO_3)_2$, NaOH, and NH_4OH. The synthesis was started by mixing 10 mM $Cu(NO_3)_2$ solution with 0.4 M NaOH then stirred in which the mixture would turn into a dark blue suspension. This change indicates the formation of $Cu(OH)_2$ deposits (Xue *et al.*2016). The next step was adding 10 mM NH_4OH as a complexing agent to form a complex of $Cu(NH)^{2+}$ ions (Shenoy & Shetty 2012). The color of the mixture changed to light blue, and then the mixture was added with 37% sugar as a reducing agent and heated at 80°C for 10 minutes. The mixture's color will turn brown, indicating the formation of Cu_2O (Yu *et al.* 2015). The copper oxide formed in the solution was then rinsed using ethanol and distilled water. The neutral Cu_2O precipitate was then dried using an oven at 105 °C to obtain Cu_2O solids. A reducing sugar is a sugar that contains a free aldehyde or ketone group. Reducing sugars can donate electrons to electron-acceptor molecules. All monosaccharides, such as glucose and fructose, reduce sugars. Sucrose is a disaccharide and has no free aldehyde or ketone groups. Sucrose cannot be a reducing agent in metal oxide synthesis because it has a glycosidic bond that prevents the ring opening of glucose and fructose monomers (components of sucrose) (Filippo *et al.*2010). However, sucrose can become a reducing sugar through hydrolysis, breaking the glycosidic bond, producing glucose and fructose (Hurtado *et al.* 2016).

The presence of carbonyl groups, especially aldehydes, in the structure of glucose and ketones in fructose can facilitate the synthesis of metal oxides. Glucose is more easily oxidized than fructose due to the presence of a hydrogen atom in its primary group, so glucose is responsible for reducing metal ions to their metallic state (Naika *et al.* 2015). Glucose acts as a reducing agent that will form gluconic acid or gluconic acid salts in the metal oxide synthesis process (Agudelo *et al.* 2018). This study used a reducing agent of pure sucrose, white, and brown sugar. White sugar and brown sugar are sugars often used by the public. Each sugar is made up of a different combination of ingredients. White sugar and brown sugar contain different levels of sucrose. White sugar is made from sugarcane juice that has undergone a purification process. The purification process aims to remove molasses so that white sugar is obtained. Molasses are by-product of the sugar processing industry. Unlike white sugar, brown sugar still contains molasses. The presence of molasses causes the sugar to turn brown. Brown sugar is the result of processed sap. The sap used is derived from sugarcane, sap, or palm juice.

Copper oxide is a semiconductor material. The color of the light reflected, absorbed, transmitted, or emitted by a semiconductor material highly depends on the bandgap energy value. A material's chemical composition, structure, size, and shape affect bandgap energy values. Therefore, the resulting color variations indicate that materials synthesized using different reducing agents possess distinct chemical composition, structure, size, or shape. Usually, Cu_2O absorbs light in the wavelength range from UV to red (Markina *et al.*2016). Cu_2O is a semiconductor material whose bandgap energy depends on the size and shape of the crystal. The synthesis of Cu_2O in this study used a reducing agent of 37% sugar. The synthesis in various types of sugar gave different Cu_2O colors. Synthesis by reducing sucrose, white sugar, and brown sugar gave orange-brown, orange, and brown (Figure 1). It showed that Cu_2O produced by different reducing agents had different sizes and crystal shapes.

Different functional groups in reducing agents, such as ketones, aldehydes, hydroxyl, amine, and carboxyl, are responsible for reducing metal ions to metal atoms (Devi *et al.* 2014). Aldehyde groups and their concentration in reducing sugars are essential in forming metal oxide particles (Shukla & Iravani 2016). In general, carbohydrates can act as reducing agents due to carbonyl groups, which can lead to the formation of metal oxides with specific shapes. However, their specific influence depends on the presence of other functional groups. Different types of sugar affect the shape of metal oxide particles (Filippo *et al.* 2010). Sucrose, white sugar, and brown sugar have different functional groups. It causes differences in the growth of Cu_2O crystals, so the size and shape of the resulting crystals are different. Differences in the size and shape of Cu_2O crystals affect the bandgap energy values, resulting in different Cu_2O colors. The morphology of all Cu_2O synthesized with three sugars used as reducing agents needs to be further characterized to determine the size and shape of the crystals.

