

SILVER NANOPARTICLE IMPREGNATED ON THE COMPOSITE OF BACTERIAL CELLULOSE-CHITOSAN-GLYCEROL AS ANTIBACTERIAL MATERIAL

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Abstract

The objective of this research were to study the characteristic of silver nanoparticles, the characteristic of silver nanoparticle impregnated on the bacterial cellulose, and the effect of silver nanoparticles toward antibacterial activity of bacterial cellulose and its composites. Bacterial cellulose of rice wastewater is fermented by *Acetobacter xylinum* for 7 days. The water containing bacterial cellulose is removed by heating in the oven. Bacterial cellulose, bacterial cellulose-chitosan composites and bacterial cellulose-chitosan-glycerol composites had been prepared by immersing dry bacterial cellulose in chitosan and glycerol solution. The silver nanoparticles was prepared by chemical reduction with using silver nitrate solution and tri-sodium citric as reducing agent. UV-Vis spectrometry indicated formation of nanoparticles. To achieve antibacterial activity, silver nanoparticles were impregnated into bacterial cellulose-chitosan-glycerol composites by immersing method. Morphology of nanoparticles impregnated in bacterial cellulose-chitosan-glycerol composites was examined by SEM. The antibacterial activity of silver nanoparticles which was impregnated on the bacterial cellulose-chitosan-glycerol was examined by shake flask turbidimetry and clear zone method. The UV-Vis spectroscopy revealed the formation of silver nanoparticles by exhibiting the typical surface plasmon absorption maximum at 419 nm. The SEM image showed that the silver nanoparticles has been impregnated in the surface of bacterial cellulose. The nanoparticles of silver-impregnated bacterial cellulose-chitosan-glycerol composites exhibited antibacterial activity against *S.aureus* and *E.coli*.

Key words: antibacterial activity, bacterial cellulose, bacterial cellulose-chitosan, bacterial cellulose-chitosan-glycerol composite, silver nanoparticles.

INTRODUCTION

Nanoparticles are particles that have dimensions less than 100 nanometers. The surface area per unit weight nanoparticles is greater than the width of the particles. This nanoparticle is more reactive than some other molecule. Silver nanoparticles have been shown to have good ability as the anti- microbial against bacteria, viruses and microorganisms eukaryotic structural. Colloidal silver is particularly interesting because it has unique properties and a good conductivity material, chemically stable, can act as a catalyst, and has an antibacterial activity. In low concentrations of silver is not toxic to human cells [1].

Colloidal silver has long been known as an antibacterial. The shape and size of nanoparticles is critical in determining the optical properties, electrical, magnetic, catalytic, and antimicrobial. The smaller size will parikel greater antimicrobial effect . Factors that affect the size of the particles in the synthesis is the temperature, reducing agents, concentration and

reaction time. Studies have shown that the particle size, morphology, stability and properties of metal nanoparticles is influenced by the operating conditions of the experiment, the kinetics of the interaction among the metal ions, reductor, and the absorption of stabilizer [2].

Cellulose is the most abundant natural biopolymer on the earth, synthesized by plants, algae, and also some species of bacteria [3]. Bacteria also synthesized cellulose, including the genera *Agrobacterium*, *Rhizobium*, *Pseudomonas*, *Sarcina*, and *Acetobacter* [15]. Bacterial cellulose is produced by *Acetobacter xylinum* is markedly different from cellulose obtained from trees and cotton. From the culture medium a pure cellulose network free of lignin and hemicellulose is obtained as a highly hydrated pellicle made up of a random assembly of ribbon shaped fibers less than 100 nm wide. These fibers themselves are composed of a bundle of much finer microfibrils of nanometric size [3, 4]. The unique properties provided by the nanometric structure have lead to a number of commercial products including tires, headphone membranes, special papers, and textiles [4], medical applications including temporary skin substitution [4, 7].

