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Biodegradable Packaging Based on Poly(vinyl Alcohol) and Carboxymethyl Cellulose Films Incorporated with Ascorbic Acid for Food Packaging Applications

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ABSTRACT: Petroleum-based plastics are used as packaging materials because of their low cost and high availability; however, continuous use of these nondegradable materials especially in the food industry has led to environmental pollution. The present study aimed to synthesize antibacterial and biodegradable films based on natural biopolymers carboxymethyl cellulose (CMC), poly(vinyl alcohol) (PVA), and ascorbic acid (AA) cross-linked in the presence of glutaraldehyde (GA). The films were synthesized in two different concentrations, 60PVA:40CMC:AA and 70PVA:30CMC:AA with a fixed amount of AA. Films with smooth texture and overall uniform thickness were obtained. Fourier transform infrared spectroscopy (FTIR) confirmed the cross-linking between the aldehyde group of GA and hydroxyl of PVA through detection of acetal and ether bridges. The synthesized films were thermally stable in the temperature range of 180−300 °C; however, 70PVA:30CMC:AA showed higher weight loss in this range as compared to the 60PVA:40CMC:AA film. Soil burial test demonstrated that the 60PVA:40CMC:AA film was more degradable (71% at day 15) as compared to the 70PVA:30CMC:AA film (65% at day 15). The films exhibited excellent antimicrobial activity against Gram-positive *staphylococcus aureus* (inhibition zone of 21 mm) and Gram-negative *Escherichia coli* (inhibition zone of 15 mm). In comparison, the 60PVA:40CMC:AA film showed better results in terms of high mechanical strength, uniform morphology, higher soil burial degradation, and lower water vapor transmission rate. Therefore, the prepared film could be used as a promising candidate in the food packaging industry.
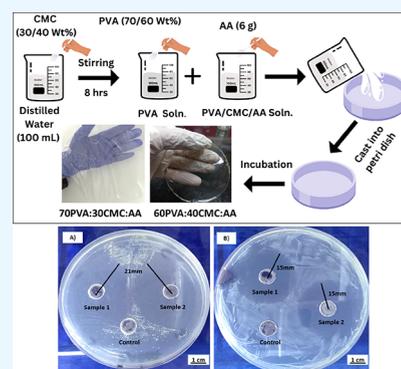
1. INTRODUCTION

Disposable and fossil-based plastics have added to environmental pollution for the last few decades. The driving forces behind shifting fossil-based disposable polymers to biodegradable polymers are the depletion of fossil resources, environmental pollution, and global warming.[1−3] The use of nonbiodegradable and nonrenewable petrochemical-based packaging in food preservation has caused a great hazard to the environment.[4] Many struggles have been to overcome the problems caused by nondegradable plastic waste. The need is to find an eco-friendly and biodegradable material of low cost, availability, and nontoxicity. Biodegradable is defined in terms of the action of naturally occurring microorganisms to break down the materials by giving the organic byproduct with no release of any harmful gases.[2] These biopolymers are renewable and biodegradable, all together for packaging materials to qualify as biodegradable, and they should break down completely and crumble into normal elements without releasing any harmful gases after disposal.[5]

A novel, eco-friendly material should be characterized by adequate durability. It should limit the growth of microorganisms accelerating food spoilage and maintain high food quality.[6] Polymer-based materials are best to use among other

packaging materials as they have some advantageous properties comparatively such as easy handling, lightweight, transparency, water impermeability, and others. Polymers of natural and synthetic origin, such as polysaccharides (cellulose, starch, chitosan), proteins (gelatin), polylactic acid (PLA), polyhydroxyalkanoates (PHAs), polyethylene (PE), etc., seem most promising as edible, intelligent, and biocompatible materials, which also fulfill the packaging industry requirements.[7−9]

The present study is focused on the investigation of biodegradable polymers poly(vinyl alcohol) and carboxymethyl cellulose proposed for use in the food packaging industry. Carboxymethyl cellulose (CMC) is a widely available, well-utilized, and superior polysaccharide-based biopolymers. It is being in biotechnological and pharmaceutical applications for its vast advantageous properties.[9] CMC is a natural polymer and a derivative of cellulose. Due to its biocompatibility,
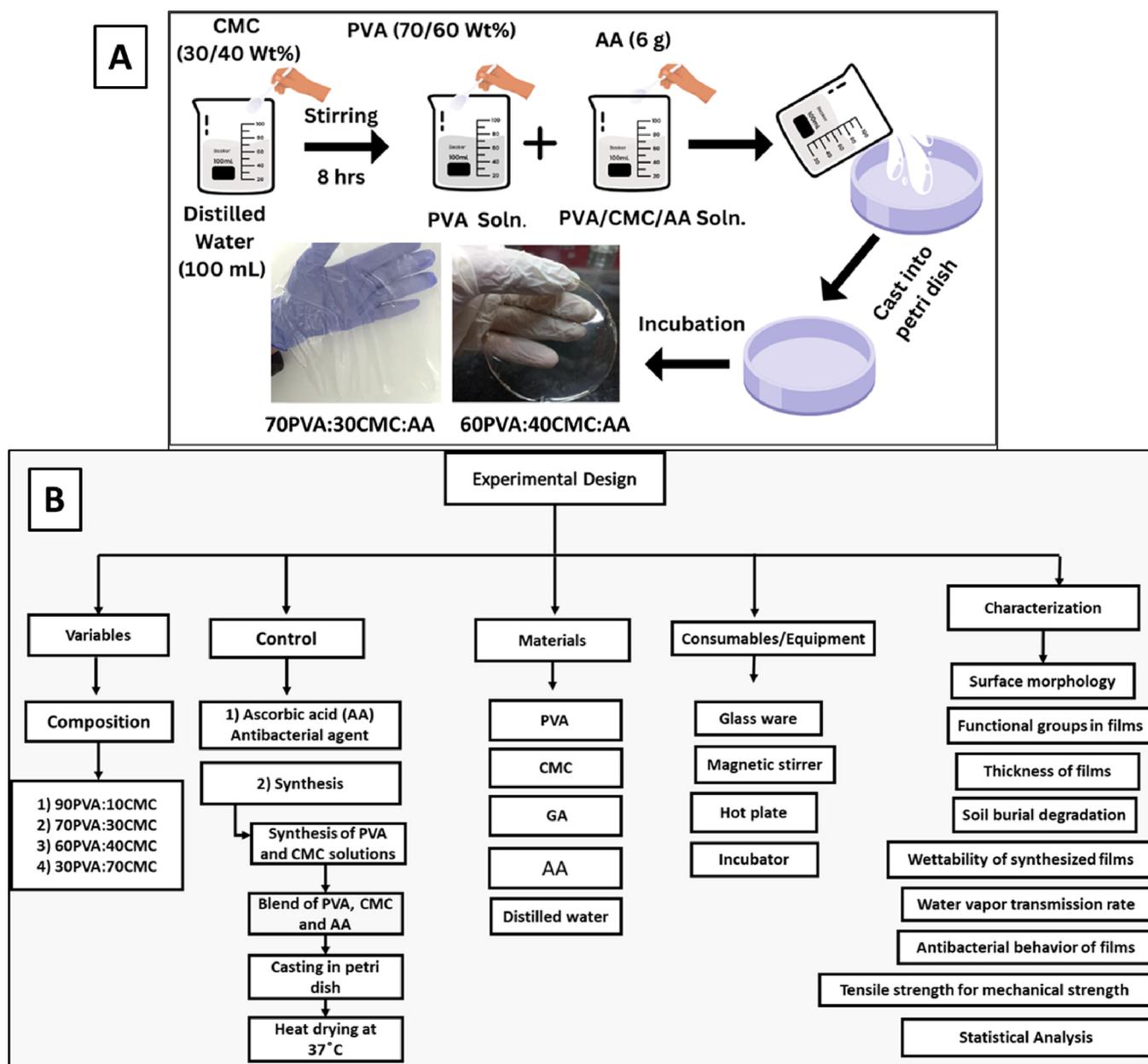
Figure 1. (A) Synthesis of 60PVA:40CMC:AA and 70PVA:30CMC:AA films. (B) Experimental design for the present study.