Figure 1 Cu_2O products with various types of reducing agents, sucrose (a), white sugar (b), and brown sugar (c).

Cu_2O/charcoal and Cu_2O/activated Charcoal Composites

This experiment synthesized Cu_2O composites with two charcoal types: activated charcoal and charcoal. Activated charcoal is made through carbonization and activation, while charcoal only goes through carbonization. The difference in the activation process causes differences in size, number of pores, surface area, and active side in charcoal. The activated charcoal that goes through an activation process has more surface area, active side, size, and number of pores than charcoal. This difference will affect the resulting composite. When added to charcoal or activated charcoal, copper oxide that was orange or brown formed a composite and changed color to black-brown (Figure 2).

Charcoal and Activated Charcoal Specific Surface Area

The specific surface area is the area covered by a solid surface per unit of material. The surface area of the adsorbent is one of the critical factors that affect the adsorption ability. The larger surface area possesses

Figure 2 Cu_2O composite with activated charcoal (1) and charcoal (2) reducing variations, sucrose (a), white sugar (b), and brown sugar (c).

more active adsorbent sites to interact with the adsorbate, which is material that gets adsorbed. The adsorption ability of an adsorbent can be determined by comparing its surface area. This study used methylene blue dye to determine the surface area of the adsorbent. The methylene blue adsorption method in the liquid phase to determine specific surface area has been carried out on various solids such as charcoal, graphite, and silica. The adsorbents used in this study were charcoal and activated charcoal. Methylene blue (MB) is an organic cationic dye with the molecular formula $C_{16}H_{18}ClN_3S$. The methylene blue molecule has an area of about 130–135 $Å^2$. The adsorbed methylene blue molecule must follow the Langmuir isotherm model, assuming only one layer is formed. The number of adsorbed methylene blue molecules can be used to determine the specific surface area (Itodo *et al.* 2010). Based on the calculations, the charcoal had a surface area of 30, and the activated charcoal was 720 m^2/g (Table 1). The activated charcoal has a larger surface area than charcoal. It is because activated charcoal goes through an activation process, while charcoal does not. The activation process will open the pores of the activated charcoal so that the activated charcoal's surface area increases and the adsorbent's adsorption capacity increases. The larger the surface area of the adsorbent, the larger the contact area for the adsorbate will be so that more adsorbates can be adsorbed.

Adsorption Isotherm

The adsorption isotherm shows the distribution of molecules between the liquid and solid phases. Solid-liquid phase adsorption generally refers to the isotherm type of Freundlich and Langmuir (Hameed *et al.* 2007). Langmuir isotherm model shows that monolayer adsorption occurs on a homogeneous surface. The Langmuir model assumes that the adsorbent has a homogeneous structure, and that the monolayer adsorption process occurs with uniform adsorption energy. The Langmuir adsorption model uses a linear relationship equation between equilibrium concentration (Ce) and equilibrium concentration per adsorption capacity (Ce/Qe). The Freundlich isotherm assumes that the surface energy is heterogeneous. Freundlich's model uses a linear equation that relates log Ce to log Qe (Xiong *et al.* 2017). The analysis showed that the charcoal and activated charcoal followed the Langmuir isotherm type. It is indicated by the constant value and coefficient of determination of the Langmuir isotherm, which was higher than that of the Freundlich isotherm (Table 2). It showed that the adsorbent used in this study has a homogeneous surface (Sahara *et al.*2017). The maximum adsorption capacity of activated charcoal was higher than that of charcoal. The maximum adsorption capacity of activated charcoal was 294.118 mg/g, while that of charcoal was only 12.107 mg/g.