Unlike cellulose from plants, bacterial cellulose (BC) is chemically pure and free of lignin and hemicelluloses. Bacterial cellulose does not require remedial processing to remove unwanted polymers and contaminants, and therefore, retains a greater degree of polymerization [3]. BC has high crystallinity, 2 common crystalline forms of cellulose, designated as I and II. Cellulose I is synthesized by the majority of plants and also by *A. xylinum* in static culture, parallel β -1,4-glucan chains are arranged uniaxially. Whereas β -1,4-glucan chains of cellulose II are arranged in a random manner. They are mostly antiparallel and linked with a larger number of hydrogen bonds that results in higher thermodynamic stability of the cellulose II. [1, 4] Bacterial cellulose is also used as a source of dietary fiber (nata-de-coco) [2], as binding or thickening agents. Interesting composite materials may also be obtained from cellulose. The water uptake depends on the crystalline state of cellulose and the amount of grafted acrylic counterpart [2]. Cellulose phosphate membranes were also studied concerning their biomedical applications. Phosphorylated cellulose seems to be interesting material for bone regeneration and osteointegration.

Bacterial cellulose has unique structural and mechanical properties as compared to higher plant cellulose, BC is expected to become a commodity material in various fields. The BC fibers have a high aspect ratio with a diameter of 20-100 nm. As a result, BC has a very high surface area per unit mass. This property, combined with its highly hydrophilic nature, results in a very high liquid loading capacity. Moreover, biocompatibility makes it an attractive candidate for a wide range of applications in different fields, especially those related to biomedical and biotechnology applications [5]. However, in most practical applications, BC may not be of perfect quality and its cost may not be suitable for industrialization either. For economical mass production, it is essential to design a culture aeration and agitation process [10].

Media that can be used as a place to live bacteria is coconut water, wastewater of cassava and rice wastewater and other sources. Rice wastewater is household waste which still contains starch and other compounds content carried by water during the process of washing rice [18], so that the rice wastewater can be used as a medium of live bacteria *acetobacter xylinum*. The use of rice wastewater is an effort to add value to the household waste as materials for natural polymers that can be degraded in the soil so it does not pollute the environment .

Bacterial cellulose had structure linear, highly crystalline, and degree of intermolecular hydrogen bonding is very high, so that many bacterial cellulose had be applied in papermaking, textile, and food industries as cosmetics and biomaterials in the medical field [16]

In the medical field, bacterial cellulose is used as a wound covering material and other medical materials. Antibacterial properties needed to maximize the usefulness of cellulose as a wound covering material because it can reduce bacterial contamination on cellulose and on wounds . The antibacterial properties can be obtained by using a cellulose material deposited

silver nanoparticles. Nanoparticles of silver have been known as an antibacterial material because of its ability to make the bacterial cell wall lysis and bacterial death [1]. In addition to the silver nanoparticles can serve as a barrier to the growth of mold and bacteria that often occurs in cellulose. Research on the antibacterial properties of silver nanoparticles and silver nanopartikel applications in the field of medical and textiles have been carried out [17]. the use of silver nanopartikel have an advantage because they are toxic to microbes but is safe for humans in low concentrations. With the presence of silver nanoparticles that are antibacterial, wound closure will be effective to protect the wound and reduce the occurrence of infections. Preparation of antibacterial cellulose can be combined with several materials to improve the antibacterial properties and mechanical properties of the cellulose. In addition to the deposition of nanoparticles, the antibacterial properties of the cellulose can be obtained with the addition of the chitosan material. Chitosan has long been known as an antibacterial polymer material [9] that are widely applied in the fields of medical and food preservation. The addition of chitosan on cellulose aims to increase the antibacterial properties of the cellulose, cellulose - chitosan composite nanoparticles deposited silver is expected to provide optimal antibacterial properties. There are shortcomings in the manufacture of cellulose - chitosan composite is. The addition of chitosan to cellulose will reduce the mechanical properties of cellulose and antibacterial properties of chitosan itself [10].

Mechanical properties are reduced due to the addition of chitosan on cellulose materials can be overcome with the addition of plasticizers such as glycerol. Glycerol is used as a plasticizer to improve the profile of alginate and chitosan films, preserving the integrity, avoiding holes and cracks [13]. Glycerol was added to a certain concentration to obtain the structure of cellulose are quite elastic so that it can be easily applied as a wound covering material.

In this paper, we focus on preparing bacterial cellulose membranes from rice wastewater with addition chitosan and a plasticizer. Rice wastewater still contains nutrient which needed *Acetobacter xylinum* in synthesizing bacterial cellulose. In this work, new composite membranes were prepared from rice wastewater and chitosan. Their characterization have been done using scanning electron microscopy, X-ray diffraction, tensile tester, FTIR, and antibacterial activity.