nontoxicity, and degradability, CMC is fundamentally used in food packaging applications.[10] Moreover, as a highly hydrophilic derivative of cellulose, CMC is extensively used as a suspending and thickening agent in the pharmaceutical and food industry. Due to its good modifiability, swell ability, and nontoxicity, CMC has engrossed the attention of scientists in the field of hydrogels for drug delivery applications.[8]

A conventionally used polymer, poly(vinyl alcohol) (PVA), has exceptional chemical and physical qualities, as well as high tensile and compression strengths when used to create polymer nanocomposites.[11−13] PVA is a hydrophilic synthetic polymer with sound mechanical properties, a planar zigzag structure, and semicrystalline nature. It possesses chemical and thermal stability and is resistant to degradation in most physiological environments. Moreover, it is water-soluble due to its high polarity, high biocompatibility, and nontoxicity. Therefore, the

mixture can be easily processed. PVA has also the great capability for producing biodegradable films.[8]

A variety of food products contain foodborne bacteria, which can be human pathogens and may spread foodborne illnesses over the globe. In this regard, there has been a great deal of interest in exploring packaging films with antimicrobial aspects. Shelf life of food can be increased by using antimicrobial packaging films, which serve as an excellent physical barrier against bacterial penetration. The development of food packaging films based on natural biomaterials including gelatin, cellulose, sodium alginate, and chitosan has received a great deal of attention.[6,8,9]

Lipid oxidation, microbial growth, and enzymatic browning cause food degradation triggered by oxygen. For this purpose, antioxidant compounds can be incorporated into food packaging materials to control the negative impact of reactive oxygen species. Ascorbic acid (AA), also termed vitamin C, of

plant origin, is well-known for its antioxidant and antimicrobial properties.[2,6,14]

Various combinations of PVA and CMC with different antimicrobial agents have been used. Youssef et.al[13] reported on antimicrobial packaging film based on biodegradable CMC/PVA-zeolite (ZA) doped with noble metal (i.e., gold: Au and silver: Ag) cations. The tensile strength of the synthesized films from CMC/PVA was improved by the addition of ZA-Au. Moreover, the prepared nanocomposite film possessed an enhanced water vapor transmission rate (WVTR) due to the characteristic porous nature of ZA. The films showed good antimicrobial and mechanical properties with the addition of ZA-Au or ZA-Ag. Amaregouda et al.[1] developed biodegradable PVA/CMC films incorporated with magnesium oxide nanoplates, previously functionalized with L-alanine for food packaging. The developed films showed excellent antimicrobial properties against *Escherichia coli* (*E. coli*) and *Candida albicans* (*C. albicans*) and also showed enhanced antioxidant potential. Ebrahimi et al.[15] used metallic Ag, zinc oxide, and copper oxide nanoparticles in combination with CMC for food packaging application. Films incorporated with nanoparticles showed antibacterial activity as compared to the pure CMC film.

In the present study, we have chosen a novel combination of AA with PVA and CMC for food packaging application, as it is an easily available and cheap antimicrobial agent with no adverse effects. The AA is a safer option to induce the antibacterial properties as compared to that of the metallic ions (may give the coloring effect) and conventional drugs. Thus, antibacterial ions (e.g., Ag, Fe, Cr, etc.) may not be a suitable choice if the release of antibacterial ions is not controlled.[16] Vitamin C, specifically L-AA has an excellent antimicrobial potential against various strains like *Bacillus subtilis*, *Corynebacterium diphtheria*, *Enterococcus faecalis*, and *Staphylococcus aureus* (*S. aureus*).[17] It is an organic compound that can be easily incorporated into packaging films. Additionally, it is a food additive, used as an antioxidant, food improver, and acidity regulator in various types of food products such as frozen food, meat, flour, juice, fresh produce, etc.,[18] which makes it an ideal choice among other antimicrobial agents. Two different compositions of PVA and CMC incorporated with a fixed amount of AA are made and compared for the best mechanical strength, water vapor transmission rate, biodegradability, microbial growth inhibition, and wetting behavior. As PVA is a biodegradable polymer and is susceptible to chemical hydrolysis, this may result in the breakdown of the polymer chain. This phenomenon is expected to weaken the films and result in the degradation of synthesized films.[19] However, PVA degradation may take longer; hence, the addition of CMC will accelerate the degradation rate of the films as it provides a source of carbon and energy for the microbes as they play a major role in the degradation process.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents. Poly(vinyl alcohol) (PVA; MW 146,000−186 000 g/mol, 98% hydrolyzed), carboxymethylcellulose sodium salt (CMC; MW 263.20 g/mol) with 0.75 degrees of substitution, and L-ascorbic acid (AA) were used for the film formation. Glutaraldehyde (GA) solution (50 wt % in H$_2$O) was used as a cross-linking agent. All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Synthesis of PVA-CMC-AA Film. PVA (10 g) was slowly added in 50 mL of preheated (~95 °C) distilled water