Antibacterial Activity

Gram-positive and Gram-negative bacteria have a negatively charged surface (Slavin *et al.* 2017). Gram-positive bacteria have a thick peptidoglycan layer formed by alternating linear chains of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) residues linked together by crosslinked 3 to 5 amino acid sequences. The negative charge on Gram-positive bacteria comes from the phosphate group on lipoteichoic acid on the surface of the bacteria. Gram-negative bacteria have a slightly more complex structure. Gram-negative bacteria have a thin peptidoglycan layer and a phospholipid outer membrane in lipopolysaccharide (LPS), increasing the negative charge on the bacteria's surface (Lopez *et al.* 2020). The negatively charged bacteria cell wall attracts positively charged metal particles to its surface due to electrostatic interactions. Copper oxide can significantly inhibit bacteria growth because it can directly contact the surface of bacteria cells. Positively charged copper-based particles form strong bonds with bacteria cell membranes (Letchumanan *et al.*2021). It causes the permeability of the bacteria cell membrane to increase. Cells will rupture, and intracellular components will leak. Leakage of intracellular components causes shrinkage of the bacteria cell membrane (Li *et al.* 2015). Disturbances in the bacteria cell membrane can open or close channels to the cell membrane. It damages membrane proteins and lipid bilayers, disrupting cell metabolism (Matai *et al.* 2014). The interaction of bacteria with Cu_2O also causes changes in bacteria cells from regular to irregular shapes (Raffi *et al.* 2010).

Copper oxide and its composites have better antibacterial activity against *E. coli* bacteria than *S. aureus*. It was indicated by the value of the inhibition zone and arbitrary unit of *E. coli* (Table 3), which was higher than that of *S. aureus* (Table 4). The same result was also shown by Hong *et al.* (2018), that Cu_2O can

Table 1 Surface area of charcoal and activated charcoal

Sample	Surface area (m^2/g)
Charcoal	30
Activated charcoal	720

Table 2 Constant value and linearity of charcoal and activated charcoal adsorption isotherm

Sample	Freundlich		Langmuir	
	k (mg/g)	R2	a (mg/g)	R2
Charcoal	3.539	0.717	12.107	0.999
Activated charcoal	2.447	0.760	294.118	0.999

Table 3 Effect of reducing agent on the antibacterial activity of *E. coli*

Sample	*E. coli*	
	Inhibitory Unit (mm)	AU (mg^{-1})
Cu_2O (1)	6.65 ± 0.16 ^{b}	30.14 ± 3.38 ^{b}
Cu_2O (2)	8.26 ± 0.19 ^{a}	67.82 ± 4.99 ^{a}
Cu_2O (3)	6.69 ± 0.01 ^{b}	31.09 ± 0.23 ^{b}
Cu_2O (1)/activated charcoal	6.23 ± 0.03 ^{d}	21.62 ± 0.49 ^{c,d}
Cu_2O (2)/ activated charcoal	6.47 ± 0.05 ^{b,c}	26.54 ± 0.94^{b,c}
Cu_2O (3)/ activated charcoal	6.33 ± 0.06 ^{c,d}	23.73 ± 1.15 ^{c,d}
Cu_2O (1)/charcoal	5.69 ± 0.02 ^{e}	11.58 ± 0.31 ^{e}
Cu_2O (2)/charcoal	6.23 ± 0.06 ^{c,d}	21.76± 1.13^{c,d}
Cu_2O (3)/charcoal	6.09 ± 0.03 ^{d}	19.04± 0.62^{d}
Activated charcoal	5.07 ± 0.03 ^{f}	1.06 ± 0.46^{f}
Charcoal	5.04 ± 0.01^{f}	0.58 ± 0.18 ^{f}

Descriptions: *Numbers in the same column and row followed by different letters are significantly different (p<0.05).