RESEARCH METHOD

Preparation of Bacterial Cellulose and Its Composites

20 grams of sucrose, 1 gram of urea, and 1,0 gram glycerol were mixed in 200 mL rice wastewater. Rice wastewater was poured into Erlenmeyer that has been equipped with a magnetic stirrer, then stirred until dissolved. When the pH of the mixture solution was ranged between 5-6, the mixture was acidified by addition of glacial acetic acid to a pH range from 3-4. Subsequently the mixture was cooled briefly and then poured in a warm state into a tray that has been sterilized with alcohol cooled to room temperature is reached. After chilling the mixture was added 40 mL of *Acetobacter xylinum* and trays sealed properly using newspaper and fermented for 7 days at room temperature. After 7 days, the newspaper cover is opened and formed pellicle layer was taken and washed several times with tap water, then with distilled water, then with hot water, then this pellicle layer weighed. Then a solution of 2% chitosan with deacetylation degree of 73.78% is poured onto the pellicle layer and dried in an oven with a temperature between 37- 40 ° C.

Application of Silver Nanoparticle to Bacterial Cellulose and its Composites

100 mL of AgNO_3 (aq) 1×10^{-3} M is put into the three neck flask and mixed with 0.5 grams of PVA and than refluxed at 100 °C by stirring. Tri-sodium citric is added until reach pale yellow color. The silver nanoparticle is characterized by using uv-vis spectrophotometer.

Bacterial cellulose and its composite is impregnated with silver nanoparticle over 60 minutes at 145 rpm.

Characterization of Bacterial Cellulose and Its Composites

Analysis of the physical properties of macroscopic and organoleptic. This analysis includes the observation of color, texture, shape and transparency of each sample.

Analysis of functional group using FT-IR instrument. This analysis uses a set of tools FTIR and performed at the Laboratory of Organic Chemistry of the Faculty of Mathematics and Science, UGM. A thin layer is clamped in place and then put the device in the direction of the infrared beam. The result will be recorded onto paper in the form of the intensity of wave numbers.

Analysis of morphological using SEM instrument. SEM image of bacterial cellulose and its composites is measured using SEM instrument. This test is performed in Laboratory at the Center for Borobudur Conservation Agency. Sample is cut in such a way, then the is coated with ion coater apparatus for approximately 5 minutes before vacuum process. Sample is introduced into the electron gun. Then the sample set with microstage to get the right focus. The main key in the ON position and set Accelerate voltage detector sets, 20 kilo volts.

Analysis of mechanical properties. The analysis was performed in Laboratory of Biotechnology, Faculty of Agricultural Technology, UGM. The dried sample material is cut to the size of 11 cm x 2 cm. Results pieces/specimen put in dumbbelle cutting. Then do the appropriate cutting pattern formed on the sample. Then the sample was measured with a micrometer Mitutoyo thickness and both sides of the cutting result is then attached to a Universal Testing Machine. Power and panel in the ON position. Fill the sample data fit standard size. Specification tool used is taking a knife to the ASTM standard. Then the device is turned on and set to the test speed = 10 mm/min and the specimens were observed to drop out, the test is stopped when the specimen is broken. Data obtained in the form of percent elongation, tensile strength, and F max.

Analysis of thermal properties using Differential Thermal Analysis (DTA). Thermal properties is performed in ATK Yogyakarta. As much as ± 15 mg samples were put into the crucible where the sample that had previously been filled little by standard alumina manufactured by Perkin-Elmer DTA-TGA. Then loaded alumina is also standard that has been put into the crucible and placed on the reference in the DTA-TGA analyzer tool and the weight of the instrument is calibrated using a reference sample in order to obtain the desired weight. Then the condition of temperature measuring devices operated at 30-400^o C with heating rate 10 ^oC per minute and appliance turned on. On the computer, set to a program to read DTA thermogram.

Analysis of crystallinity by X-Ray Diffraction (XRD). Crystallinity was analyzed by using XRD instruments in Department of Geological Engineering, UGM. Samples are cut to size sheet 2x2 cm². The sample is then placed in the sample holder and the sample flat on the cultivated sample holder. Further cooling equipment XRD, XRD instrument is turned on and then set conditions for devices with rotary angle $2\theta = 2^{\circ}$ to 80° , scan step = 0.04, and a scan speed = 4 ^o/min.

