DEVELOPMENT OF AG NANOPARTICLE BIOCHAR EMBEDDED PVA/ALGINATE BEADS AND THEIR APPLICATION AGAINST GRAM-POSITIVE BACTERIA.

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| ABSTRACT:-

The development of antibacterial materials derived from renewable resources is of increasing interest for environmental and biomedical applications. In this study, polyvinyl alcohol/silver nanoparticle-loaded biochar/sodium alginate (PVA/Ag-BCH/SA) composite beads were fabricated using corn husk as a carbon source to produce biochar. Silver nanoparticles were synthesized tea polyphenols as reducing agent and were subsequently immobilized on the surface of corn husk biochar. The Ag-BCH was uniformly embedded into the PVA/SA hydrogel network through ionic crosslinking, resulting in the spherical beads with good mechanical properties. Structural and physicochemical properties of composite beads confirmed the successful incorporation of silver nanoparticles and strong intermolecular interactions between the polymer chains and biochar. The composite beads exhibited a lower swelling ratio while maintaining structural stability in aqueous and simulated sweat solution. Antibacterial activity was evaluated against gram-positive bacteria, including *Staphylococcus aureus* and *Staphylococcus epidermidis*. The PVA/Ag-BCH/SA beads displayed clear inhibition zones and revealed a good stability for antibacterial activity, indicating their potential as an antibacterial material for biomedical applications or related applications.

| KEYWORDS

Silver nanoparticles, Corn husk biochar, Composite bead, PVA, sodium Alginate, Gram-positive bacteria

Introduction:-

Silver nanoparticles (Ag-NPs) have attracted considerable attention as antibacterial agents because of their broad-spectrum antimicrobial activity and their capability to inhibit the growth of pathogenic microorganisms through multiple mechanisms. In recent years, Ag-based nanomaterials have been increasingly incorporated into polymeric matrices and porous supports in order to improve the stability, dispersion, and practical applicability of silver species. Compared with conventional antibiotics, silver nanoparticles exhibit lower susceptibility to bacterial resistance and can interact with bacterial membranes, proteins, and intracellular components, resulting in the suppression of microbial growth (Qing et al., 2018), (Slavin et al., 2017). Although numerous studies have reported the antibacterial activity of Ag-NPs against gram-negative bacteria, investigations involving gram-positive bacteria remain relatively limited due to the more complex and thicker peptidoglycan layer of gram-positive cell walls, which may influence the antibacterial performance of silver species (Wang et al., 2017).

Biochar derived from agricultural biomass has recently emerged as an attractive supporting material for metal nanoparticles owing to its porous structure, surface functionality, and environmental sustainability (Dong et al., 2023), (Zhao et al., 2025). In particular, agricultural residues are considered promising low-cost carbon precursors for preparing functional biochar materials with high added value. Corn husk is one of the major agricultural wastes generated in large quantities from maize processing; however, its utilization remains limited in many regions. Previous studies have demonstrated that biochar obtained from lignocellulosic biomass can serve as an effective support for immobilizing silver nanoparticles and enhancing antibacterial performance (Eltaweil et al., 2022). The incorporation of Ag-NPs onto biochar surfaces not only improves nanoparticle dispersion but may also reduce particle aggregation and facilitate controlled release of silver species during application.

Despite these advantages, the direct use of powdered Ag-loaded biochar still presents several practical limitations, particularly in aqueous systems, including difficulty in separation, poor handling

characteristics, and limited reusability. To overcome these drawbacks, hydrogel-based immobilization systems have been widely investigated (Lee et al., 2012), (Dragan et al., 2014), (Hu et al., 2020). Sodium alginate (SA), a naturally derived polysaccharide, has been extensively employed for bead fabrication because of its biocompatibility, hydrophilicity, and ability to form three-dimensional networks through ionic crosslinking with divalent cations such as Ca^{2+} (Lee et al., 2012). Nevertheless, alginate-based hydrogels generally exhibit insufficient mechanical stability and excessive swelling under prolonged exposure to aqueous environments, which may restrict their practical applications (Dragan et al., 2014).

Polyvinyl alcohol (PVA) is a hydrophilic synthetic polymer widely recognized for its excellent film-forming ability, chemical stability, and mechanical properties. The incorporation of PVA into alginate matrices has been reported to improve structural integrity and dimensional stability of hydrogel systems through intermolecular hydrogen bonding and polymer chain interactions (Saad et al., 2024). In addition, embedding functional carbonaceous fillers within PVA/SA hydrogel networks may further enhance mechanical performance and reduce excessive swelling behavior.

