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Cite as: AIP Conference Proceedings 2554, 070002 (2023); <https://doi.org/10.1063/5.0103724>  
Published Online: 25 January 2023

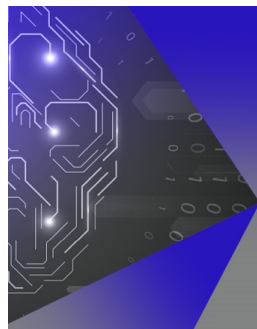
A'yunil Hisbiyah, Lilik Nurfadlilah and Khoirun Nisyak



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# The Nanocellulose Addition's Effect on Chitosan Composites Mechanical Properties and Antibacterial Activity Against *S. aureus*

A'yunil Hisbiyah<sup>1, a)</sup> Lilik Nurfadlilah<sup>1, b)</sup> and Khoirun Nisyak<sup>1, c)</sup>

<sup>1</sup>Chemistry Laboratory, STIKES RS Anwar Medika, Indonesia

<sup>a)</sup> Corresponding author: yuihisbi@gmail.com

<sup>b)</sup> liliknf@gmail.com

<sup>c)</sup> nisachemist@gmail.com

**Abstract.** Nowadays, chitosan has the potential as a biodegradable composite with good antibacterial activity. Therefore, research related to the development of the mechanical stability of chitosan as a biodegradable composite was needed. This research focuses on the effect of sugarcane bagasse-nanocellulose addition on the mechanical and antibacterial activity of chitosan membrane against *S. aureus*. Chitosan Composite with the addition of sugarcane bagasse-nanocellulose were prepared on acrylic molds and dried by oven. Mass ratio of sugarcane bagasse-nanocellulose in the chitosan Composite was varied to 2:10 and 4:10. The surface morphology of composite was evaluated by scanning electron microscopy. The interaction between nanocellulose and chitosan was monitored by X-Ray Diffraction and Fourier Transform-Infra Red Spectroscopy. The obtained result suggested that there are hydrogen bonding between nanocellulose and chitosan. The mechanical properties was observed, include Composite thickness, transparency, moisture content, and thermal properties. The nanocellulose addition can decreased chitosan Composite moisture content. Analyses of thermal properties showed that nanocellulose addition on chitosan Composite have effect increasing stability of Composite under high temperature, and the degradation occurred above 250°C.

## INTRODUCTION

Chitosan (CHIT) is a biopolymer compound produced from crustacean class animal chitin such as shrimp and crab which can be used as food packaging material because it is easily degraded, edible (edible Composite), antioxidant, and antibacterial[1]. Chitosan was easily dissolved at low pH. Chitosan can be composited with various polymeric materials. Due to their biocompatibility, biodegradability, non-toxicity, and their antibacterial activity, their application were studied in various scientific fields, such as in the food industry as a stabilizer and thickener, in agriculture as a fertilizer, and in the field of biomedical engineering as antibacterial material[2,3]. The main disadvantage of chitosan composites is their poor mechanical properties[4]. Therefore, research related to the development of Chitosan mechanical properties is needed. Chitosan polymers are easily brittle so that nanocellulose is combined to increase the stability of the structure[5]. Several studies have proven the ability of nanocellulose as an antibacterial support material[6,7,8]. Nano cellulose can be bio composite with natural materials to increase its usefulness[5,9,10]. Nanocellulose can increase the antibacterial activity of chitosan against *B. cereus*, *Salmonella typhimurium*, *S. Aureus*, and *E. Coli*[4]. Nanocellulose is a new type of cellulose material characterized by an increase in crystallinity, aspect ratio, surface area, and increased dispersion and biodegradability [10,11,12]. Nanocellulose has a large number of hydroxyl groups so that it has a stable structure in water[13].