RESULT AND DISCUSSION

Figure 1 shows that there is a widening of the spectra in the region 3400 cm⁻¹ when cellulose was added to the chitosan. The addition of chitosan can improve the reading group at wave number 3400 cm⁻¹ due to the -NH₂ in chitosan and -OH in cellulose overlap. All three spectra are still visible in the area around wave number 1570 cm⁻¹ indicating the presence of aromatic rings. In the cellulose contained chitosan shows amino group the vibrations at 1558.48 cm⁻¹ which are characteristic of chitosan [9]. The characteristic absorption alleged overlap with the aromatic ring absorption at wave number 1570 cm⁻¹, cellulose and chitosan have aromatic rings. In both control no appearance of the spectra in the region 1635 cm⁻¹. These spectra indicate the presence of group C = O. It shows that with the addition of chitosan which has a C = O group in the cyclic as well as the possibility of residual chitin are not going to increase the absorption at 1635 cm⁻¹. Bacterial cellulose has functional group -OH, -CH,

CH₃ bending vibration, and β -1,4-glucan. The cellulose-glycerol-chitosan composites shows –OH, –CH, CH₃ bending vibration, C=O stretching, β -1,4-glucan, –NH stretching (amide I) and –NH bending (amide II). The adding chitosan can cause the presence of C = O stretching (Amide I) and–NH bending (Amide II) that are characteristic of group C = O amide I and amide II NH group contained chitosan. This indicates that chitosan has interacted with bacterial cellulose and glycerol, but amide groups of chitin are not deacetylated perfectly.

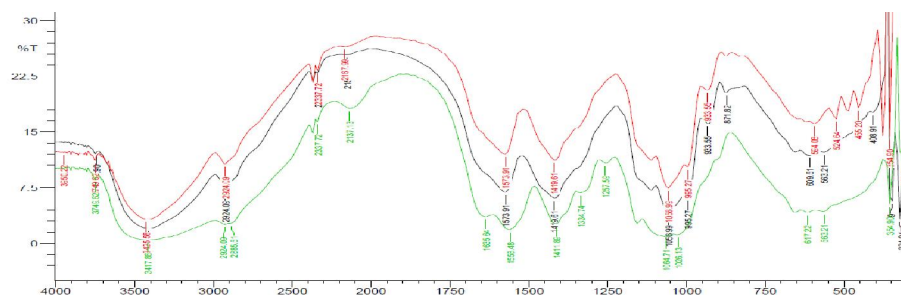


Figure 1. FTIR spectrum of bacterial cellulose-glycerol/BC-G (upper), BC (medium), and bacterial cellulose-glycerol-chitosan/BC-G-Ch (lower)

Table 1 shows absorbance of –OH and –NH in bacterial cellulose and its composites. The adding glycerol and chitosan in preparation bacterial cellulose can cause the decreasing absorbance of the–OH group. This will support the decreasing in percent elongation of bacterial cellulose when added with glycerol and chitosan as well as increasing thermal stability of bacterial cellulose-glycerol-chitosan.

Table 1. The Absorbance of bacterial cellulose from rice wastewater and its composites

Wave number (cm ⁻¹)	Functional group	Absorbance		
		BC	BC-G	BC-G-Ch
3400	-OH	0.360	0.320	0.329
1635	-NH	-	-	0.116

The addition of chitosan will lead to widening and sharpening of the peak–OH group of cellulose bacteria around the wave number 3400 cm⁻¹. The broadening of this peak indicates a potential for overlap between the–OH group with –NH₂ groups. This is indicates the possibility of overlap between the interaction of hydrogen from the–OH with –NH₂ [9]. Based on Table 1, the visible presence of 1635.64 cm⁻¹ and 1566.20 cm⁻¹ indicating the presence of C = O stretching (Amide I) and–NH bending (Amide II), shows amide groups of chitin are not deacetylated perfectly [9].

Figure 2 presents a SEM photograph of the surface of bacterial cellulose and bacterial cellulose-glycerol -chitosan. However, the second image is sufficient to prove the existence of differences between chitosan and cellulose. It is proved that chitosan is able to coat the entire surface added from bacterial cellulose.