Several previous studies have reported antibacterial hydrogel composites containing silver nanoparticles or biochar-based materials (Xie et al., 2023). However, most investigations mainly focused on antibacterial activity against gram-negative bacteria or adsorption-related applications, while studies involving gram-positive bacterial inhibition together with structural stability evaluation under simulated physiological conditions are still limited as summarized in Table 1. Furthermore, only a few reports have explored the utilization of agricultural waste-derived biochar as a supporting material for green-synthesized silver nanoparticles within PVA/alginate hydrogel bead systems.

In the present work, silver nanoparticle-loaded biochar derived from corn husk was successfully prepared through a green synthesis approach using tea polyphenols as a reducing agent and subsequently incorporated into PVA/sodium alginate hydrogel beads via ionic crosslinking. The resulting PVA/Ag-BCH/SA composite beads were systematically characterized in terms of physicochemical properties, swelling behavior, structural stability, and mechanical performance. In addition, the antibacterial activity of the prepared composite beads against gram-positive bacteria, including *Staphylococcus aureus* and *Staphylococcus epidermidis*, was investigated. The present study demonstrates an alternative approach for converting agricultural waste into functional antibacterial composite materials with potential applications in biomedical and related fields. Although Ag-containing PVA/alginate hydrogel systems have previously been reported, the present work differs from earlier studies in terms of the utilization of corn husk-derived biochar prepared from agricultural waste, green synthesis of Ag nanoparticles using tea polyphenols, and evaluation of antibacterial stability against gram-positive bacteria under simulated sweat conditions.

Table 1. Comparison of previously reported Ag-based hydrogel composites and the present work.

Material	Biochar source/support	Polymer matrix	Target bacteria	Key feature	Ref.
AgNP-loaded hydrogel beads	–	Alginate	*E. coli*	Antibacterial activity in aqueous system	(Lee et al., 2012)
Ag/biochar composite beads	Wood-derived biochar	PVA/SA	Gram-negative bacteria	Improved antibacterial activity	(Xie et al., 2023)

Biochar-supported AgNP composite	Agricultural biomass biochar	–	Mixed bacterial strains	AgNP stabilization on porous carbon	(Eltaweil et al., 2022)
PVA/alginate hydrogel beads	–	PVA/SA	–	Enhanced mechanical stability	(Candry *et* al., 2022)
Present work	Corn husk biochar	PVA/SA	*S. aureus, S. epidermidis*	Green synthesis, gram-positive antibacterial activity, simulated sweat stability	This work

Methodology:-

Chemicals and reagents

Corn husk residue (CH) was collected from Chiang Mai province, Thailand. Silver nitrate (analytical grade 99.9%, $AgNO_3$), Sodium Alginate (analytical grade 99%, SA) and Calcium chloride (analytical grade ≥ 97 %, $CaCl_2$) were purchased from Sigma-Aldrich. Tea polyphenol extract (98%) was obtained from Word-way Biotech Inc. All chemicals were of analytical grade and were used without further purification.

Preparation of polyvinyl alcohol/Ag-NPs biochar/sodium alginate (PVA/Ag-BCH/SA) composite bead

Corn husk residue was used as the raw material for biochar production. The residue (40 g) was washed thoroughly with deionized water and dried at 110 °C overnight, followed by grinding and sieving to obtain particle sizes smaller than 10 mm. The prepared material was subsequently pyrolyzed in a tube furnace (Sante STG-100) at 600 °C for 4 h with a heating rate of 5 °C min^{-1} to produce corn husk biochar (BCH), which was stored in a desiccator. For the preparation of silver loaded biochar (Ag-BCH), a green synthesis approach was employed using polyphenol extracted from green tea as a reducing agent. In summary, 100 mL of 3 mM green tea extract was combined with 100 mL of 1.25 mM $AgNO_3$ solution, followed by the addition of 10 g of BCH to the mixture (Tran et al., 2022). The solution was agitated at 50 °C for 1 hour, following which the pH was incrementally raised to above 8 using 0.1 N NaOH to promote the deposition of silver nanoparticles onto the surface of biochar. The mixture was further heated for 1 h, and the resulting Ag-BCH was filtered, oven-dried at 80 °C, and stored in a desiccator prior to further use.

A 2% sodium alginate (SA) solution and a 4% polyvinyl alcohol (PVA) solution were prepared, and 2.0 g/L of silver corn husk biochar (Ag-BCH) was added to the 2% SA solution, stirred evenly, sonicated, and left to stand for defoaming (PVA/Ag-BCH/SA). Subsequently, the PVA/Ag-BCH/SA mixture was drawn up into a syringe and dropped into 45 mL of 10% $CaCl_2$ solution. The composite bead was placed in a refrigerator at 4 °C for 12 h, then washed with deionized water many times.

In addition, the polyvinyl alcohol/sodium alginate (PVA/SA) composite bead was synthesized as the same procedure as PVA/Ag-BCH/SA but without adding silver-corn husk biochar.