Due to wide range application of nanocellulose, various method of nanocellulose extraction have been developed. Li et al. (2012) succeeded in making nanocellulose with a diameter of 10-20 nm from bagasse through high pressure mechanical methods. Camargo et al. (2016) succeeded in making bagasse nanocellulose using the enzymatic hydrolysis method while Saputri et al. (2018) made nanocellulose from bagasse with a simpler technique, through a

blending technique using a household blender. The chemical synthesis method can be done by hydrolysis[14]. Several studies have used nanocellulose as the base material for bionanocomposites because Nano cellulose can be obtained from renewable and biodegradable natural materials [15]. Various methods of nanocellulose extraction have been developed, until now the discovery of an environmentally friendly, energy efficient and cost-effective nanocellulose isolation method remains a challenge. Therefore, it is necessary to conduct research on the method of extraction of nanocellulose-based antibacterial materials that are environmentally friendly, energy efficient, and cost effective.

Nanocellulose can be extracted from agricultural waste, one of which is sugarcane bagasse[16,17,18]. Nanocellulose extraction methods from bagasse that have been successfully carried out include the high pressure mechanical method[17], the enzymatic hydrolysis method, the acid hydrolysis method, and the ultrasonication method[13]. Each method has its weaknesses, the high pressure mechanical method requires considerable energy, the enzymatic hydrolysis method requires a lot of cost and the extraction time is longer, the acid hydrolysis method is less environmentally friendly because of the use of high concentrations of acid, while the ultrasonic method is environmentally friendly, however Nanocellulose from acid hydrolysis is better than that of ultrasonication. Based on this, this study used a combination method of acid hydrolysis with ultrasonication to extract Nanocellulose form sugarcane bagasse. Furthermore, sugarcane bagasse-nanocellulose was added to chitosan Composite with various mass ratio. Focus of this study is the effect of sugarcane bagasse-nanocellulose addition on mechanical properties of chitosan Composite and antibacterial activity against *S. aureus*.

## MATERIAL AND METHODS

### Instrumentation and Materials

The materials used in this research are nanocellulose from sugarcane bagasse (NC-SB) that obtained from the author's previous study[19], Chitosan (CV. Biochitosan Indonesia), acetic acid (Merck), H<sub>2</sub>SO<sub>4</sub> (Merck), NaOH (Merck), demineralized aqua (Bratachem), Filter paper (Whatmann No.44), Dimethyl sulfoxide (Merck), Natrium agar media (Merck). *S. aureus* isolate (ATCC 23235). The instruments that used in this study include Ultrasonicator, analytical balance (Ohaus px224/E), Oven (DGG 9053A), sentrifuge, microplate reader, Laminar air flor, autoclave. Interaction between nanocellulose and chitosan was analysed by Fourier transformed-Infrared spectroscopy (Flexible benchtop FT-IR spectrometer Carry 360). Meanwhile cristallinity of material was analyzed by X-Ray diffraction (XRD Philip analytical). Surface morphology of material was analyzed by Scanning Electron Microscopy (SEM SU3500). Thermal properties of material was characterized by Thermogravimetry/Differential Thermal Analysis (TG/DTA Hitachi STA 7300). Moisture content was analyzed by Moisturizer analyze (MOC63u).

### Isolation Nanocellulose from Sugarcane Bagasse (NC-SB)

The nanocellulose isolation method that used in this study was Mandal and Chakrabarty (2011) method with some modification. Ultrasonication was used in this study to carry out delignification reactions, bleaching, and acid hydrolysis. Sugarcane bagasse-nanocellulose that obtained was dried at 60°C until constant weight [7,20].

### Preparation of Sugarcane Bagasse Nanocellulose/Chitosan Composite (NC-SB/CHIT)

**TABLE 1.** Mass ratio of Sugarcane bagasse nanocellulose and chitosan in composite

Material	Mass ratio Chitosan:Nanocellulose
Composite 0	10:0
Composite 1	10:2
Composite 2	10:4

3% w/v chitosan solution was obtained by dissolving chitosan in 2% acetic acid solution. Furthermore, the nanocellulose was added to the 3% chitosan solution with various mass ratio (Table 1). Then, the mixture was ultrasonicated for 8 hours at 45°C[5]. Furthermore, the mixture was poured into (7.5x10) cm acrylic moulds. The Composite was dried for 72 hours at 60°C. Furthermore, Composite was characterised by FT-IR, XRD, TG/DTA, and SEM.