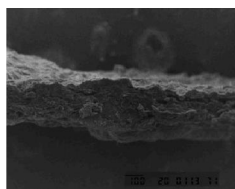


Fig 2.a. SEM Image of Bacterial Cellulose from rice wastewater

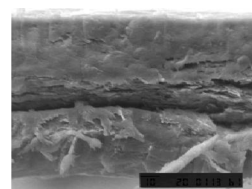


Fig 2.b. SEM Image of Bacterial Cellulose-Glycerol-Chitosan Composite

Table 2. Mechanical Properties of Bacterial Cellulose from Rice Wastewater and Its Composites

Parameter	BC	BC-G	BC-G-Ch
Tensile strength (MPa)	22.48	15.60	17.01
Elongation (%)	22.18	28.12	8.01

Table 2 shows that bacterial cellulose control (BC) has a tensile strength is 22.48 MP , whereas the cellulose-glycerol (BC-G) has a tensile strength that is 15.60 MPa . Decrease in tensile strength is due to the nature of glycerol as a plasticizer that makes rigidity of the material decreases. Result of the decrease in rigidity of this material is lower tensile strength but has the advantage of a higher elongation. Percentage of elongation of BC-G is 28.12 % while in the control cellulose (BC) only 22.18 % . It is proved that the addition of glycerol as a plasticizer cause decreased in tensile strength but increased in elongation [10]. This decrease was due to reduced intermolecular interactions were replaced with polymer bonding interactions with glycerol resulted properties of the polymer becomes more elastic but brittle.

When comparing the cellulose (BC and BC-G) with BC-G-Ch, tensile strength of BC with addition of chitosan will be lower than BC but higher than BC-G. The decreasing of tensile strength of BC-G-Ch is caused chitosan has a high amorphous, whereas the cellulose has high crystallinity. A strong structural material due to its high crystallinity naturally resilient to higher pressure, than the irregular structure of materials and provide lots of space around it. The addition to the amorphous nature of the material which has a high crystallinity will make the strength of the pressure is reduced [14]. The addition of the chitosan, elongation decreased very significantly. Elongation of BC-G's were initially 28.12%, with the addition of chitosan led to a 8.01%. The use of corn starch films happen intermolecular bonds form hydrogen bonds. This bond increases tensile strength but lower elongation [15].

The existence of the rigid structure of the polymer chain will cause the polymer chains become increasingly difficult to move when it is pulled so that elongation will decline [10]. This is reinforced by the results of XRD analysis, a decline in the crystallinity of bacterial cellulose when added to the chitosan. A decrease in the crystallinity showed the addition of amorphous regions in bacterial cellulose. Chitosan is able to reduce the crystallinity of bacteria cellulose due to the presence of amorphous region in chitosan. Bacterial cellulose has a high crystallinity, this is relationship with the high mechanical properties of bacterial cellulose [11]. The addition of chitosan concentration on bacterial cellulose from 12 to 45%, its tensile strength tends to decrease from 130 MPa to 54 MPa while the value of percent elongation dropped from 12% to 6.8% [11].

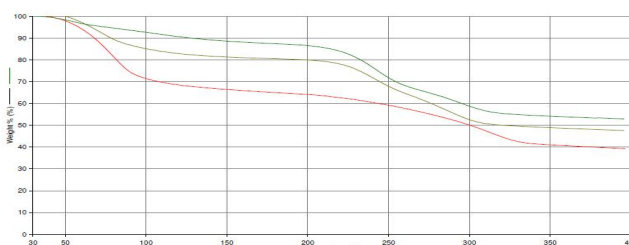


Figure 3. The TGA Thermogram of BC-G (upper), BC (medium), and BC-G-Ch (lower)

Based on Fig. 3, cellulose is added with glycerol and chitosan has a high stability compared with both BC and BC-G. If the data is quantified by taking the final point on the importance of the temperature of 400°C, % mass loss of cellulose (BC), BC-G, and BC-G-Ch respectively 33.54%, 35.56%, and 27.11%. Decrease in thermal stability of BC-G compared to BC might be due to differences in the polymerization of the cellulose. Polymerization on cellulose without glycerol forming linear polymers whereas the cellulose polymer is added to the glycerol producing branched polymers. This is explained by the strong bond that is formed. Bonding in the linear polymer β - 1,4 - glycosidic bonds which are covalent, while the branched polymer to form hydrogen bonds. The covalent bond will be much stronger than hydrogen bonds so that the linear polymer is more difficult to decompose than branched polymers [16]. Another possibility, because glycerol is evaporated of polymers. Composite of glycerol-a polymer will evaporated at 200°C temperature [12].