Characterization of composite bead

Fourier-transform infrared spectroscopy (FTIR) was used to identify functional groups in the composites, using a Perkin-Elmer spectrometer with the KBr method. Sixty-four scans throughout the range of 800-4000 cm^{-1} at a resolution of 4 cm^{-1} were averaged for each spectrum. The measurement of N_2 adsorption-desorption was performed at -196 ºC utilizing an Anton Paar Nova 600 to determine the textural characteristics of the materials. The mass of the sample, initially approximately 40 mg, was precisely recorded following pretreatment at 150 ºC for a duration of 2 h. The X-ray fluorescence analysis (XRF) was performed on a M4 Tornadoplus Bruker, X-ray fluorescence spectrometer for quantitatively analyzing the Ag content of the samples. The SEM micrographs were captured with a magnification of 120× using a JEOL JSM-IT800. The compressive strength of the optimal swollen composite bead was assessed utilizing Universal Testing Machines, INSTRON/5566. The sample was meticulously prepared in a cylindrical shape. Subsequently, it was submerged in distilled water for a duration of one hour. The swollen cylinder exhibited a surface area of 15.89 cm^2, with a length measuring 2 cm. The enlarged sample was placed into the machine for the compression test at room temperature.

Antibacterial activity tests

The obtained PVA/Ag-BCH/SA and PVA/SA composite bead have been tested for anti-bacterial activity against gram positive bacteria such as *S. aureus* and *S. epidermidis* by the zone of inhibition test.

Results and Discussion:-

Characterization of composite beads

The FT-IR spectra of PVA/SA and PVA/Ag-BCH/SA composite beads are shown in Figure 1. A broad absorption band observed around 3350 cm^{-1} is attributed to hydrogen bonding interactions between the hydroxyl groups of the PVA matrix and surface functional groups of the biochar, which is commonly reported in PVA based composite systems (Xie et al., 2023), (Candry et al., 2022). The absorption bands at approximately 2910 cm^{-1} and 1433 cm^{-1} correspond to the stretching and bending vibrations of C–H bonds, respectively (Xie et al., 2023). Furthermore, the peaks located near 1680 cm^{-1} and 1388 cm^{-1} are assigned to the asymmetric and symmetric stretching vibrations of carboxylate groups ($–COO^–$), indicating intermolecular interactions between PVA and sodium alginate chains through hydrogen bonding [14]. A characteristic band appearing around 1600 cm^{-1} is associated with N–H stretching vibrations, which may originate from nitrogen-containing functional groups on the biochar surface (Candry et al., 2022). The absorption peaks at 1080 cm^{-1} and 1020 cm^{-1} can be attributed to the vibrational modes of C–N/O and C–O–C bonds, respectively (Candry et al., 2022). These spectral features suggest the occurrence of intermolecular interactions and crosslinking between PVA and SA, leading to the formation of stable composite bead structures.