## Test of the Composite Thickness and Moisture Content

The thickness of the composite is measured using digital micrometre ( $\pm 0.001$  mm). Measurement done at 5 random points[21]. Moisture content was analyzed by Moisturizer analyze (MOC63u).

## Test of Thermal Properties of Composite

Thermal Properties of composite was analysed by TGA Hitachi STA 7300. 5–10 mg of the film's samples sealed in aluminium pans and a 20–400°C heating increase under a nitrogen flux of 50 mL/min. The heating rate was 10°C/min. Analyses were preceded by scans in the heat-cool-heat mode with a maximum temperature at 200°C to eliminate water from the sample but not cause its decomposition [5].

## Antibacterial Activity Test Against *S. aureus*

Antibacterial activity test was using the pour plate method and carried out in triplicates, *S. aureus* was used in this study as gram-positive bacteria. *S. aureus* with concentration  $10^6$  cell/mL was inoculated in Natrium agar media. Furthermore, composite with various mass ratio was cut to size (7x7) mm. Then, bio composite were placed into the well and incubated for 24 hours at 37°C. After that, the clear zone was measured. The clear zone indicated the inhibition zone of bio composite against *S. aureus*.

## RESULT AND DISCUSSION

### Preparation of Composite

The preparation of composite with various mass concentration was through ultrasonication process to homogenizing the mixture. The higher mass ratio of nanocellulose in the mixture, the higher the viscosity of the mixture. This causes the composite to rapidly aggregate during the membrane imposing process so that the surface of the bio composite membrane is not homogeneous. The temperature during the stirring of the bio composite mixture and drying of the membrane plays an important role because it can cause sublimation and phase separation so that the membrane formed will agglomerate and make the membrane porous. Bio composite was printed on acrylic mould (7.5x10) cm<sup>2</sup> with the casting method. It can be seen that the higher mass concentration of nanocellulose, the lower the transparency of bio composite and the colour of bio composite more yellow (Figure 1).