and magnetically stirred until the complete dissolution of PVA. At the same time, 2 g of CMC was dissolved in 50 mL of distilled water at room temperature and stirred overnight. Then, PVA and CMC solutions were mixed by magnetic stirring to form a binary blend solution in two different ratios, i.e., 60PVA:40CMC:AA and 70PVA:30CMC:AA. Afterward, 6 g of AA (MIC: 60 mg/mL) was added to 100 mL of both blends to form a ternary blend. Later on, the ternary blend was cross-linked by dropwise addition of 100 μL of GA. The homogeneous mixture was then poured into plastic Petri dishes, sealed with parafilm, and dried at 37 °C. Figure 1 shows a schematic of the synthesis process of the films.

2.3. Characterization of Synthesized Films. *2.3.1. Functional Groups in Films.* Films of PVA-CMC-AA were assessed by an attenuated total reflection Fourier transformation infrared (ATR-FTIR) spectroscope (Thermofisher Nicolet Summit Pro) equipped with a diamond crystal having a unique spectrum and the OMNIC paradigm software. The spectrum was obtained in transmittance mode in the range of 4000−400 cm^{-1} at a resolution of 4 cm^{-1}.

2.3.2. Morphology of Films. PVA-CMC films of both compositions were further characterized by field emission scanning electron microscopy (FESEM) (Mira 3 TESCAN) integrated with an energy dispersive spectroscopy (EDS) (Oxford Instruments) analyzer. Images were collected in secondary electron (SE) mode at a 10 kV accelerating voltage. SEM-EDS was employed to judge the surface morphology and elemental composition of the synthesized films.

2.3.3. Thickness of Films. The thickness of the prepared PVA-CMC films was determined by a micrometer screw gauge (Fujisan Digital micrometer) with the least count value of 0.001 mm. The thickness was noted at six different points around the films, and the mean value with standard deviation was calculated.

2.3.4. Thermal Stability of Films. The thermal stability of films was characterized via thermogravimetric analysis (TGA 1 STAR System, METTLER TOLEDO). Ten mg of each film was analyzed at a heating rate of 10 °C/min in an N$_2$ environment in the temperature range of 25−500 °C. TGA gives the variation in the mass of the sample, measured over time with temperature changes, such as phase changes, solid−gas reactions, and thermal decomposition.

2.4. Soil Burial Degradation (SBD). Soil burial degradation (SBD) of the prepared films was evaluated by using the reported method in the literature.[20] The film sample (1.415 cm × 1.415 cm) was weighed after drying. The weighed dry films were then buried at a depth of 10 cm in black soil. Ten mL of water was supplied every day for 15 days. After the desired period, the buried films were carefully removed from the soil, washed with water, and dried. The degraded samples were weighed again and the percentage degradation was calculated using eq 1.

$$\%SBD = (W_i - W_f) \times 100 \tag{1}$$

2.5. Wettability of Synthesized Films. The wetting behavior of synthesized packaging was determined by placing a 10 μL drop of distilled water on the surface of 60PVA:40CMC:AA and 70PVA:30CMC:AA films using a microliter pipet following the protocol reported in the literature.[21,22] The images of the drop were captured by using a digital camera within 5 s of placing the drop. The contact angle between the surface of the films and the water drop was obtained by analyzing the images in ImageJ. The angles were measured in

triplicate for each film, and the contact angle was obtained as the mean value of the three measurements.

2.6. Water Vapor Transmission Rate (WVTR). The developed films were evaluated for their water vapor transmission rate (WVTR) reported in the literature.[23] To perform the test, 10 mL of DI water was placed in a glass bottle with a 20 mm inner diameter. The mouth of the bottle was enclosed with the prepared films and tightened with Teflon tape. The weight (W_i) of the bottle was recorded before heating, and it was then kept in a hot air oven at 40 °C for 24 h. After the desired period, the sample bottle was weighed (W_f) again, and the WVTR was estimated using eq 2.

$$WVTR = [W_i - W_f/A] \times T \qquad (2)$$

where A is the area of the mouth of the bottle and T is the time.

2.7. Antibacterial Behavior of Films. The antibacterial behavior of the prepared films was assessed using the well diffusion method. The prepared films were tested against Gram-positive *S. aureus* and Gram-negative *E. coli*. To begin the test, the pure colonies of the two bacterial strains were inoculated into nutrient broth culture, followed by 24 h incubation. The blend of polymers containing AA was made for the agar well diffusion technique. After that, an aliquot of 20 μL of each bacterial strain having an OD_{600} of 0.015 was spread on sterilized nutrient agar plates. An 8 mm biopsy bore was used to make wells for each test and control samples without AA in the sterile agar plates. Wells were filled with the blends of test samples containing PVA-CMC-AA and control sample PVA-CMC only, followed by incubation for 24 h at 37 °C. The zone of inhibition was measured against both strains of bacteria after 24 h to assess the antibacterial efficacy of the films.

2.8. Tensile Test for Mechanical Strength of Films. The mechanical strength of synthesized films was measured via a tensile test (ASTM D882) using a tensile testing machine (Linkam TST350 UK). Films were cut into 26 × 7 × 0.5 mm pieces for the test. A load cell of 200 N was applied at a crosshead speed of 83.35 μm/s. The films were stretched to the fracture point.

