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BIO-MICROBEADS FROM BACTERIAL CELLULOSE INCORPORATED WITH ANTIMICROBIAL OF CHITOSAN AND MORINGA LEAVES FLAVONOID

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ABSTRACT

Microbeads are micro-sized plastics generally used to manufacture cosmetic and hygiene products. Their micron size makes the plastic microbeads potentially dangerous to the ecosystem and has non-degradable properties. This research goal determined the optimum concentration of microbeads made from bacterial cellulose (BC) and antimicrobial agent in the form of chitosan and flavonoid from Moringa leaves releasing inhibition diameter of bacteria. This also characterized the resulting BC microbeads through the Fourier-transform infrared (FTIR) and Brunauer–Emmett–Teller (BET) analysis. An antimicrobial test was conducted by applying BC microbeads to hydrogel soap and testing its inhibition on *Staphylococcus aureus*. Furthermore, as evidenced by FTIR analysis, BC microbeads were synthesized through NaOH-PEG dissolution and drying under vacuum pressure. The BET analysis showed that the addition of chitosan reduces the physical properties of BC microbeads, i.e. the pore volume and the surface area. It also implies that the addition of flavonoids reduces the pore volume and specific surface area of the BC microbeads. The optimum chitosan and flavonoid concentrations observed are 2.5%, with *Staphylococcus aureus* inhibition of 11 mm and 10 mm, respectively.

Keywords: Microbeads, Bacterial Cellulose, Chitosan, Flavonoid, Moringa Leaves, Antibacterial Activity.
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INTRODUCTION

Plastics are polymer derivatives comprising harmful chemical additives, such as lead and tributyl group substances. It is very difficult to decompose and has a negative impact on the environment and health.[1] Therefore, several investigations in the fields of biodegradable films have been extensively conducted to determine and overcome the problem associated with non-degradable plastic usage.[2] Plastic in its micron size gives rise to pollution and accumulates significantly in soil, waterways, rivers, and sea,[3] thereby producing microplastics, an exfoliating agent in the form of microbeads.[4] Microbeads in cosmetic and hygiene products have been banned in various European and Asian countries.[5] This is because its micron size makes it difficult for microbeads to be treated with ordinary sewage procedures. Microbeads discharged into the sea can be consumed by marine biota, thereby endangering their lives and of humans.[6] Therefore, it is imperative to determine an alternative replacement to plastic microbeads. Some research has been conducted on making microbeads from other materials such as polyvinyl-alcohol mixed with chitosan and titanium,[7] or calcium alginate,[8] calcium sulfate,[9] alginates,[10] to enhance their characteristics, however, they did not decompose properly in the environment. Other substitute materials used to produce microbeads include silica,[11] carobs,[12] and cellulose.[13–15] Cellulose can be sourced from biomass such as wood,[16] cotton,[17] wheat straw,[18] *Nerium oleander*,[19] *Populus tremula*,[20] starch,[21–26] and bacterial cellulose (BC).[27] BC is usually produced by *Gluconacetobacter xylinum* (also known as *Acetobacter xylinum*),[28–32] *Acanthamoeba, Achromobacter*, and *Zoogloea.*[33] It has numerous advantages, such as high mechanical strength, crystallinity, nanofibril structure, purity, and biocompatibility. Furthermore, BC has the potential to be used in biomedicine,[34] audio membrane components,[35] electronic paper,[36] and biocomposites.[37,38] It can also be applied as a substitute for plastic in manufacturing microbeads with environmentally friendly technology. As opposed to plastic, its biocompatibility will help prevent environmental pollution when turned into microbeads. Efforts to develop BC microbeads have been conducted by cultivating hydrogel fibers,[39] alginate fiber,[40] and microfluidic in wound treatments.[41]

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However, these processes are too complicated and involve alginate as the core of microparticles and agarose as the coating. More environmentally friendly methods have been investigated using ultrasonication and dried with an oven, producing nano-sized BC.[42] Other methods such as cellulose dissolution and freeze-drying were also used, yet it was impossible to obtain micron size BC.[43] To overcome these shortcomings, research has been conducted on combining these processes by making BC microbeads through freeze-drying with pre-treatment ultrasonication. Low antibacterial property is one of the weaknesses of this material, hence, the addition of antibacterial agents can counterbalance this weak point. Some antibacterial agents such as ZnO[44] are highly advantageous, while others, such as flavonoids contained in mulberry leaves, performed better.[45]