Table 4 Effect of reducing agent on the antibacterial activity of *S. aureus*

Sample	*S. aureus*	
	Inhibitory Unit (mm)	AU (mg^{-1})
Cu_2O (1)	6.12 ± 0.03 ^{b,c}	19.49 ± 0.56 ^{b,c}
Cu_2O (2)	6.76 ± 0.05 ^{a}	32.43 ± 1.09 ^{a}
Cu_2O (3)	6.13 ± 0.10 ^{b,c}	19.76 ± 1.92 ^{b,c}
Cu_2O (1)/activated charcoal	6.08 ± 0.03 ^{b,c}	18.85 ± 0.55 ^{b,c}
Cu_2O (2)/ activated charcoal	6.22 ± 0.04 ^{b}	21.43 ± 0.81 ^{b}
Cu_2O (3)/ activated charcoal	6.10 ± 0.04 ^{b,c}	19.17 ± 0.83 ^{b,c}
Cu_2O (1)/charcoal	5.41 ± 0.02 ^{e}	6.70 ± 0.30 ^{e}
Cu_2O (2)/charcoal	6.04 ± 0.05 ^{c}	18.09 ± 0.97 ^{c}
Cu_2O (3)/charcoal	5.62 ± 0.10 ^{d}	10.29 ± 1.70 ^{d}
Activated charcoal	5.07 ± 0.03 ^{f}	1.06 ± 0.46 ^{f}
Charcoal	5.00 ± 0.00 ^{f}	0.00 ± 0.00 ^{f}

Descriptions: *Numbers in the same column and row followed by different letters are significantly different (p<0.05).

inhibit *E. coli* better than *S. aureus*. The peptidoglycan layer of *E. coli*, a Gram-negative bacterium, is thinner than that of *S. aureus* (a Gram-positive bacteria). Copper oxide is easier to damage and penetrate the outside of *E. coli*, so the antibacterial activity of Cu_2O on *E. coli* is higher. The thick peptidoglycan layer of *S. aureus* makes it difficult for Cu_2O to penetrate the outside of the bacteria, making it more difficult to enter the internal bacteria organelles. Based on this, Cu_2O is more effective in inhibiting *E. coli*, a major pathogen associated with waterborne diseases. *E. coli* in food or wa ter indicates poor food or water processing hygiene. *E. coli* contamination can cause gastrointestinal infections, such as stomach cramps, vomiting, and diarrhea (Odonkor & Mahani 2020). The resulting copper oxide can be used in water treatment processes. Copper oxide will kill *E. coli*, improving water quality.

Different types of reducing agents and adding activated charcoal and charcoal as composites resulted in different antibacterial activities. The order of antibacterial from the best, namely Cu_2O (2), Cu_2O (3), Cu_2O (1), Cu_2O (2)/activated charcoal, Cu_2O (3)/activated, Cu_2O (1)/activated charcoal, Cu_2O (2)/charcoal, Cu_2O (3)/charcoal, and Cu_2O (1)/charcoal. Copper oxide with white sugar reducer gave the best inhibition zone and activity unit. The best order of Cu_2O antibacterial was based on the reducing sugars: white sugar, brown sugar, and sucrose. Different types of sugars have varying reduction potentials due to differences in their chemical structures and impurities. Brown sugar, which contains molasses, may have a higher reducing potential than refined white sugar. This difference in reducing potential can influence the rate and extent of reduction reactions. Furthermore, the presence of impurities in sugar can act as nucleation sites or surface modifiers during metal oxide crystal growth. That may affect the nucleation kinetics of the resulting Cu_2O crystals, so the size and shape of the Cu_2O crystals produced are different. The smaller size of metal oxide particles will increase the size of the surface area. The large surface area increases the interaction of metal oxides with bacteria, increasing the antibacterial activity. On the other hand, the large metal oxide particle size causes the surface area to decrease so that the antibacterial activity decreases (Slavin *et al.* 2017).

The composites produced lower antibacterial activity than Cu_2O. Cu_2O/activated charcoal composites were better than Cu_2O/charcoal, while the activated charcoal and charcoal produced the lowest antibacterial activity. Bacteria have different strengths when attached to different types of charcoal. The difference is due to the number of active sites in the charcoal due to the interaction with the outer layer of the bacteria surface (Karnib *et al.* 2013). Bacteria attach to the activated charcoal particles through strong van der Waals forces between the bacteria and the activated charcoal surface (Akasaka & Watari 2009). The number of metal oxide particles in charcoal also affects the antibacterial activity. The more metal oxides in activated charcoal, the higher the antibacterial ability (Zhao *et al.* 2013).