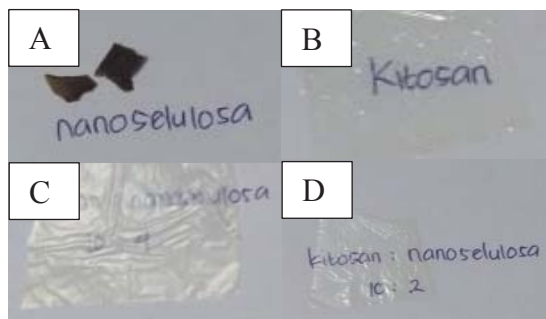
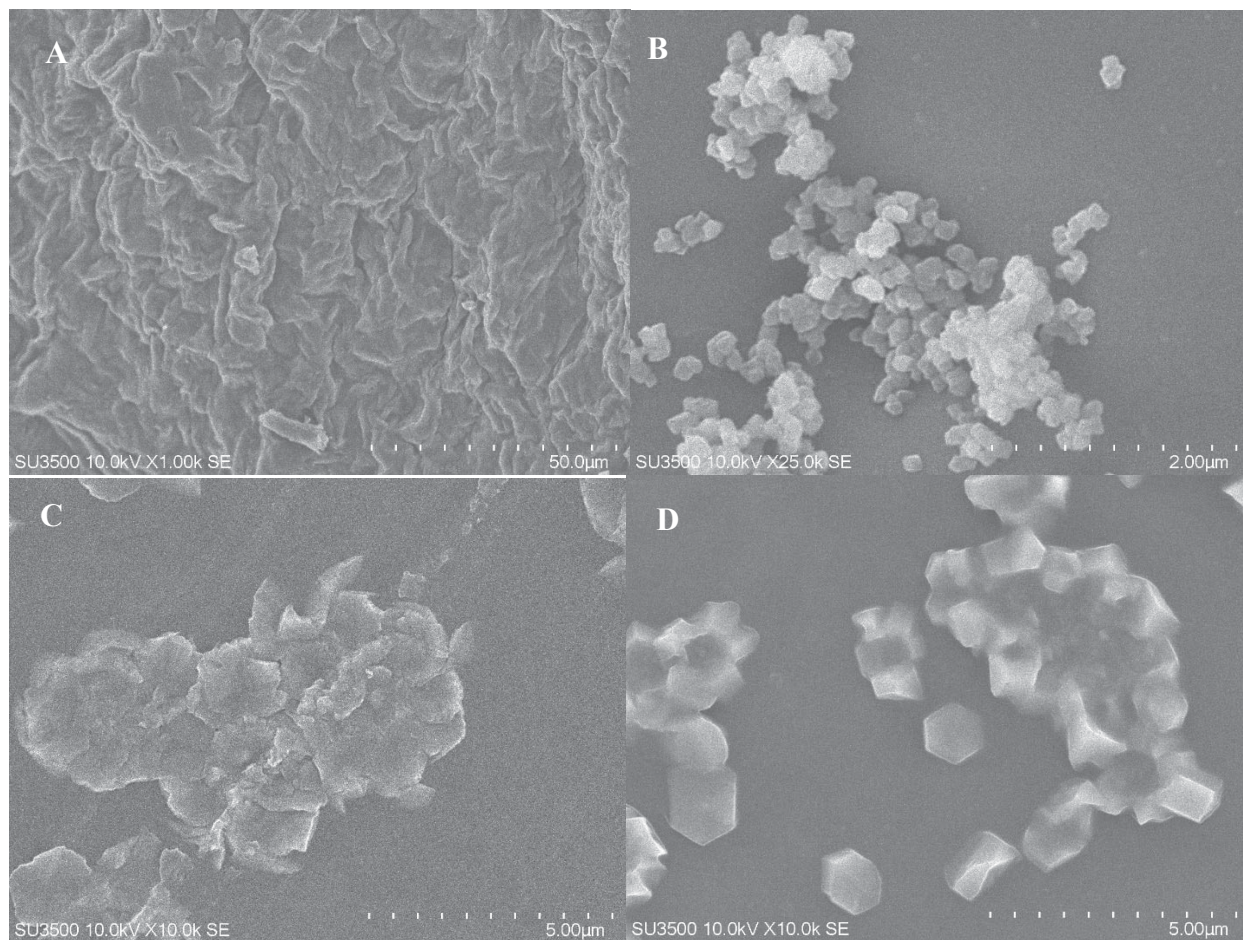


FIGURE 1. A. NC-SB; B. Composite 0; C. Composite 1; D. Composite 2

### Morphology of Composite

Morphological differences of each composite and NC-SB can be seen on Figure 2B, 2C and 2D at 10,000x magnification. In the composite 0, separate granules are visible, while in the Composite 1 and 2, the grain structure is closer. This showed that the addition of nanocellulose to the chitosan layer can affect the stability of the layer structure so that it can affect the mechanical properties of the layer. Therefore, the increase in antibacterial activity in these composites can be caused by the structural stability of the film layer.





**FIGURE 2.** Micrograph of A. NC-SB, B. Composite 0, C. Composite 1, D. Composite 2

### FT-IR Analysis

The effect of NC-SB addition on functional group of composite was analysed by FT-IR. The shifts of FT-IR bands are showed the interactions between molecules. The spectra of NC-SB was showed to highlight the differences among chitosan (Figure 3). The NC-SB spectra showed commonly bands for cellulose[5]. Comparing to NC-SB, the composite have bands around  $1556\text{ cm}^{-1}$ , this region was the peak of NH- bending vibration, while NC-SB does not have a band in that area. In composite 1 and 2, the bands at  $2924\text{ cm}^{-1}$  and  $2855\text{ cm}^{-1}$  have a higher % transmittance than composite 0. This can be attributed to the intermolecular interaction between NC-SB and Chitosan. The region between  $1180$  and  $800\text{ cm}^{-1}$  has the same pattern for the NC-SB and composite due to their similar structure. This region is also sensitive for the C–O and C–C stretching vibrations. The main characteristic feature is the presence of bands at  $1610\text{ cm}^{-1}$  (overlapped by water band at  $1640\text{ cm}^{-1}$ ) related to carbonyl group stretching (C=O) from cellulose oxidation as an outcome bleaching process with sodium hypochlorite. This result suggested that there was interaction between the oxidized surface of the NC-SB and the amide groups of CHIT.