2.9. Statistical Analysis. The statistical analysis was carried out on obtained data to determine the variances in the test groups. One-way analysis of variance (ANOVA) with a significance value ($p < 0.05*$) was employed. Tukey's range test was utilized for the post-hoc analysis. The analysis was carried out using OriginPro 8.5 software. The reported results are mean ± standard deviation for experiments carried out in triplicates.

3. RESULTS AND DISCUSSION

3.1. ATR-FTIR Spectroscopy. FTIR spectra of the PVA, CMC, AA, and PVA/CMC/AA blends are shown in Figure 2. PVA showed a broad range of transmittance peaks between 3200 and 3530 cm^{-1} (−OH stretching). The peaks of −CH stretching, −CH bending, and −C−O stretching appeared at 2973, 1406, and 1055 cm^{-1}, respectively.[24] The FTIR spectra of CMC showed a strong broad band of −OH stretching at 3200−3510 cm^{-1}.[25] The CMC spectra showed −CH asymmetric stretching and −COO ester asymmetric stretching at 2906 and 1590 cm^{-1}, respectively.[26] In addition, the CH_2 and −OH bending peaks appeared at 1417 and 1325 cm^{-1}, respectively.[27] The FTIR spectra of AA showed an unsaturated C=C bond peak at 1653 cm^{-1}. Moreover, the enol hydroxyl

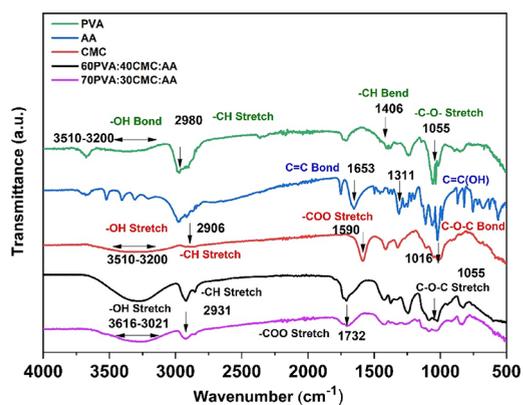**Figure 2.** FTIR spectra of PVA, AA, CMC, 60PVA:40CMC:AA, and 70PVA:30CMC:AA films.

(C=C(OH)) peak appeared at 1311 cm^{-1}.[28] PVA and CMC are hydrophilic individually. The 60PVA:40CMC:AA film spectra showed a band at 3000−3600 cm^{-1} attributed to the stretching of the hydroxyl group due to the strong cross-linking between the aldehyde group of GA and hydroxyl of PVA, forming highly intense sharp peaks corresponded to acetal and ether bridges.[29] However, in the case of weakly cross-linked 70PVA:30CMC:AA film, less intense peaks were observed for acetal and ether bridges.

The cross-linking of the film components in the presence of GA was also confirmed by the appearance of a −COO stretch with a slight shift at 1732 cm^{-1}, and C−H and C=O stretching of PVA at 2931, and 1091 cm^{-1}, respectively, while CMC was entrapped inside the network.[30,31] The characteristic peak of CMC at 1590 cm^{-1} was not detected in the synthesized film which could be due to the hindrance of the peak by the GA-cross-linked structure of the PVA.[32]

3.2. Surface Morphology of Films. To investigate the surfaces of the synthesized films, SEM was employed. Figure 3 shows SEM images of the synthesized films at different magnifications. The low magnification images (Figure 3A,C) shows that the synthesized films have a uniform and smooth surface with adequate structural integrity. The 60PVA:40CMC:AA film showed a continuous matrix with no cracks or porosity at high magnification (Figure 3B); however, a little porosity was observed in 70PVA:30CMC:AA film as shown in Figure 3D. The absence of any visible porosity maintains the water content of the packaged food restricting the water transmission from the films and keeping it fresh for an extended period. In this scenario, the 60PVA:40CMC:AA films seem more suitable for our application. The EDS analysis revealed that the films are composed of C and O mainly which are essentially the backbone of the polymers.

The thickness of the synthesized films was determined by a micrometer screw gauge. Average thickness value of 60PVA:40CMC:AA film was 0.36 ± 0.04 mm, and that for 60PVA:40CMC:AA film was 0.37 ± 0.05 mm. Hence, it was confirmed that the synthesized films had a uniform thickness all over.