Through activation, activated charcoal has a larger surface area, active site, size, and number of pores than charcoal. Based on this, activated charcoal can interact with Cu_2O and more bacteria. The higher the amount of Cu_2O, the higher the antibacterial activity. On the other hand, charcoal, without an activation process, has a smaller surface area, active site, size, and number of pores. Thus, the number of bacteria and Cu_2O caught was less, so the antibacterial activity was lower than that of activated charcoal. Metal particles can also release metal ions from the extracellular space, be able to enter cells, and interfere with biological processes. Metal ions can induce the production of reactive oxygen species (ROS). Under normal conditions, bacteria have defense mechanisms against ROS, such as glutathione, superoxide dismutase, and catalase, which act as antioxidant enzymes and remove ROS. High concentrations of metal ions result in extreme levels of oxidative stress, although antioxidant enzymes can remove some metal ions. It is not sufficient to neutralize the number of metal particles that enter. The resulting oxidative stress causes glutathione oxidation, suppressing the antioxidant defense mechanisms of bacteria against ROS. Metal ions entering bacteria cells are free to interact with cellular structures, such as proteins, membranes, and DNA, so cell function is disrupted (Stensberg *et al.* 2011).

Analysis of Scanning Electron Microscope

SEM analysis showed that the synthesized Cu_2O morphology was spherical and some were octahedral (Figure 3a). Figure 3b shows the morphology of activated charcoal has small pores, while charcoal has large pores (Figure 3c). The number of pores in activated charcoal is more than charcoal because activated charcoal is prepared through an activation process that causes the hydrocarbon bonds to break and the molecules on the surface are oxidized. This causes there are physical and chemical changes so that the number of active sites and surface area of activated charcoal increases (Ajayi & Olawale 2009).

Activated charcoal or charcoal combined with Cu_2O will form a composite. The morphology of the activated charcoal/Cu_2O composite differed from that of charcoal/Cu_2O because the morphology and surface structure of the precursors were different. The interaction of charcoal or activated charcoal with Cu_2O is affected by the surface area, active site, and pore size. The large surface area, active site, and pore size will increase the amount of Cu_2O that sticks to and occupies the pores of charcoal or activated charcoal (Shu *et al.* 2017). Figures 3d and 3e show that spherical Cu_2O molecules scattered randomly occupy the pores and surfaces of charcoal and activated charcoal. The amount of Cu_2O attached to activated charcoal is more than charcoal because the number of pores and the active side of activated charcoal is much more.

CONCLUSIONS

Copper oxide (Cu_2O) and composites of Cu_2O/charcoal, Cu_2O/activated charcoal can be synthesized by the Tollens-like reaction method.