This research explores the incorporation of an antimicrobial agent into microbeads in the form of chitosan and flavonoids, which are natural substances with antimicrobial and antioxidant properties. Flavonoids are polar and can easily penetrate the polar peptidoglycan layer in microbes' membranes, making them very effective in inhibiting microbial growth.[46] They are usually found in buckhorn textured plant leaves,[47] *Gossypium hirsutum,*[48] honey,[49] and 5.53% in moringa leaves.[50] Another substitute material for microbeads is chitosan [β-(1–4)-2-amino-2-deoxy-*D*-glucopyranose], the largest available biomaterial after cellulose.[51] It is the deacetylation of protein, minerals, and chitin derived from crustacean marine biota waste.[52] Chitosan has superior properties such as biodegradable, biocompatible, non-toxic, antibacterial, antifungal, and the potential to heal wounds.[53] It has three functional groups consisting of primary OH, secondary OH, and NH_2 groups, which can inhibit the growth of most microorganisms, such as bacteria and fungi.[54] The properties of chitosan that are good for the environment are quite contradictory to plastic. Chitosan has abundant availability and antibacterial potential as BC.[55] Furthermore, this research is aimed to obtain an optimum concentration of microbeads made from bacterial cellulose (BC) and antimicrobial agent in the form of chitosan and flavonoid from Moringa leaves releasing inhibition diameter of bacteria.

EXPERIMENTAL

This research was carried out using bacterial cellulose commercially supplied from the Wong Coco brand. The solvents used were NaOH (> 99%) and PEG-400 from Merck and Sigma Aldrich, while distilled water, HCl, and 70% ethanol were used for regeneration. An antimicrobial agent of 15% liquid chitosan was obtained from CV. Nura Jaya, and flavonoids were acquired from Moringa leaves. It was also obtained from a commercial rose water solution. The dissolving and freeze-drying methods were used and carried out at the Department of Chemical Engineering at Diponegoro University. Furthermore, FTIR and BET analyses, as well as antimicrobial tests were conducted at Integrated Laboratory at Diponegoro University and Central Java Provincial Health Office, respectively. The commercial BC was washed with distilled water, blended at 2000 rpm for 5 minutes, and then sterilized with 1 N NaOH for 1 hour at 90 °C. The BC was mixed with water at a concentration of 1% by weight and homogenized at 150 W 20 kHz using homogenizer probe F250N. Afterward, it was mixed into a 10% NaOH/PEG and stirred for 5 hours to obtain a homogeneous solution. It was further frozen for 12 hours and thawed in the open air for 30 minutes. This process was repeated 3 times in accordance with previous research[43], followed by the addition of 1% commercial chitosan.[55] The BC solution previously mixed with the antibacterial agent was regenerated by dripping 70% ethanol into it, which was later frozen for 6 hours.[43] This BC regeneration was freeze-dried at 100 °C for 48 hours under vacuum pressure (25 kPa). The assessments of the product were confirmed by FTIR and BET analysis and antibacterial test by *Staphylococcus aureus*.

RESULTS AND DISCUSSION

FTIR Analysis of BC Microbeads

The FTIR spectrum of the BC microbeads, BC/Chitosan, and BC/Flavonoid shown in Figure-1 was conducted over the 400-4000 cm^{-1} wave range. The characteristics peak of the BC microbeads is at 3448.69 cm^{-1}, with an OH stretching vibration of 1645 cm^{-1}, indicating the deformation vibration of the adsorption of water molecules. Furthermore, the H-C-H and O-CH vibrations at 1431.03 cm^{-1} and 1153.58 cm^{-1} indicate deformation of C-H and asymmetric stretching of C-O-C. A C-H bending vibration at 667.35cm^{-1} indicates the bending vibration of C-OH.[15,56] Similar wave peaks are found for the BC/Chitosan microbeads, while additional wavelengths of 3343.38 cm^{-1} (N-H stretching) and 1605.89 cm^{-1} (amine N-H bending) denoted the characteristics of the chitosan structure.[57]