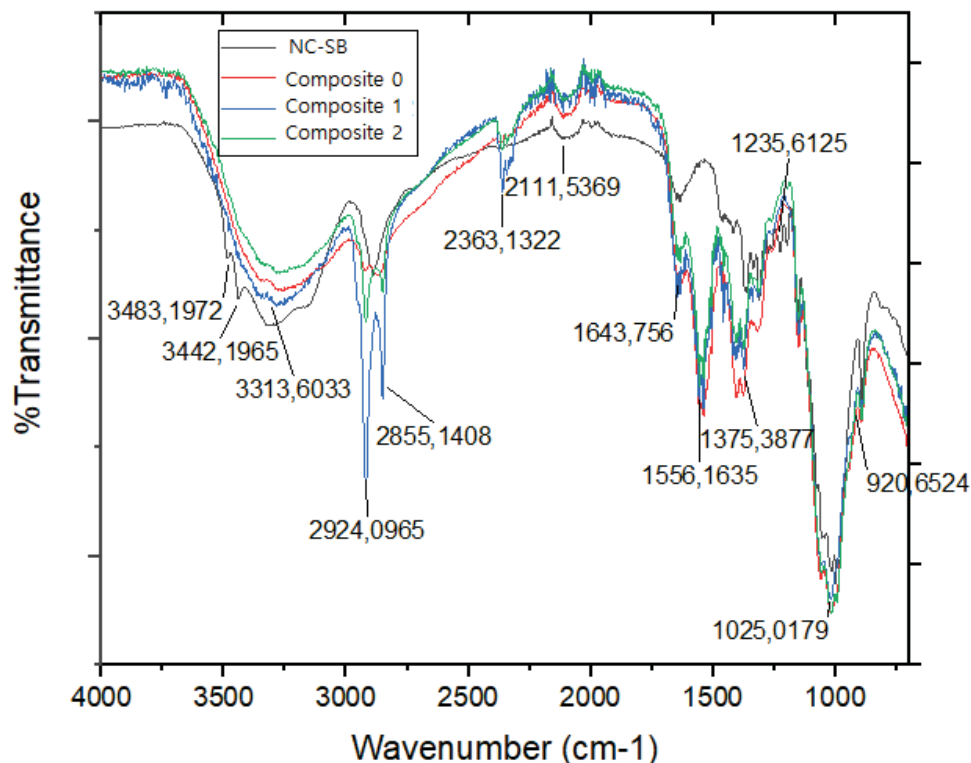


FIGURE 3. FT-IR Spectrum of NC-SB, composite 0, composite 1, and composite 2.