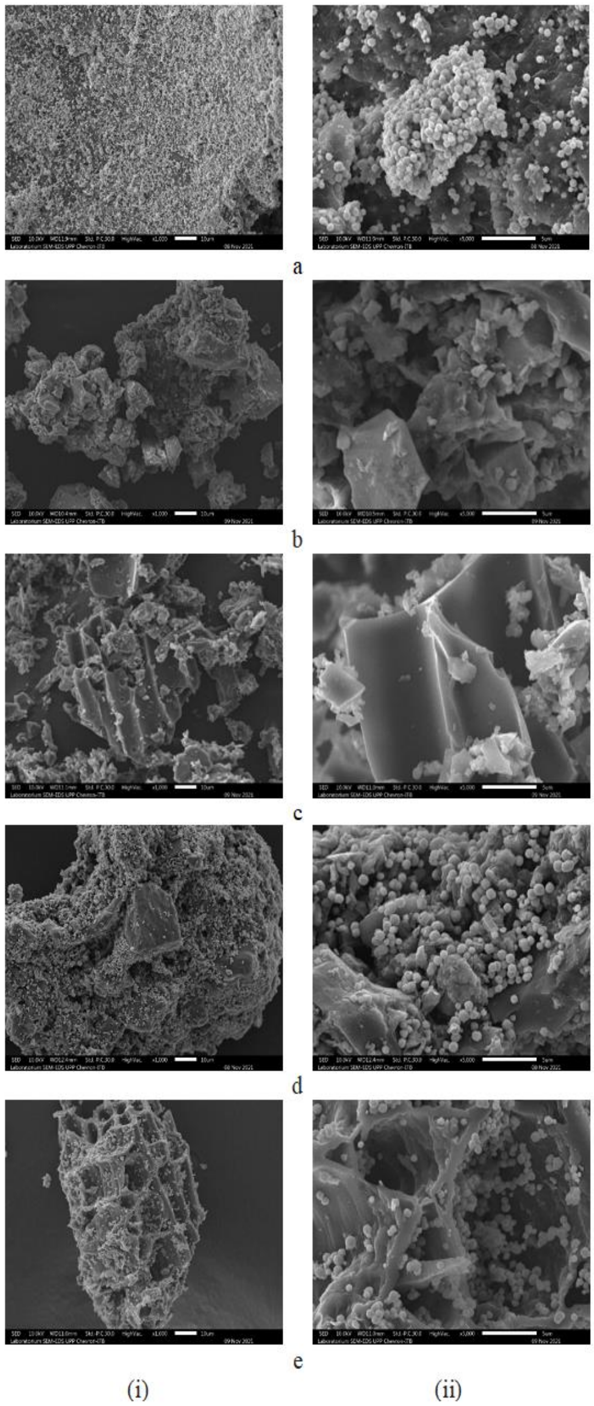

Figure 3 Cu_2O (a), activated charcoal (b), charcoal (c), Cu_2O/activated charcoal (d), Cu_2O/charcoal (e) at 1000X (i) and 5000X (ii).

Sucrose, white sugar, and brown sugar can be used in supporting environmentally friendly agents. Various in reducing agents affect the morphology of Cu_2O. Differences in preparing charcoal and activated charcoal affect the surface area, pores, and active sites. The activated charcoal can capture more Cu_2O than charcoal. Copper oxide and the resulting composite have higher antibacterial properties in *E. coli* than *S. aureus*. The best antibacterial activity is the Cu_2O (2).

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REFERENCES

Agudelo W, Montoya Y, Bustamante J. 2018. Using a non-reducing sugar in the green synthesis of gold and silver nanoparticles by chemical reduction method. *DYNA.* 85(206): 69–78. https://doi.org/10.15446/dyna.v85n206.72136

Allaker RP, Memarzadeh K. 2014. Nanoparticles and the control of oral infections. *Journal Antimicrobial Agents.* 43(2): 95–104. https://doi.org/10.1016/j.ijantimicag.2013.11.002

Akasaka T, Watari F. 2009. Capture of bacteria by flexible carbon nanotubes. *Acta Biomaterialia.* 5(2): 607–612. https://doi.org/10.1016/j.actbio.2008.08.014

Bezza FA, Tichapondwa SM, Chirwa EMN. 2020. Fabrication of monodispersed copper oxide nanoparticles with potential application as antimicrobial agents. *Scientific Reports*. 10: 1–18. https://doi.org/10.1038/s41598-020-73497-z

Devi RB, Sharma NHK, Radhapiyari W, Brajakishor C. 2014. Green synthesis, characterization and antimicrobial properties of silver nanowires by aqueous leaf extract of piper betle, *International Journal of Pharmaceutical Sciences Review and Research.* 26: 9–13.

Fan W, Wang X, Cui M, Wang X, Zhang D, Liu H, Guo L. 2012. Differential oxidative stress of octahedral and cubic Cu_2O micro/nanocrystals to *Daphnia magna. Environmental Science and Technology*. 46(18): 55–62. https://doi.org/10.1021/es3011578

Filippo E, Serra A, Buccolieri A, Manno D. 2010. Green synthesis of silver nanoparticles with sucrose and maltose: morphological and structural characterization. *Journal of Non-Crystalline Solids.* 356(6–8): 344–350. https://doi.org/10.1016/j.jnoncrysol.2009.11.021

Hurtado RB, Valadez MC, Rodriguez LPR, Rodriguez EL, Alvarez RAB, Rocha OR, Beleno YD, Nunez CEM, Chavez HA, *et al.* 2016. Instant synthesis of gold nanoparticles at room temperature and SERS applications. *Physics Letter A.* 380(34): 1–6.