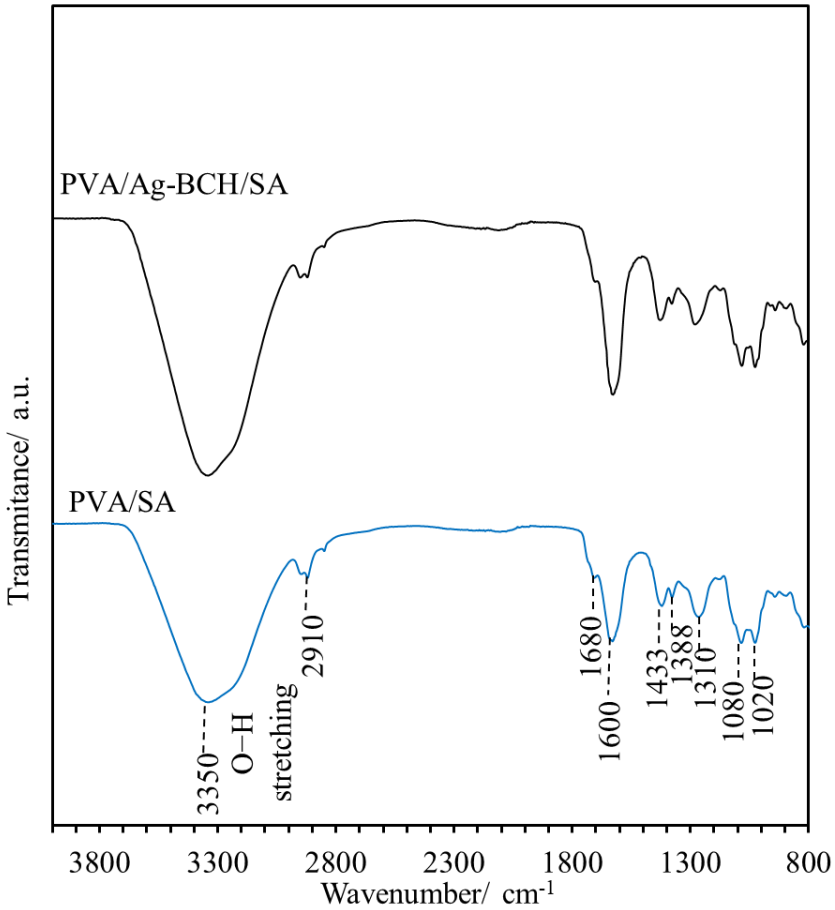

Figure 1. FTIR spectra of PVA/SA and PVA/Ag-BCH/SA composite bead.

Table 2 shows the physicochemical parameters of the hydrogel bead composites that were prepared. The findings demonstrate that the PVA/Ag-BCH/SA composite beads exhibited a greater BET surface area in comparison to the PVA/SA beads. The addition of Ag-modified biochar to the hydrogel matrix caused this increase by creating more accessible pores. Biochar particles inside the polymer network disrupt polymer chain arrangement, leading to a less compact internal structure and an increased number of sites for nitrogen adsorption (Eltaweil et al., 2022), (Xie et al., 2023).

XRF analysis indicated that silver species were successfully added to the composite beads. The silver concentration in the PVA/Ag-BCH/SA beads was found to be around 8.2 wt.%. This amount of silver is close to what has been reported for other silver-modified carbon-based composites made using similar methods. The relatively stable silver concentration indicates that the Ag-BCH particles were evenly spread out throughout the PVA/SA matrix instead of only being on the surface of the beads. This internal distribution is anticipated to affect the textural properties and functional performance of the composite material, especially regarding adsorption capacity and antibacterial efficacy (Eltaweil et al., 2022).

It is also noted that the BET surface area values of both hydrogel bead samples were inferior to those typically documented for standard porous adsorbents. This is a common feature of hydrogel-based materials, where the swelling of the polymer and the density of the network make it hard to get to the interior surfaces. Despite this intrinsic constraint, the significant augmentation of surface area attained with the integration of Ag-BCH illustrates its dual function as both a structural filler and a porosity-enhancing agent within the hydrogel system. These attributes are advantageous for applications necessitating a balance of surface functioning, mechanical stability, and overall material performance, particularly in environmental and biomedical domains (Xie et al., 2023).

Table 2. Physicochemical properties of the composite beads.

Sample	S_{BET} [a]	% Ag [b]
	(m^2 g^{-1})	
PVA/SA	1.78	*n.d.*
PVA/Ag-BCH/SA	2.38	8.2

[a] BET surface area
[b] Determined from XRF

In order to represent circumstances pertinent to skin-contact applications, the hydrogel bead composites' swelling behavior was investigated in deionized water and simulated sweat solution, as shown in Figure 2. Since swelling behavior has a direct impact on overall performance and dimensional stability, it is a crucial property of hydrogel materials. The swelling ratio progressively rose when the beads were submerged in the simulated sweat solution for both PVA/SA and PVA/Ag-BCH/SA. This behavior shows that the liquid phase is gradually absorbed into the polymer network, causing the hydrogel structure to expand. Deionized water showed a similar pattern, although the swelling was often less severe.

The PVA/SA beads exhibited a greater swelling ratio than the PVA/Ag-BCH/SA composite beads when evaluated under identical experimental circumstances. The addition of biochar particles may have led to a more constrained polymer network, which inhibited chain mobility and decreased excessive expansion, as seen by the lower swelling seen for the Ag-BCH-containing beads [16]. Ag-BCH's reinforcing function inside the hydrogel matrix is responsible for this effect. The morphological features shown by SEM examination (Figure 4), which showed that the PVA/Ag-BCH/SA beads had a denser internal structure, suggesting greater mechanical stability, are consistent with the observed swelling behavior.