Results from DTA thermogram shows endothermic peak as the release of water molecules. Temperature required for BC-G-Ch release of water molecules is higher compared with both BC and BC-G, because the cellulose pores filled by chitosan, so that water molecules trapped in the cellulose.

BC has degree of crystallinity was 73.65%, while the cellulose is added glycerol and chitosan having a degree of crystallinity of 50.15% (Fig. 4). This indicates that chitosan causes a decrease in the degree of crystallinity of cellulose. Chitosan has an amorphous nature, whereas cellulose has a high crystalline properties. The existence of the amorphous nature of the cellulose into the cause deterioration of crystallinity. Decrease the degree of crystallinity affects the mechanical properties of cellulose. Cellulose that has a low degree of crystallinity tend to have a low tensile strength. The addition of chitosan will increase the tensile strength due to the formation of hydrogen bonding between cellulose with chitosan that increases rigidity, but there was also a decrease in the degree of crystallinity of cellulose so that the tensile strength decreases.

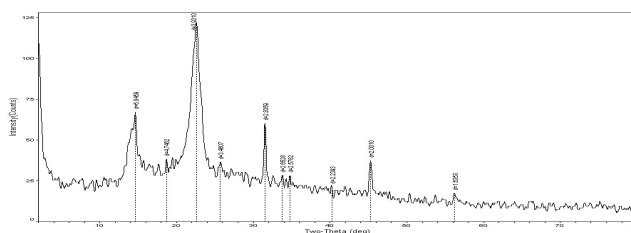


Figure 4. The XRD Diffractogram of BC from rice wastewater-Glycerol-Chitosan Composite

Results of characterization of colloidal silver nanoparticles by using UV - Vis Spectroscopy Shimadzu 1601 is showed by Fig. 5. Figure 5 showed the maximum wavelength of 10^{-3} M AgNO_3 solution at 216.20 nm region.

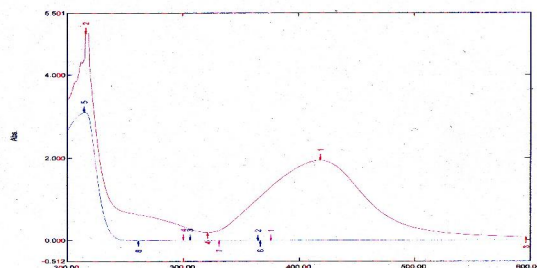


Figure 5. Spectrum of UV - Vis of 10^{-3} M AgNO_3 solution and colloidal silver nanoparticles

Colloidal silver nanoparticles showed two largest peaks in the maximum length of 423.40 nm and at 225.80 nm at the maximum length. Based on the studies that have been done by Sileikaite et al (2006) showed that colloidal silver nanoparticles having a maximum wavelength range 350-550 nm with the analysis using visible light spectrophotometer. This research was conducted in the wavelength range of 200-500 nm. Silver nitrate solution has an absorbance spectrum peaks at a wavelength of 215 nm. After the silver nitrate solution is mixed with trisodium citrate, UV-Vis spectra obtained are very much different and the obtained peak absorbance at a wavelength of 350-500 nm. After the reduction process, obtained absorbance of visible light at a wavelength of 419 nm of 0.187. The results of spectrophotometric analysis of the silver nanoparticles at a wavelength of 400-450 nm particles formed is Ag. Based on these data it can be concluded that silver nanoparticles has been obtained by reduction silver nitrate solution.

The addition of silver nanoparticles on cellulose did not change the physical characteristics of cellulose previously. Fig. 6 and Fig. 7 showed SEM image of bacterial cellulose and composite of bacterial cellulose – chitosan impregnated silver nanoparticles.