Gawande MB, Goswami A, Felpin FX, Asefa T, Huang X, Silva R, Zou X, Zboril R, Varma S. 2018. Cu and Cu-based nanoparticles: synthesis and applications in catalysis. *Chemical Reviews*. 116(6): 3722–3811. https://doi.org/10.1021/acs.chemrev.5b00482

Gopalakhrisnan K, Ramesh C, Ragunathan V, Thamilselvan M. 2012. Antibacterial activity of Cu_2O nanoparticles on *E. coli* synthesized from *Tridax procumbens* leaf extract and surface coating with polyaniline. *Journal of Nanomaterials and Biostructures.* 7(2): 833–839.

Hameed BH, Ahmad AL, Latiff KNA. 2007. Adsorption of basic dye (methylene blue) onto activated carbon prepared from rattan sawdust. *Dyes and Pigments*. 75(1): 143–149. https://doi.org/10.1016/j.dyepig.2006.05.039

Hemmati S, Roberts ER, Scott C, Harris MT. 2018. Artificial sweeteners and sugar ingredients as reducing agents for green synthesis of silver nanoparticles. *Journal of Nanomaterials*. 2019: 1–16. https://doi.org/10.1155/2019/9641860

Hong D, Cao G, Qu J, Deng Y, Tang J. 2018. Antibacterial activity of Cu_2O and Ag co-modified rice grains-like ZnO nanocomposite. *Journal of Materials Science and Technology*. 34(12): 2359–2367. https://doi.org/10.1016/j.jmst.2018.06.011

Itodo AU, Itodo HU, Gafar MK. 2010. Estimation of spesific surface area using langmuir isoterm method. *Journal Applied Science and Environmental Management*. 14(4): 141–45. https://doi.org/10.4314/jasem.v14i4.63287

Karnib M, Holail H, Olama Z, Kabbani A, Hines M. 2013. The antibacterial activity of activated carbon, silver, silver impregnated activated carbon and silica sand nanoparticles against pathogenic *E. coli* BL21. *International Journal of Current Microbiology and Applied Sciences.* 2(4): 20–30.

Kiaune L, Singhasemanon S. 2011. Pesticidal copper (I) oxide: environmental fate and aquatic toxicity. *Environmental Contamination Toxicology*. 213: 1–26. https://doi.org/10.1007/978-1-4419-9860-6_1

Lopez ES, Gomez D, Esteruelas G, Bonilla L, Machado ALL Galindo R, Cano A, Espina M, Ettcheto M, Camins A, Silva AM, Durazzo A, Santini A, Garcia ML, Souto EB. 2020. Metal-based nanoparticles as antimicrobial agents: an overview. *Nanomaterials.*

10(292): 1–39. https://doi.org/10.3390/nano10020292

Letchumanan D, Sok SPM, Ibrahim S, Nagoor NH, Arshad NM. 2021. Plant-based biosynthesis of copper/copper oxide nanoparticles: an update on their application in biomedicine, mechanism, and toxicity. *Biomolecules.*11(564): 1–28. https://doi.org/10.3390/biom11040564

Li Y, Cai R, Lu R, Gao L, Qin S. 2018. Template synthesis of the Cu_2O nanoparticle-doped hollow carbon nanofibers and their application as a non-enzymatic glucose biosensors. *Royal Society Open Sciences*. 5: 1–12. https://doi.org/10.1098/rsos.181474

Markina NE, Pozharov MV, Markin AV. 2016. Synthesis of copper (I) oxide particles with variable color: demonstrating size-dependent optical properties for high school students. *Journal of Chemical Education.* 93(4): 704–707. https://doi.org/10.1021/acs.jchemed.5b00563

Matai I, Sachdev A, Dubey P, Kumar SU, Bhushan, Gopinath P. 2014. Antibacterial activity and mechanism of Ag-ZnO nanocomposite on *S. aureus* and GFP-expressing antibiotic resistant *E. coli. Colloids and Surface B: Biointerfaces.* 115: 59–67. https://doi.org/10.1016/j.colsurfb.2013.12.005

Naika HR, Lingaraju K, Manjunath K, Kumar D, Nagaraju G, Suresh D, Nagabhushana H. 2015. Green synthesis of CuO nanoparticles using *Gloriosa superba L*. extract and their antibacterial activity. *Journal Taibah University for Science.* 9(1): 7–12. https://doi.org/10.1016/j.jtusci.2014.04.006