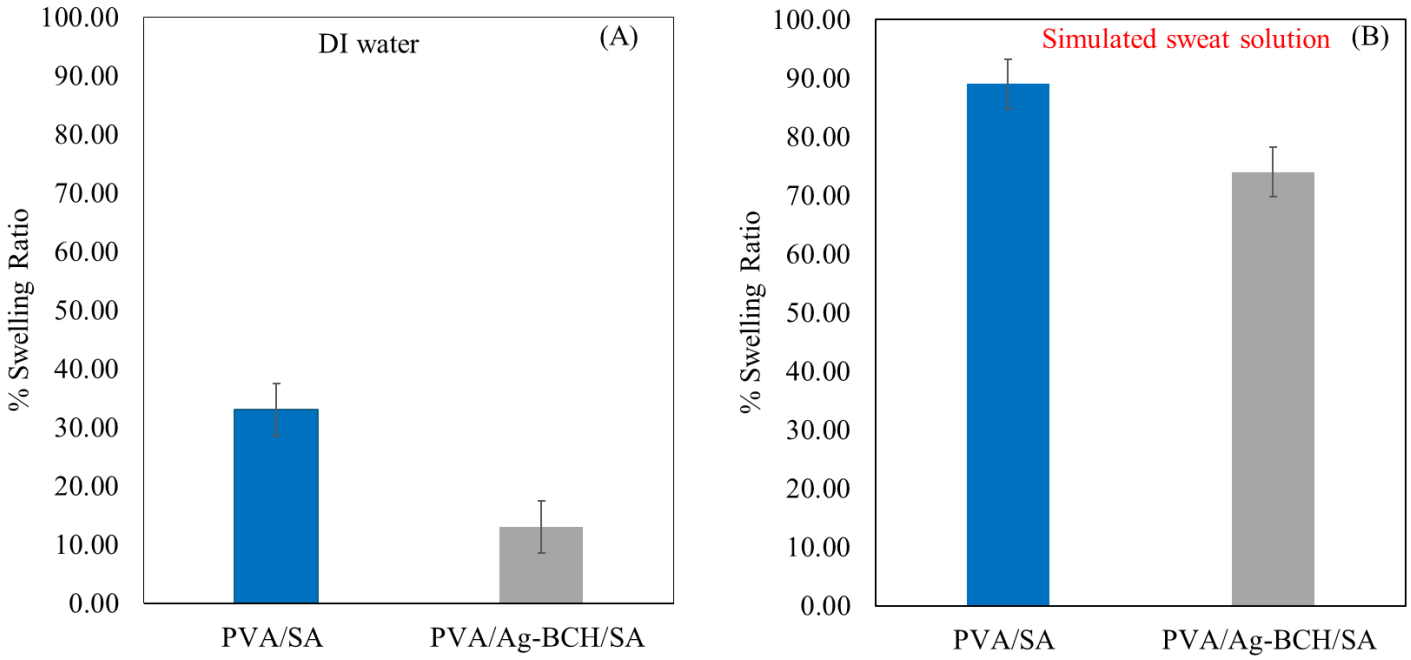

Figure 2. Swelling ratio of PVA/SA and PVA/Ag-BCH/SA hydrogel bead composites in (A) DI water and (B) simulated sweat solution

The hydrogel beads' macroscopic appearance after 94 hours of immersion in a sweat simulation solution is shown in Figure 3. Both kinds of beads showed no signs of breaking or disintegrating under these circumstances, remaining whole and round. After extended exposure to the simulated environment, there was no discernible change in the size of the PVA/Ag-BCH/SA beads, which had an average diameter of around 1.25 mm. The steady bead morphology indicates that the addition of Ag-BCH improved the hydrogel matrix's structural integrity, enabling the beads to keep their physical shape over time.

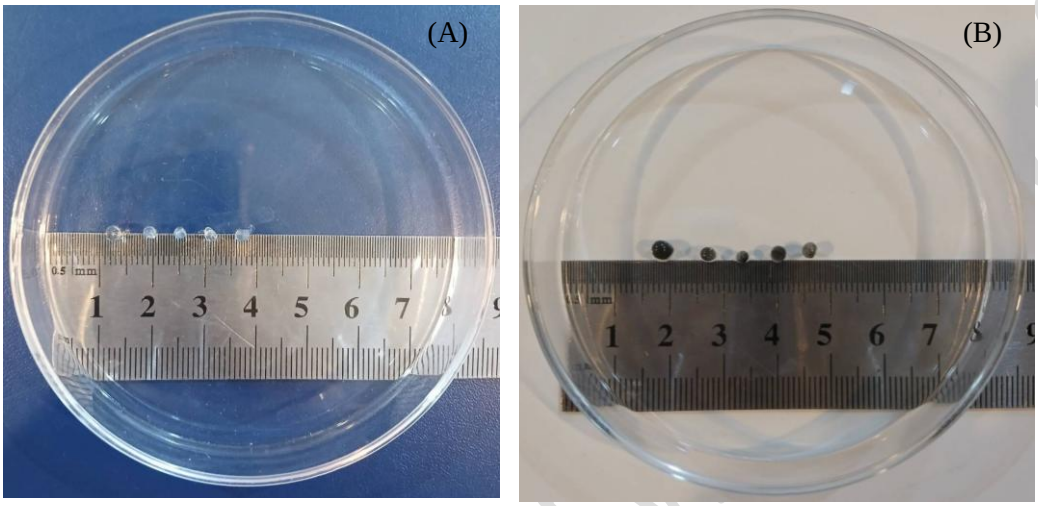

Figure 3. Stability of (A) PVA/SA and (B) PVA/Ag-BCH/SA hydrogel beads following extended immersion (94 h) in a simulated sweat solution.