3.3. Thermal Analysis. The thermal stability of films was characterized using TGA. In TGA, the variation in the weight of the films over the temperature change was measured. The TGA analysis of pure PVA (Figure 4A) showed two degradation steps. The first step indicated the degradation of PVA in the temperature range of 290−415 °C with a weight loss of 62%. The second degradation step showed 23% weight
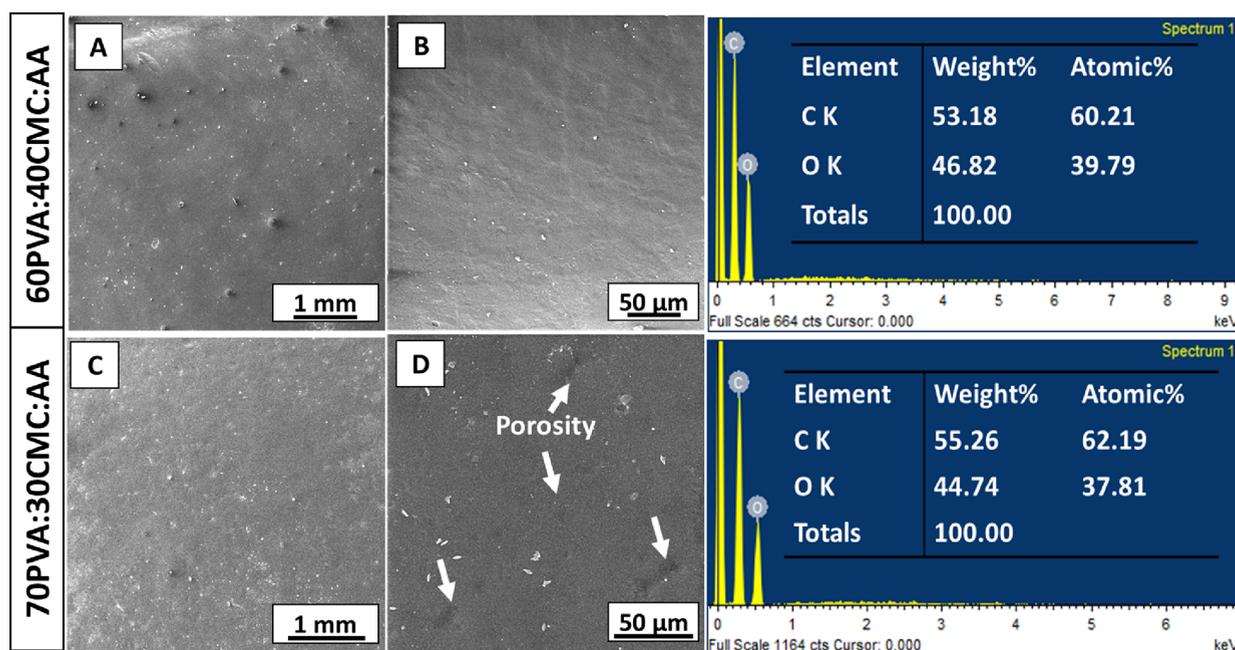
Figure 3. SEM images and EDX analysis at high and low magnification for (A, B) 60PVA:40CMC:AA and (C, D) 70PVA:30CMC:AA films.
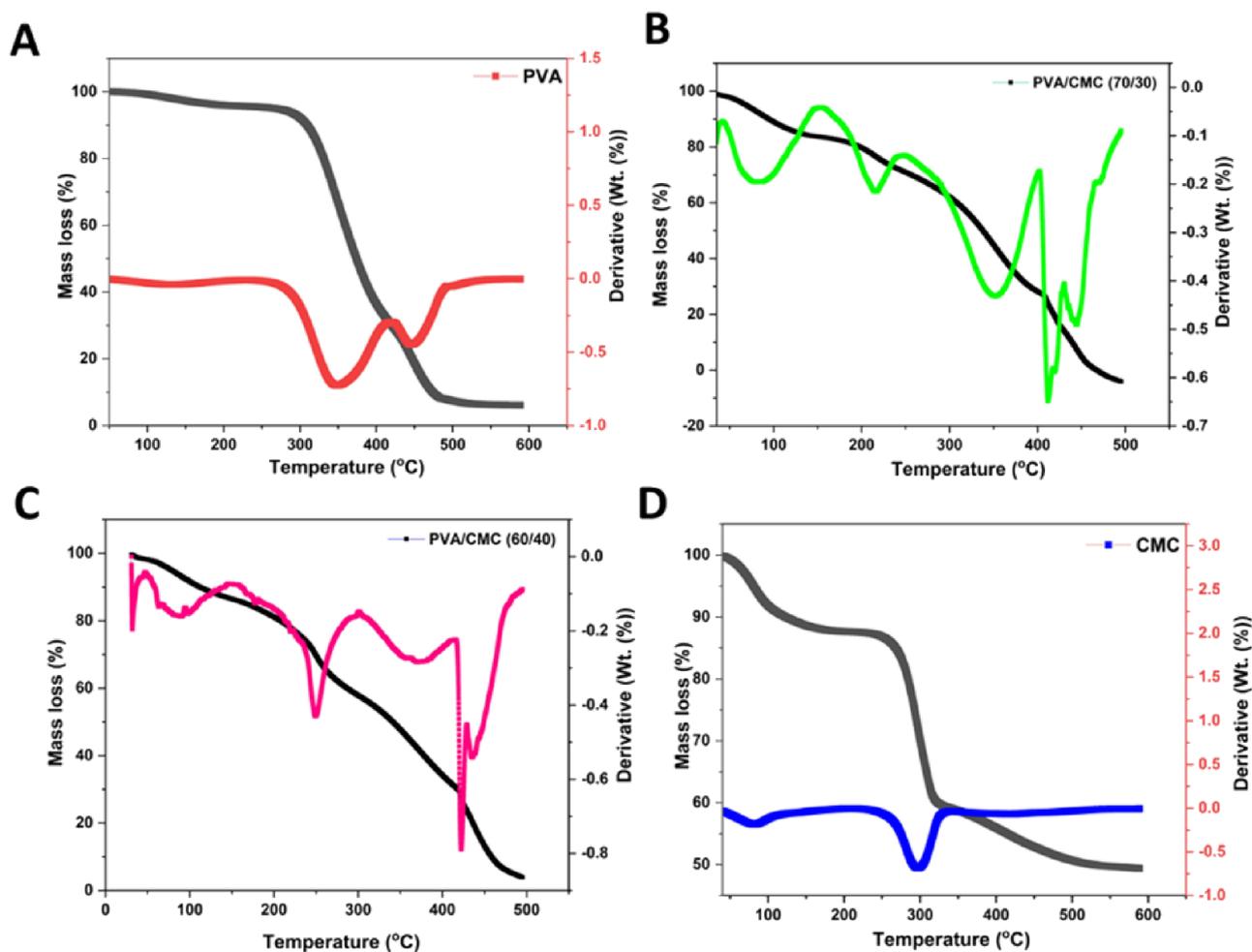

Figure 4. TGA% and DrTGA% of synthesized films over temperature change; (A) PVA, (B) 70 PVA:30 CMC:AA, (C) 60 PVA:40 CMC:AA, and (D) CMC.

loss in the temperature range of 415−488 °C attributed to the degradation of low molecular weight fragments. Similar results were reported by.[33] The thermogravimetric analysis (TGA) of carboxymethyl cellulose (CMC) revealed two distinct degradation stages. The first step occurred in the temperature range of 41−110 °C and resulted in a weight loss of 9%. This initial stage was attributed to the evaporation of the moisture content present in the CMC sample. The second degradation step was observed in the temperature range of 250−326 °C and led to a weight loss of 30%. This second stage corresponded to decomposition of the CMC polymer itself. The CMC is generally stable below 280 °C while PVA shows stability below 400 °C.[34] Figure 4 shows the TGA curves with their derivatives for PVA, 60PVA:40CMC:AA, and 70PVA:30CMC:AA films.