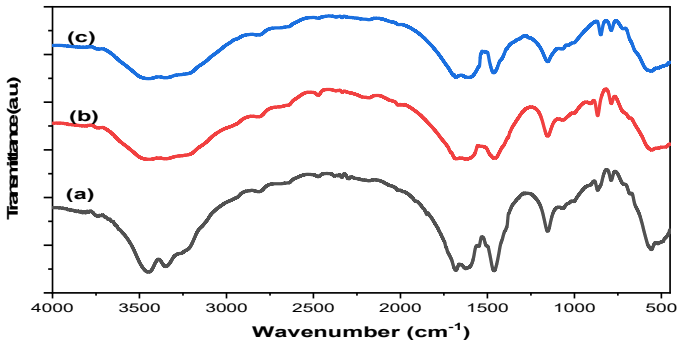

Fig.-1: FTIR Analysis for Prepared (a) BC, (b) BC/Chitosan, and (c) BC/Flavonoid

From the data obtained, it can be concluded that the BC microbeads incorporated with chitosan antibacterial agent were successfully synthesized. Both exhibit similar structural characteristic peaks with the FTIR spectra of BC and BC/Flavonoid microbeads illustrated in the spectrum (a) and (c). Similar wave peaks are found in the BC/Flavonoid spectra, while some additional values were in the FTIR. A peak in the wavelength of 1611.53 cm^{-1} indicates the benzene ring's absorption equivalent to the flavonoid structure.[45] Therefore, from the data obtained, it can be concluded that the antibacterial incorporated BC microbeads were successfully synthesized. The BC/Flavonoid possesses the same characteristic peaks corresponding to the structures of flavonoids and pure BC microbeads shown in the FTIR spectra.

BET Analysis of BC Microbeads

BET analysis was performed to evaluate the pore properties of the synthesized composite by determining some physical parameters in the following Table-1. BC/Chitosan has a smaller surface area and pore volume than pure BC microbeads. The decrease in the physical properties is caused by the large chitosan molecule leading to the closure of pores in its structure, thereby reducing all the parameters, including specific surface area, pore-volume, and diameter.[58] The BC/Flavonoids microbeads also have smaller surface areas than pure BC microbeads. This phenomenon is instigated using van der Waals forces and the hydrophobic nature of the flavonoids, thereby causing a diminish in value of the pore volume and specific surface area of BC/Flavonoids microbeads.[59]

Table-1: BET Results of the Three Different Composites of Microbeads

Microbeads	Specific Surface Area (m^2/g)	Pore Volume (cc/g)	Pore Diameter (nm)
BC	64.380	0.052	3.4184
BC/Chitosan	14.597	0.015	3.4164
BC/Flavonoid	45.688	0.039	3.0542

Antimicrobial Test of BC Microbeads

Table-2 displays the extent of the inhibition zone of the microbeads against *S. aureus* bacteria represented through the diameter of the inhibition area. The samples tested were hydrogel soaps that were pre-added with the microbeads at various concentrations ranging from 0% to 2.5%. Based on the data shown, it can be observed that the microbeads revealed a high concentration releasing a great in the zone of inhibition against bacteria. Chitosan and flavonoid have their antibacterial characteristics against various bacteria. Chitosan has a positive charge on its amino group. While microbial cell membranes have a negative charge resulting in the disruption of the intercellular constituents of the microorganism.[60–62] This prompts an increase in the inhibitory zone of the BC/Chitosan microbeads against *S. aureus* bacteria as with the increase in its concentration used in the soap. While flavonoid inhibits the synthesis of nucleic acids by attacking the permeability of the cell membranes and also the cell organs such as lysosome and microsome. It also alters the performance of cell membranes by generating complex substances with extracellular protein compounds. Lastly, it inhibits metabolic energy by disturbing the metabolism process of the microorganisms.[46]

Preliminary discussions indicate evident antibacterial activities in the BC/Chitosan and BC/Flavonoid microbeads from the outcome of inhibition zones. This indicates the greater the concentration of the BC microbeads, the more significant the antibacterial activity of the inhibition zone of the test media.

BC/Chitosan and BC/Flavonoid microbeads are proven to be quite effective in inhibiting the activity of *S. aureus* bacteria, respectively.[46]

Table-2: Microbeads Inhibition Diameter on *S. aureus* in mm

Microbeads type	Inhibition diameter on *S. aureus* (mm)					
	0%	0.5%	1%	1.5%	2%	2.5%
BC	7	7	7	7	7	7
BC/Chitosan	7	8	8	10	10	11
BC/Flavonoid	7	7	8	9	10	10

CONCLUSION

Based on this research, BC microbeads were successfully synthesized through dissolving and freeze-drying combined methods, as evidenced by the results of the FTIR analysis. Furthermore, its incorporation with chitosan and flavonoid antibacterial agent was successfully synthesized as portrayed by the appearance of another peak in the FTIR spectra correlating to the characteristics of chitosan structure. The presence of chitosan reduces the physical properties of the BC microbeads in terms of the volume of pores and the specific surface area because its molecules are large enough to close the inherent pores. The presence of flavonoids reduces the physical properties of BC microbeads releasing trapped flavonoids in the pore of BC microbeads. This implies that the microbeads used high concentration in the soap media producing a great antimicrobial activity.

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