Figure 4 displays SEM micrographs of the PVA/Ag-BCH/SA composite beads both before and after swelling. After immersion, there were no visible indications of surface degradation, and the bead surfaces seemed undamaged. After swelling, there was a little increase in surface roughness, but the overall shape of the bead remained same. These findings imply that the hydrogel matrix did not significantly deteriorate morphologically while maintaining enough structural resilience to tolerate swelling.

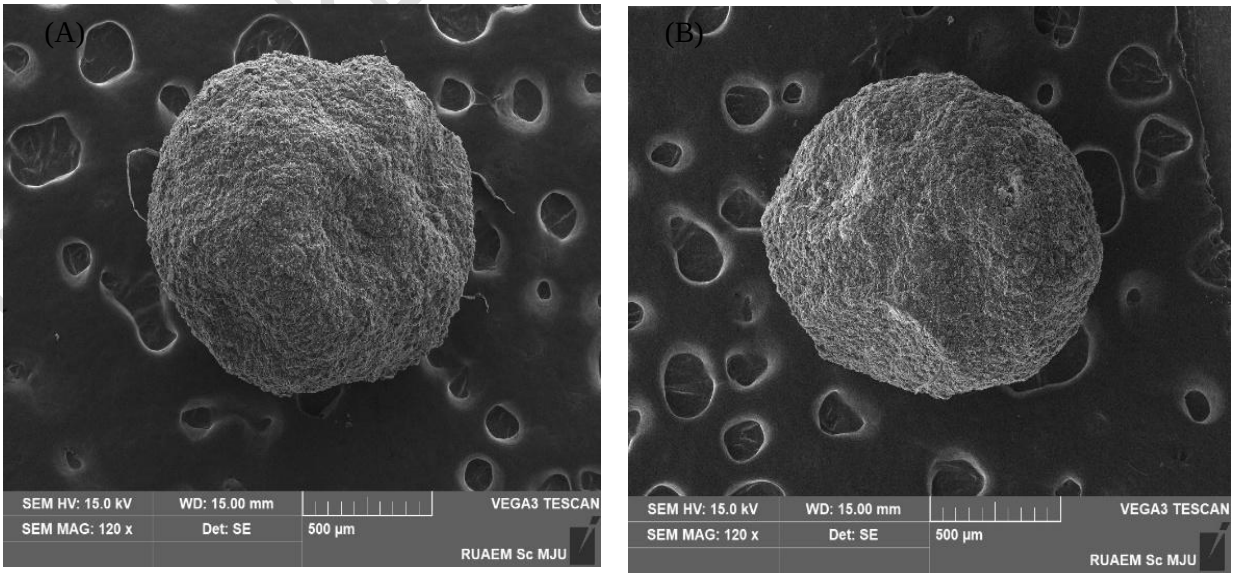

Figure 4. SEM images (120 x magnification) of PVA/Ag-BCH/SA composite bead (A) before and (B) after immersing in simulated sweat solution.

Compressive strength measurements were used to assess the mechanical behavior of the swelled hydrogel beads, as seen in Figure 5. With no discernible shape change during unloading, the PVA/SA beads demonstrated a compressive strength of 19.14 kN m^{-2} and could withstand compressive deformation of up to about 50% of their initial height. These findings show that the PVA/SA beads have enough flexibility to regain their form while still being able to support loads.

Under the same testing circumstances, the PVA/Ag-BCH/SA composite beads showed a slightly lower compressive strength of 16.64 kN m^{-2}. Despite a little decrease in compressive strength due to the addition of Ag-BCH, the composite beads' mechanical stability and structural integrity were still satisfactory (Xie et al., 2023). Although the compressive strength slightly decreased after Ag-BCH incorporation, the composite beads still retained adequate mechanical stability for handling and aqueous applications. Applications require prolonged usage in aqueous settings, where resistance to deformation is necessary to maintain material functioning, benefiting from such mechanical performance (Eltaweil et al., 2022).