### XRD Analysis

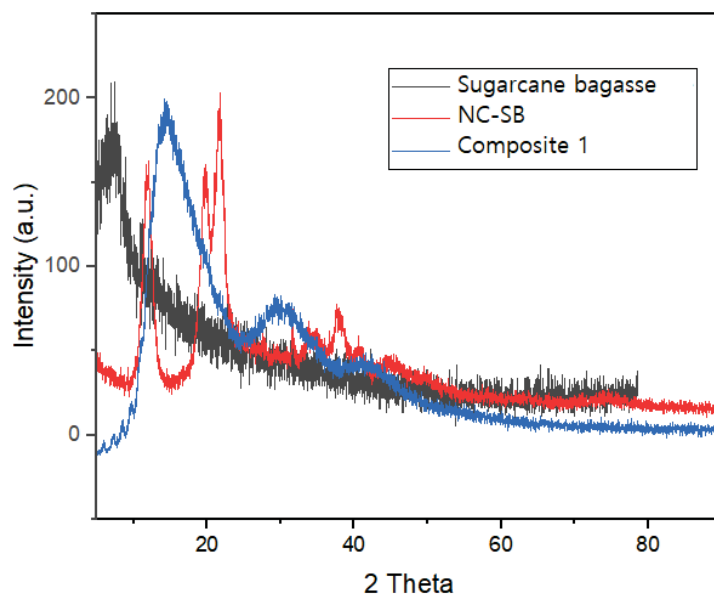


FIGURE 4. Diffractogram of sugarcane bagasse, NC-SB, and composite 1

XRD analysis aims to see the crystallinity of a material. XRD analysis Figure 4 showed the XRD patterns of sugarcane bagasse, NC-SB, and composite 1. All of XRD patterns (Figure 4) showed peaks around  $2\theta$  of  $5-22^\circ$  indicating the typical cellulose I structure. The only difference is slight intensity change in the peaks that indicating

some change in the crystallinity index of samples [22]. The NC-SB's peak at 5-25° more defined than Sugarcane Bagasse peak. This is indicated that acid hydrolysis and ultrasonic wave can increase crystallinity index of cellulose. During the acid hydrolysis, hemicelluloses and lignin were dissolved and the remaining pure of crystallin were isolated. The particle can increases peak intensity and give narrower crystalline peaks[23]. Composite 1 showed different XRD pattern at 2θ range from 5° to 40° that indicates the presence of chitosan in composite. it means that NC-SB has succeeded in binding to chitosan in the composite 1. Composite 1 have less crystallinity than NC-SB, it can be seen from peak intensity of composite 1. Composite 1 give broad peak, it indicated that composite 1 have more amorphous region than NC-SB.

### The Thickness of composite

The composite thickness test was measured using a micrometre with a minimum scale of 0.01 mm. Due to the very small thickness of the membrane, to facilitate measurement, eight membranes were stacked and measurements were made at five different points. The thickness measurement results are then divided by eight and averaged. Based on the measurement results (Table 2), it is known that the composite 1 thickness was 0.0255 mm ± 0.005 mm, while the composite 2 thickness was 0.0302 mm ± 0.005 mm. This showed that the higher the NC-SB concentration in the composite, the thicker the membrane formed. This is influenced by the presence of composite aggregation factors during the film printing process. In addition, the increase in thickness is due to the addition of mass of NC-SB biopolymer which binds to CHIT which causes the thickness of the composite to increase. The results of the thickness value measurement are shown in Table 2.

TABLE 2. The Composite Membrane Thickness

Material	Thickness (mm)
Composite 0	0,0135 ± 0,005
Composite 1	0,0255 ± 0,005
Composite 2	0,0262 ± 0,005

### The Moisture Content of Composite

Moisture analysis aims to see the effect of chitosan addition on the physical properties of the membrane. The % moisture of sample showed on Table 3. Based on the data in Table 3, composite 0 has the highest % moisture than other materials at 7.75%. The addition of NC-SB in the chitosan film in composite 2 can cause the material moisture decreased to 5.24%. This decrease in% moisture content affects the antibacterial activity of a substance. Bacteria easily grow in places with high humidity. Therefore, in this study the% moisture of the material is thought to affect its antibacterial activity. It is evident that composite 2 has a larger zone of inhibition against the growth of *S. aureus* than composite 0 film (Table 3).

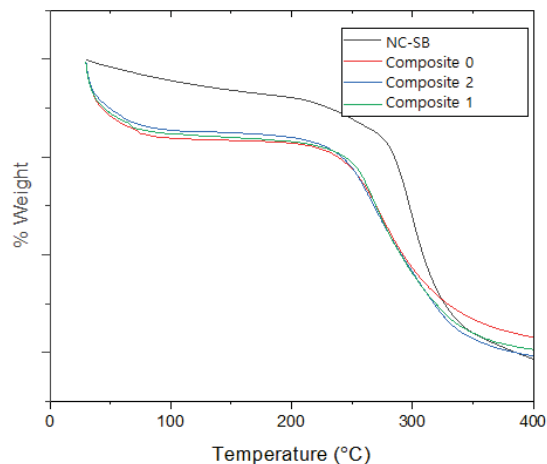
TABLE 3. Moisture Content Analysis

Material	% Moisture Content
Sugarcane bagasse	1.47
NC-SB	3.85
Composite 0	7.75
Composite 1	6.35
Composite 2	5.24