The TGA thermograms of 70PVA:30CMC:AA (Figure 4B) and 60PVA:40CMC:AA (Figure 4C) exhibited two distinct thermal degradation steps with continuous weight loss (%). The first step corresponded to the removal of moisture content in the temperature range of 50−130 °C with a slightly higher weight loss (%) for 70PVA:30CMC:AA film as compared to the 60PVA:40CMC:AA film. This effect could be due to the presence of surplus −OH groups of PVA in 70PVA:30CMC:AA film as the same amount of GA was added in both films; hence, there may remain some unreacted OH groups in 70PVA:30CMC:AA causing evaporation at first step and higher weight loss as compared to 60PVA:40CMC:AA film.[30]

The thermal stability of the synthesized films was observed in the temperature range of 180−300 °C with 10% weight loss for 70PVA:30CMC:AA and 20% weight loss for 60PVA:40CMC:AA. At this step, weight loss was higher for 60PVA:40CMC:AA, which indicated that the increased amount of CMC had a detrimental effect on the thermal stability of the film. The same behavior of CMC was observed in the work of Hashmi et al.[34] where increased concentration of the CMC in PVA electrospun fibers decreased the thermal stability of the fibers. The final step in the temperature range of 300−455 °C attributed to the complete degradation of PVA and both films with further weight loss.[35] The degradation temperatures for synthesized films (as compared to the pure PVA) shifted to lower values with the addition of CMC as it is less stable than PVA.[33] However, the obtained thermal stability of the films is plentiful for food packaging application. The char yield values are mentioned in Table 1.

Table 1. Char Yield Value of Synthesized Films

film	aT10 (°C)	cT50 (°C)	dT75 (°C)	eTmax (°C)	fYc at 455 °C (%)
PVA	75	278	403	464	8.1
60PVA:40CMC:AA	94	342	412	455	11.1
70PVA:30CMC:AA	111	338	427	454	3.35
CMC	102	390		581	45

Table 1 shows the percentage weight loss at various temperatures. The char yield values obtained at 455 °C for PVA, 60PVA:40CMC:AA, and 70PVA:30CMC:AA were 8.1, 11.1, and 3.35, respectively, where aT10 = temperatures at 10% weight loss, cT50 = temperature at 50% weight loss, dT75 = temperature at 75% weight loss, eTmax = maximum decomposition temperature, and fYc = char yield, weight of polymer remained.

3.4. Soil Burial Degradation. It is necessary to investigate the biodegradation of synthetic films due to their adverse effect on the environment.[1] A soil burial degradation test was performed to evaluate the biodegradability of the prepared films. PVA-CMC samples have shown good biodegradation activity. Films were weighed and buried in the soil for 15 days. After the desired period, the degradation rate of the films was evaluated using eq 1. A significant weight loss was observed. Both films, 60PVA:40CMC:AA and 70PVA:30CMC:AA, have shown the best degradation values of 71 and 65%, respectively, which is in agreement with the previously done study on the PVA/CMC system.[36] The presence of heterobonds in the PVA and CMC chemical structure facilitates the biodegradation process. However, a high degradation rate for 60PVA:40CMC:AA film was observed, which could be attributed to the high content of carbohydrates in the CMC, which provides a large carbon source to bacteria. The presence of moisture in soil activates bacterial activity, which plays a major role in the degradation process. Water and moisture present in the soil diffused into the films, causing them to expand, which resulted in improved biodegradability. The results of the present study are excellent as compared to the previous studies which have shown degradation rates at 36 and 40%.[1,37,38]

3.5. Wettability of Synthesized Films. The ideal food packaging requires a minimum interaction of water with the packaging material. The texture, flavor, and quality of food deteriorate if water adheres to the surface and is absorbed in the film. In this regard, the food packaging is designed to be hydrophobic and nonadhesive toward the water content.[39] Hence, it is vital to evaluate the interaction of packaging film with moisture present in the environment. The water contact angle technique is commonly used to determine the wettability of the surface of the packaging film and to investigate the role of intermolecular forces (adhesion and cohesion) involved.[40]

The PVA and CMC are both hydrophilic compounds. However, for food preservation, a water-repellent surface is required as discussed above. The cross-linking of PVA and CMC in the presence of a suitable compound can tune the wetting behavior of the synthesized film. As illustrated in Figure 5, the wettability of the PVA-CMC films changed drastically with the change in the concentration of the PVA and CMC. The 60PVA:40CMC:AA film had a contact angle of 72 ± 6° (Figure 5A) and showed moderately hydrophobic character as compared to the 70PVA:30CMC:AA film with a contact angle of 26 ± 1° (Figure 5B). The cross-linking of PVA and CMC in 60:40 ratios in the presence of GA may have removed hydroxyl groups from the PVA and CMC,[5,41] resulting in the acetal and ether linkages, thus restraining the hydrophilic nature of PVA and CMC. The increased amount of the PVA (70 wt %) in the film lowered the contact angle enhancing the hydrophilicity of the film since some of the unreacted −OH groups are still presently providing affinity for water molecules. Furthermore, SEM (Figure 3) shows that 70PVA:30CMC:AA film had a little porosity in the matrix. The hydrophilic character of the 70PVA:30CMC:AA film could also be due to the presence of pores[35] as compared to the 60PVA:40CMC:AA film, which means that it can absorb and retain moisture content and thus cannot be used for food packaging. The statistical analysis (Figure 5C) of the measured contact angles showed that the wettability of the both films had a significant difference at $p < 0.05$.