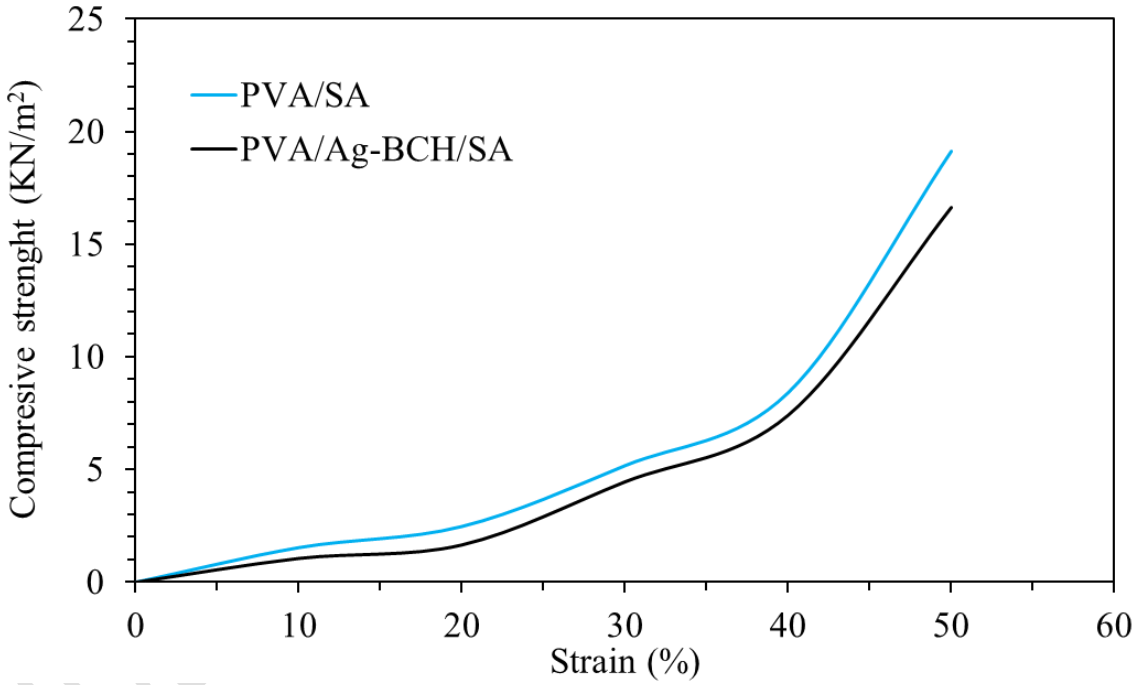

Figure 5. The compression strength test results of the swollen PVA/SA and PVA/Ag-BCH/SA composite beads.

Antibacterial activity of composite beads

The antibacterial efficacy of the synthesized hydrogel beads was assessed against *Staphylococcus aureus* and *Staphylococcus epidermidis*, two types of gram-positive bacteria. Figures 6 and 7 demonstrate the existence of inhibitory zones around the PVA/Ag-BCH/SA composite beads for both bacterial strains. However, no inhibitory zones were seen around the PVA/SA beads in the absence of Ag-BCH. This finding suggests that the integration of silver-modified biochar into the hydrogel system conferred antibacterial characteristics. The PVA/Ag-BCH/SA beads generated inhibitory zones of about 14–15 mm

in width for both bacterial species. The absence of inhibitory zones for the PVA/SA beads suggests that the polymer matrix inherently lacks antibacterial properties. The same inhibitory zone widths for *S. aureus* and *S. epidermidis* suggest a comparable antibacterial response to both gram-positive bacteria.

The antibacterial characteristics of the PVA/Ag-BCH/SA beads are attributed to the liberation of silver ions from the Ag-BCH particles embedded inside the hydrogel matrix. The PVA-based network may absorb water, allowing liquids to penetrate the beads. This facilitates the movement of silver ions from the inside to the outside. The released silver species are recognized for their interaction with bacterial cells, influencing standard biological processes and hindering bacterial growth. The biochar in the composite helps silver species spread evenly throughout the hydrogel matrix, which keeps the antibacterial effect steady (Slavin et al., 2017), (Eltaweil et al., 2022). The results show that adding Ag-BCH to the PVA/SA hydrogel beads provides them with antibacterial characteristics without changing their structure. These results suggest the potential applicability of the developed composite beads in antibacterial and biomedical-related applications.