### Thermal Properties of Composite

In this study, TGA was used to analyses thermal properties of material. TGA is a method of thermal analysis of a sample by taking into account the mass decrease during the heat treatment which is carried out in an atmospheric environment. The TGA curve of bagasse nanocellulose showed a curve with the more gentle mass reduction than chitosan composite. This shows that nanocellulose has better thermal stability than chitosan. Mass decomposition of nanocellulose began to occur drastically at 300°C, while mass decomposition of chitosan began to occur at 250°C. The addition of nanocellulose to chitosan composites can increase the thermal stability of composites at temperatures less than 250°C (Figure 5). This can be seen in the TGA curve, bio composite 2 has a higher mass% than bio composite

1, meaning that the higher the concentration of nanocellulose found in composites, the more increases the thermal stability of composites at temperatures more than 250°C.



**FIGURE 5.** TGA Curve of NC-SB, Composite 0, Composite 1, and Composite 2.

### **Antibacterial Activity of Bio composite Against *S. aureus***

The results showed the most optimum area of inhibition is on the composite 1, namely the area of inhibition of 8.5 mm<sup>2</sup> (Table 4). While the inhibition area of composite 0 was 7 mm<sup>2</sup>. This result indicated that the addition of NC-SB on CHIT film can enhance their antimicrobial activity against *S. aureus*. The composite mixture is thought to be able to inhibit microbes by destroying the peptidoglycan structure in the cell wall and denaturing proteins, resulting in enzyme deactivation. This is because the process of inhibiting the growth of microorganisms is generally caused by several things including the presence of disruptive compounds on the cell walls, causing increased cell membrane permeability resulting in loss of cell components, inactivity of enzymes in cells, and the process of destruction or damage to genetic material [24]. On the other hand, the addition of NC-SB concentration did not show an increase in the area of inhibition power. This could be due to the limited ability of CHIT to bind NC-SB. Not all NC-SB can be bound by CHIT.

**TABLE 4.** The inhibition zone test against *S. aureus*

<b>Material</b>	<b>Inhibition zone (mm)</b>
Composite 0	7,00 ± 0,05
Composite 1	8,50 ± 0,05
Composite 2	8,00 ± 0,05

### **CONCLUSION**

The NC-SB addition on chitosan composite has effect on their mechanical properties and antibacterial activity against *S. aureus*. The optical transparency of films was very good except for those with the highest content of nanocellulose. SEM showed that NC-SB was dispersed in CHIT matrix and formed a denser structure. SEM indicated the presence of aggregation. FTIR spectroscopy suggested that there was interaction between the oxidized surface of the NC-SB and the amide groups of CHIT. The addition of NC-SB to the CHIT can enhancing composite hydrophobicity. This increase is probably connected with the interaction between nanocellulose and chitosan to form a denser structure. Analyses of the thermal properties showed that composites are stable at high temperature, and the degradation occurred above 250°C. Antimicrobial properties indicated that the composite 1 has the highest inhibitory zone against *S. aureus*.



## ACKNOWLEDGMENTS

The author would like to thank to the Ministry of Research, Technology and Higher Education who has provided research grant 2020 through Beginner lecturer research schemes with contract number 083/SP2H/LT/DRPM/2020; 128/SP2H/LT-MONO/LL7/2020; 004/STIKESRSAM/SP-Penelitian/III/2020 and STIKES Rumah Sakit Anwar Medika.