3.6. WVTR (%) of Films. The WVTR is evaluated to detect water vapor diffusion from the environment to the packaging
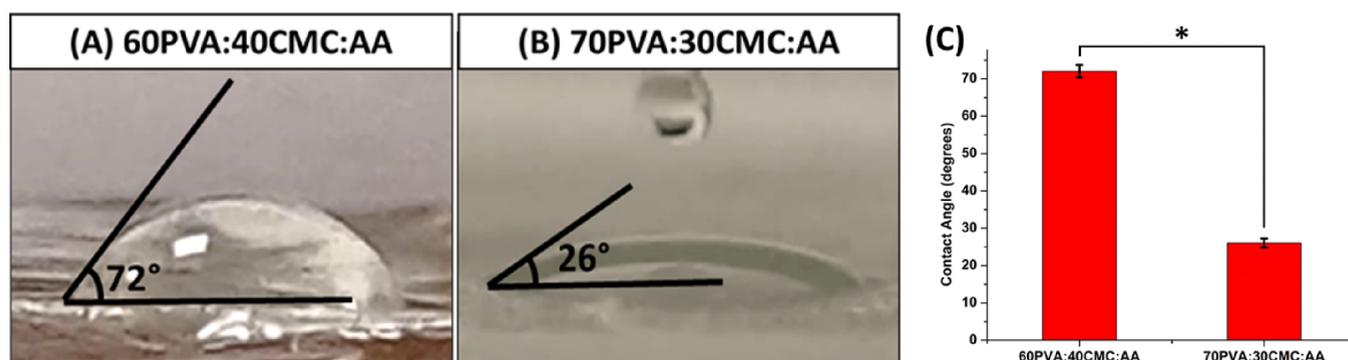
Figure 5. Digital image of the contact angles between water and (A) 60PVA:40CMC:AA and (B) 70PVA:30CMC:AA. The hydrophilic character of the film is shifted toward hydrophobic with increased CMC content. (C) One-way ANOVA showing that the wettability of the synthesized films is significantly different at $p < 0.05$.

film, as the quality of the preserved food depends on the diffusion of moisture from the surrounding. The WVTR (%) of the synthesized films was calculated. The 45 g/m² h WVTR for 60PVA:40CMC:AA and 55 g/m² h for 70PVA:30CMC:AA were recorded. The film containing higher CMC content (40CMC) showed lower WVTR as compared to the film with a lower concentration of CMC (30CMC), which could be due to the formation of the highly uniform and pore-free matrix, as shown in SEM imaging (Figure 3A,B). On the other hand, PVA has pore-free film-forming properties; however, after blending with CMC in the presence of GA, the film with higher PVA content (70PVA) showed higher WVTR as compared to the low-concentration (60PVA) film, which could be attributed to the presence of porosity in the matrix of that film (Figure 3C,D) and also due to the unreacted −OH groups. The difference in the wetting behavior of the films also directly affects the WVTR. As the 60PVA:40CMC:AA film was hydrophobic (Figure 5) and fewer hydroxyl groups were available, the moisture could not easily pass through this film. Figure 6 demonstrates the statistical difference in the WVTR% of both films. The analysis shows that the WVTR% of 60PVA:40CMC:AA significantly lower than the 70PVA:30CMC:AA at $p < 0.05$.
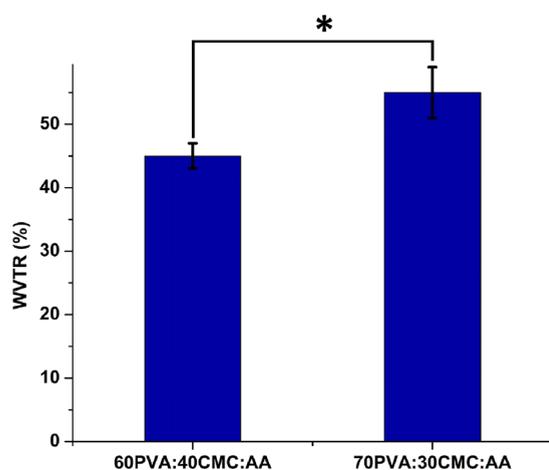

Figure 6. Graph shows the percentage water vapor transmission rate (WVTR %) of 60PVA:40CMC:AA and 70PVA:30CMC:AA films. One-way ANOVA shows that the WVTR% of the synthesized films is significantly different at $p < 0.05$.

3.7. Antibacterial Effect of Synthesized Films. In the food industry, bacterial film formation is a serious issue in which pathogens survive for a long time, hence contaminating the food. The antimicrobial activity of food packaging is an important element as it protects food from spoilage by inhibiting the growth of pathogenic organisms. Moreover, it plays a key role in extending the shelf life of food.[42] In the present study, the antibacterial activity of the prepared films was investigated against Gram-positive *S. aureus* and Gram-negative *E. coli* by using the agar well diffusion technique. AA was added as an antimicrobial agent to the blends of PVA/CMC. The antimicrobial efficacy was evaluated by measuring the zone of inhibition after 24 h, as shown in Figure 7. Both films mentioned as sample 1 (60PVA:40CMC:AA) and sample 2 (70PVA:30CMC:AA) containing an equal amount of AA have shown excellent antibacterial activity. The zone of inhibition was measured as 21 mm for both films against *S. aureus* (Figure 7A) and 15 mm for both films against *E. coli* (Figure 7B). The control sample did not show any activity as it does not contain AA, which indicated that AA is an efficient antibacterial agent in the prepared films. AA plays a key role in eradicating biofilms and creates an environment unsuitable for bacteria to grow by lowering the pH of the environment. Moreover, its antiquorum sensing activity plays a major role in inhibiting the growth of bacteria as its antiquorum sensing activity disrupts the communication of bacteria, which inhibits expression of its virulence factors, hence reducing the ability of bacteria to cause infections.[18,43−45] AA is a suitable choice to induce antibacterial properties compared to the metallic ions owing to different mechanism (lowering of pH in case of AA and penetration of reactive oxygen species in case of metallic ions) to eradicate the bacteria from the food packaging films.