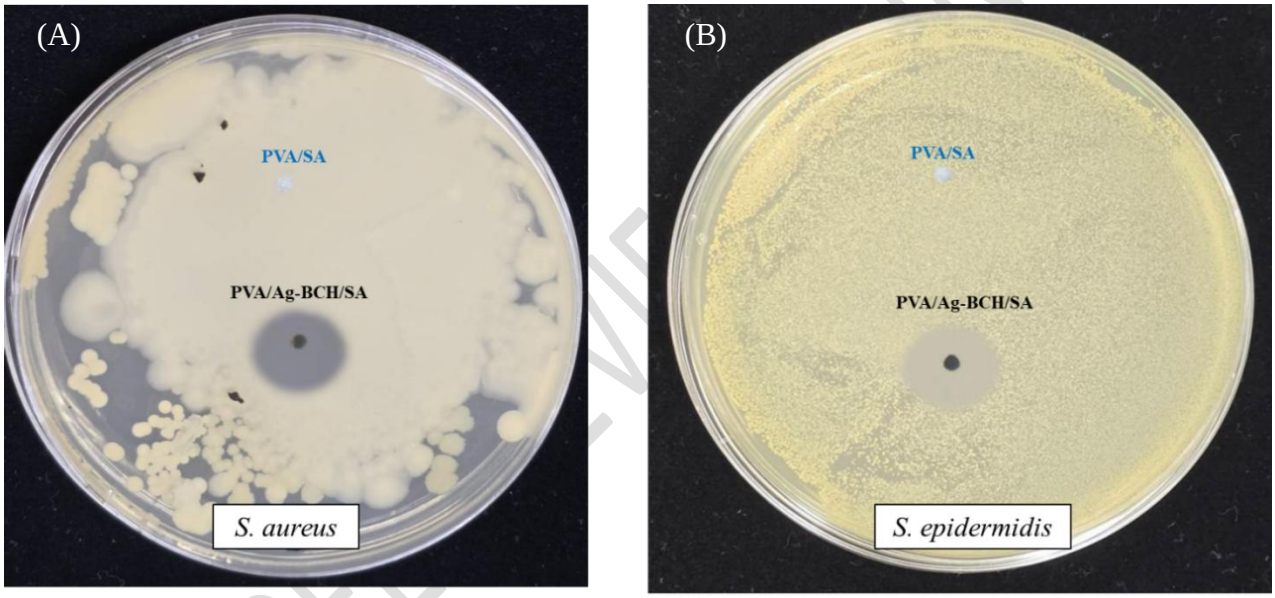

Figure 6. Representation of inhibition tests against (A) *S. aureus* and (B) *S. epidermidis* for PVA/SA and PVA/Ag-BCH/SA composite bead.

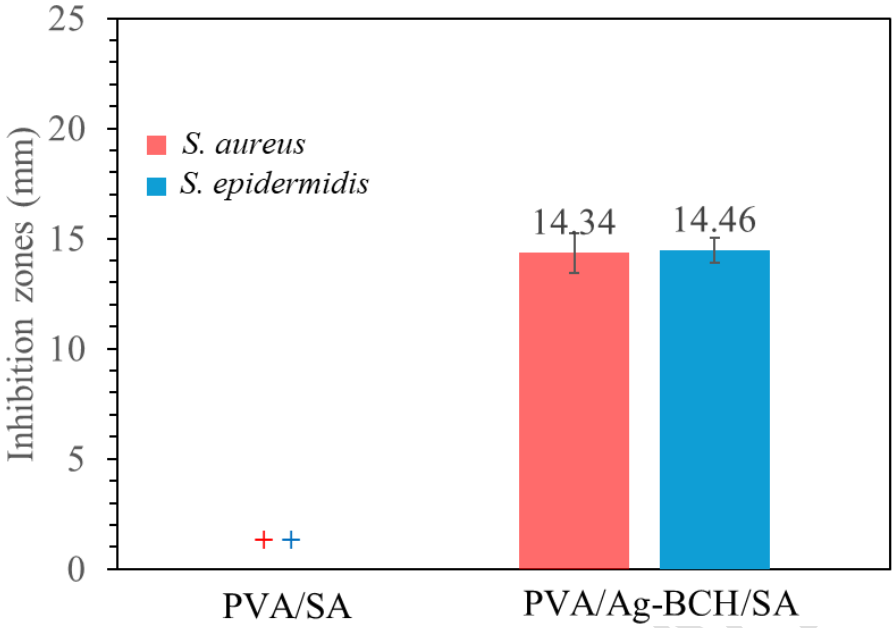

Figure 7. Antibacterial effects of PVA/SA and PVA/Ag-BCH/SA composite bead (++ no antibacterial effect).

Conclusion:-

This work developed composite hydrogel beads composed of PVA, sodium alginate, and silver nanoparticle-loaded biochar from corn husk utilizing green synthesis and ionic crosslinking methods. The addition of Ag-BCH improved the physicochemical characteristics and structural stability of the hydrogel beads, reduced excessive swelling, and maintained satisfactory mechanical performance in aqueous environments. The antibacterial test indicated that the PVA/Ag-BCH/SA beads inhibited the growth of gram-positive bacteria, specifically *Staphylococcus aureus* and *Staphylococcus epidermidis*. The PVA/SA beads without Ag-BCH did not have any antibacterial effect. The antibacterial properties of the composite beads are related to the release of silver species from the Ag-BCH embedded in the hydrophilic polymer matrix. Although the developed composite beads demonstrated promising antibacterial activity and structural stability, additional studies regarding silver ion release kinetics, cytotoxicity, long-term durability, and reusability are still required before practical biomedical applications.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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