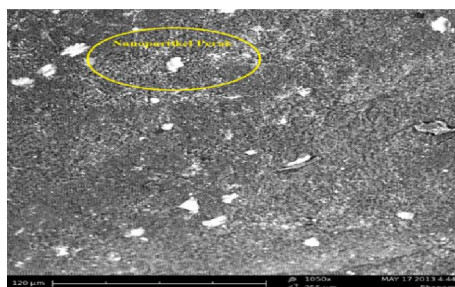


Figure 6 . SEM image of Bacterial Cellulose Composites from rice wastewater impregnated Ag (magnification 1050x)

Based on Fig. 6, we can see the surface morphology of bacterial cellulose impregnated silver nanoparticles with a magnification of 1050x. Fig. 7 showed the top surface morphology of bacterial cellulose - chitosan composite impregnated silver nanoparticles with a magnification of 8700x . Based on SEM image, we can see that silver nanoparticles successfully impregnated on the bacterial cellulose derived from rice waste water. Suspected silver nanoparticles adsorbed on bacterial. Interaction between the bacterial cellulose and silver nanoparticles occurs through chemical bonds to form a covalent bond between the-OH group on the bacterial cellulose with Ag in the silver nanoparticles.

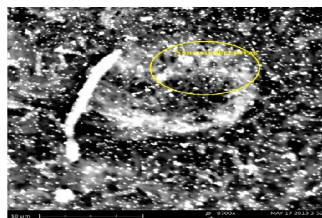


Figure 7. SEM image of Bacterial Cellulose - Chitosan Composites impregnated Ag (magnification 8700x)

Fig. 8 showed antibacterial activity of bacterial cellulose – glycerol – chitosan composite impregnated Ag against *S.aureus* and *E.coli* by using shake flash turbidimetry method. The results of testing the antibacterial activity of all the cellulose samples showed positive results. The clear zone method showed that antibacterial activity of bacterial cellulose – glycerol – chitosan composite impregnated Ag against *S.aureus* lower than antibacterial activity of bacterial cellulose – glycerol – chitosan composite impregnated Ag against *E.coli*. The diameter of clear zone in bacterial cellulose – glycerol – chitosan composite was 0.27 mm against *S.aureus*, and 1.00 mm against *E.coli*.

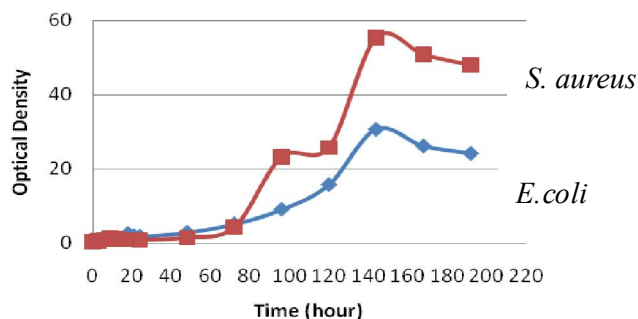


Figure 8. Antibacterial activity of Bacterial Cellulose – Glycerol – Chitosan composite impregnated Ag against *S.aureus* and *E.coli*

Mechanism of the antibacterial properties of silver nanoparticles is the presence of heavy metal ions will react with the thiol group (- SH) on the protein surface . Particles of monovalent silver ions (Ag^+) cation is able to replace the hydrogen (H^+) of the thiol groups of proteins that inactivate sulphhydryl, a decrease in membrane permeability, and ultimately leads to cell death. From these statements interaction between silver nanoparticles with cellulose membrane is important because it affects the release of silver nanoparticles will attack the bacterial wall, so that differences in the effectiveness of the antibacterial activity of each cellulose in testing can be due to differences in the interaction of silver nanoparticles with functional groups on each cellulose.

The interaction that occurs between the silver nanoparticles with functional groups on the cellulose membrane is electrostatic interaction. Electrostatic interaction between functional groups of polymers and nanoparticles depends on the charge of polymer, polymer structure and chemical content in the polymer. Any kind of cellulose samples tested had the charge of electrons (the number of pairs of electrons) and a different structure of cellulose .

CONCLUSION

The UV-Vis spectroscopy revealed the formation of silver nanoparticles by exhibiting the typical surface plasmon absorption maximum at 419 nm. The SEM image showed that the silver nanoparticles has been impregnated in the surface of bacterial cellulose. The nanoparticles of silver-impregnated bacterial cellulose-chitosan-glycerol composites exhibited antibacterial activity against *S.aureus* and *E.coli*.

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