3.8. Mechanical Strength of Films. The synthesized films were subjected to the microtensile test to determine the strength under load. The film must maintain its structural integrity throughout the processing, transportation, and distribution stages. The stress vs strain curve of the films is presented in Figure 8A. The image shows typical curves for tough plastic materials. A very small change in length (strain) is observed at the start; however, when the stress reaches above 38 and 18 MPa for 60PVA:40CMC:AA and 70PVA:30CMC:AA, respectively, a strain hardening effect is observed for both films. This type of behavior is observed in other PVA/CMC combinations as well as reported by Amaregouda et al.[19] Further evaluation of the mechanical properties of the films is presented in Table 2. The TS and %EB of 60PVA:40CMC:AA
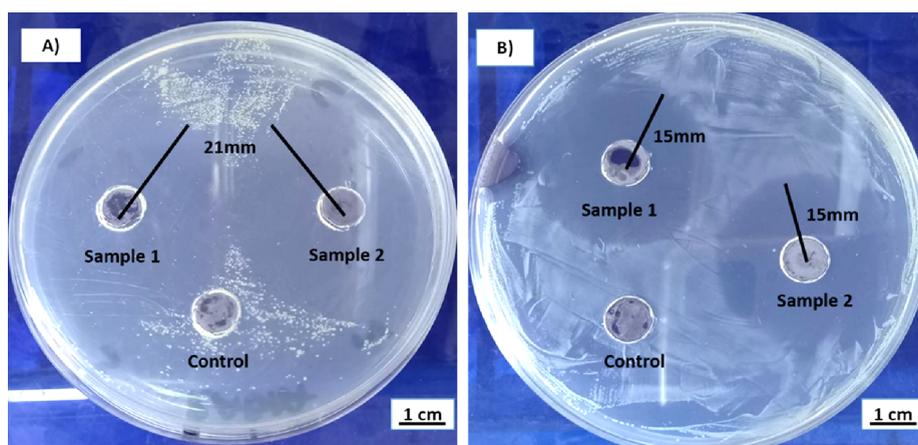
Figure 7. (A, B) Antibacterial effects of the synthesized films. Sample 1:60PVA:40CMC:AA and sample 2:70PVA:30CMC:AA against (A) *S. aureus* and (B) *E. coli*.
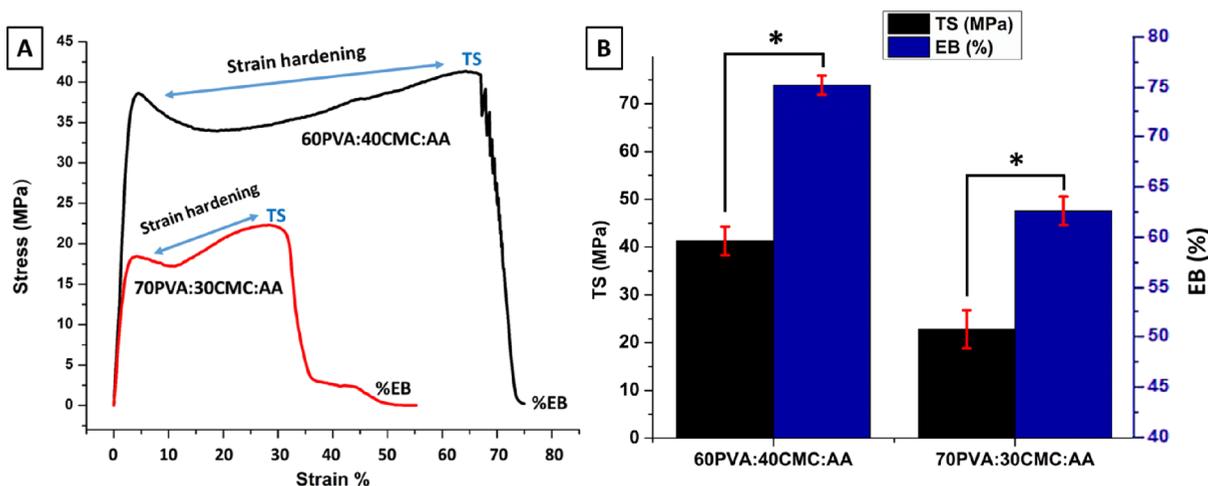

Figure 8. (A) Stress−strain curve of the 60PVA:40CMC:AA and 70PVA:30CMC:AA films. (B) One-way ANOVA depicting that the TS and %EB of 60PVA:40CMC:AA is significantly different from 70PVA:30CMC:AA at $p < 0.05$.

Table 2. Tensile Strength (TS) and % Elongation at Break (% EB) of the Films

film	TS (MPa)	% EB
60PVA:40CMC:AA	41.3 ± 0.8	73.9 ± 0.5
70PVA:30CMC:AA	22.8 ± 1.6	47.6 ± 0.8

is higher than those of 70PVA:30CMC:AA, which confirms that the higher CMC content has improved the mechanical strength and stress tolerance of the synthesized film. Figure 8B shows that the TS and %EB of 60PVA:40CMC:AA is significantly different from 70PVA:30CMC:AA at $p < 0.05$.

4. CONCLUSIONS

The PVA/CMC/AA films were synthesized with two different compositions i.e. 60PVA:40CMC:AA and 70PVA:30CMC:AA for antimicrobial food packaging application. An excellent antibacterial effect was observed against *S. aureus* and *E. coli* for both films due to the same amount of AA. Inhibition zone of 21 mm was measured against *S. aureus* and 15 mm against *E. coli*. AA provided potent antibacterial effect due to the localized change in the pH. The incorporation of AA in PVA/CMC matrix is a better choice to prevent the biofilm formation compared to that of the metallic ions (chances of

toxicity are very low with AA). The morphological analysis showed that 70PVA:30CMC:AA film had some porosity in its matrix due to which the film became hydrophilic and had a higher water vapor transmission rate, i.e., 55 g/m^2 h. However, the 60PVA:40CMC:AA was more uniform and compact, posing a barrier to water vapor transmission, i.e., 45 g/m^2 h. The hydrophobicity was improved by adding 60 wt % PVA in the film due to removal of the hydroxyl group. The results of the present degradation study of the films achieved much better results as compared to the results reported in the literature as discussed above. Both films, 60PVA:40CMC:AA and 70PVA:30CMC:AA, have shown the best soil burial degradation of 71 and 65%, respectively. The 60PVA:40CMC:AA film also had high mechanical strength and ample thermal stability; hence, according to the present study, the composition 60PVA:40CMC is more suitable for food packaging applications.

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R.H. did the design, experiments, and writing. S.A.B. and A.A. performed data analysis, review, and editing. M.A. and M.A.U.R. did the design, review, and editing.

Notes

The authors declare no competing financial interest.

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