## REFERENCES

1. R. C. F. Cheung, T. B. Ng, J. H. Wong, and W. Y. Chan, *Marine Drugs* **13**, 5156-5186 (2015)
2. N. Morin-crini, E. Lichhtfouse, G. Torri, and G. Crini, *Sustainable Agriculture Review* (Springer International Publishing, 2019), pp. 338.
3. W. Wang, Q. Meng, Q. Li, J. Liu, M. Zhou, Z. Jin, and K. Zhao, *Int. J. Mol. Sci.* **21**, 1-26 (2020).
4. A. Khalil, C. K. Saurabh, Adnan, M. R. N. Fazita, M. I. Syakir, Y. Davoudpour, M. Rafatullah, C. K. Abdullah, M. K. M. Hafiz, and R. Dungani, *Carbohydr. Polym.* 216–226 (2016).
5. M. Szymańska-Chargot, M. Chylinska, G. Pertile, P. M. Pieczywek, K. J. Cieslak, A. Zdunek, M. Frac, *Cellulose* **26**, 9613–9629 (2019).
6. J. George and S. N. Sabapathi, *Nanotechnol. Sci. Appl.* **8**, 45–54 (2015).
7. Q. Xu, Y. Ji, Q. Sun, Y. Fu, Y. Xu, and L. Jin, *Nanomaterials* **9**, 1-13 (2019).
8. J. Li, R. Cha, K. Mou, X. Zhao, K. Long, H. Luo, F. Zhou, X. Jiang, *Adv. Healthc. Mater.* **7**, 1–16 (2018).
9. A. Hänninen, E. Sarlin, I. Lyyra, T. Salpavaara, M. Kellomäki, *Carbohydr. Polym.* **202**, 418–424 (2018).
10. K. Oksman, Y. Aitomäki, A. P. Mathew, G. Siqueira, Q. Zhou, S. Butylina, S. Tanpichai, X. Zhou, S. Hooshmand, *Compos. Part A Appl. Sci. Manuf.* **83**, 2–18 (2016).
11. T. Abitbol, A. Rivkin, Y. Cao, Y. Nevo, E. Abraham, T. Ben-Shalom, S. Lapidot, and O. Shoseyov, *Curr. Opin. Biotechnol.* **39**, 76–88 (2016).
12. S. Mondal, *Carbohydr. Polym.* **163**, 301–316 (2017).
13. P. Phanthong, P. Reubroycharoen, X. Hao, G. Xu, A. Abudula, G. Guan, *Carbon Resour. Convers.* **1**, 32–43 (2018).
14. M. A. Guancha-Chalapud, J. Gálvez, L. Serna-Cock, and C. N. Aguilar, *Sci. Rep.* **10**, 1–10 (2020).
15. D. B. Effendi, N. H. Rosyid, A. B. D. Nandiyanto, A. Mudzakir, *J. Integr. Proses.* **5**, 61–74 (2015).
16. L. A. Camargo, S. C. Pereira, A. C. Correa, C. S. Farinas, J. M. Marconcini, L. H. C. Mattosoamargo, *Bioenergy Res.* **9**, 894–906 (2016).
17. J. Li, X. Wei, Q. Wang, J. Chen, G. Chang, L. Kong, J. Su, Y. Liu, *Carbohydr. Polym.* **90**, 1609–1613 (2012).
18. L. H. Saputri, R. Sukmawan, H. S. B. Rochardjo, and Rochmadi, *Prosiding Seminar Nasional Teknik Kimia “Kejuangan” Pengembangan Teknologi Kimia untuk Pengolahan Sumber Daya Alam Indonesia* [Proceeding of National Seminar on Development of Chemical Engineering for Management of Indonesian Natural Resources] (UPN Veteran, Yogyakarta, 2018), pp. 1-6. [Bahasa Indonesia].
19. A. Hisbiyah and L. Nurfadlilah, *Jurnal Kimia Sains dan Aplikasi* **24**, 146–151 (2021).
20. A. Mandal and D. Chakrabarty, *Carbohydr. Polym.* **86**, 1291–1299 (2011).
21. E. R. Amanda, K. Nisyak, and Y. A. Prasetya, *J. Kim. dan Kemasan* **42**, 29–36 (2020).
22. K. Subramanian, P. S. Kumar, P. Jeyapal, N. Venkatesh, *Eur. Polym. J.* **41**, 853–861 (2015).
23. N. A. Rosli, I. Ahmad, I. Abdullah, *BioResources* 1893–1908 (2013).
24. D. K. Paramita, N. S. Antara, I. W. Gunam, J. Rekeyasa Dan Manaj. *Agroindustri* **2**, 29–38 (2014).