Odonkor ST, Mahami T. 2020. *Eschericia coli* as a tool for disease risk assessment of drinking water sources. *International Journal of Microbiology.* 1–7. https://doi.org/10.1155/2020/2534130

Okeola OF, Odebunmi EO, Ameen OM. 2012. Comparison of sorption capacity and surface area of activated carbon prepared from *Jatopha curcas* fruit pericarp and seed coat. *Bulletin of the Chemical Society of Ethiopia*. 26(2):71–80. https://doi.org/10.4314/bcse.v26i2.2

Raffi M, Mehrwan S, Bhatti TM, Akhter JI, Hameed AH, Yawar W, Hasan MMU. 2010. Investigation into the antibacterial behavior of copper nanoparticles against *Escherichia coli. Annals of Microbiology.* 60(1): 75–80. https://doi.org/10.1007/s13213-010-0015-6

Sahara E, Kartini NPW, Sibarani J. 2017. Pemanfaatan arang aktif dari limbah tanaman gumitir (*Tagetes erecta*) teraktivasi asam fosfat sebagai adsorben ion Pb^{2+} dan Cu^{2+} dalam larutan. *Cakra Kimia.* 5(2): 67–74.

Shenoy US, Shetty AN. 2014. Simple glucose reduction route for one-step synthesis of copper nanofluids. *Applied Nanoscience*. 4(1): 47–54. https://doi.org/10.1007/s13204-012-0169-6

Shukla AK, Iravani S. 2016. Green synthesis and spectroscopic characterization of nanoparticles. *Nanoscience in Food and Agriculture*. 1: 65–99. https://doi.org/10.1007/978-3-319-39303-2_3

Slavin YN, Asnis J, Hafeli UO, Bach H. 2017. Metal nanoparticles: understanding the mechanism behind antibacterial activity. *Journal of Nanobiotechnology.* 15(65): 1–20. https://doi.org/10.1186/s12951-017-0308-z

Stensberg MC, Wei Q, Mclamore E, Porterfield DM. 2011. Toxicology studies on silver nanoparticles: challenges and oportunities in assessment, monitoring and imaging. *Nanomedicine.* (5): 79–98. https://doi.org/10.2217/nnm.11.78

Xiong L, Tong ZH, Chen JJ, Li LL, Yu H.Q. 2015. Morphology-dependent antimicrobial activity of Cu/Cu_xO nanoparticles. *Ecotoxicology.* 24(10): 67–72. https://doi.org/10.1007/s10646-015-1554-1

Xiong W, Tong J, Yang Z, Zeng G, Zhou Y, Wang D, Song P, Xu R. Zhang C, Cheng M. 2017. Adsorption of phosphate from aqueous solution using iron-zirconium modified activated carbon nanofiber: Performance and mechanism. *Journal of Colloid and Interface Science.* 493: 17–23. https://doi.org/10.1016/j.jcis.2017.01.024

Xue Z, Li M, Rao H, Yin B, Liu X, Lu X. 2016. Phase transformation-controlled synthesis of CuO nanostructure and their application as an improved material in a carbon-based modified electrode. *RSC Advances.* 6:29–36. https://doi.org/10.1039/C5RA22297D

Yadav S, Chauhan M, Mathur D, Jain A, Malhotra P. 2020. Sugarcane bagasse-facilitated benign synthesis of Cu_2O nanoparticles and its role in photocatalytic degradation of toxic dyes: a trash to treasure approach. *Environtment, Development and Sustainability.* 23(3): 1–14. https://doi.org/10.1007/s10668-020-00664-7

Yu R, Jun J, Kim Y. 2015. Synthesis and characterization of SiO_2, SnO_2, and TiO_2 metal oxide shells covering Cu_2O particles. *Archives of Metallurgy and Materials.* 60(2): 59–63. https://doi.org/10.1515/amm-2015-0089

Zhao Y, Wang ZQ, Zhao X, Li W, Liu SX. 2013. Antibacterial action of silver-doped activated carbon prepared by vacuum impregnation. *Applied Surface Science.* 266: 67–72. https://doi.org/10.1016/j.apsusc.2012.11.084