Banana Pseudo-Stem and Cattle Manure for Lactic Acid Production and the Application of Polylactic Acid-Cellulose Silver Nanoparticle-based Nanocomposite Films in Food Storage

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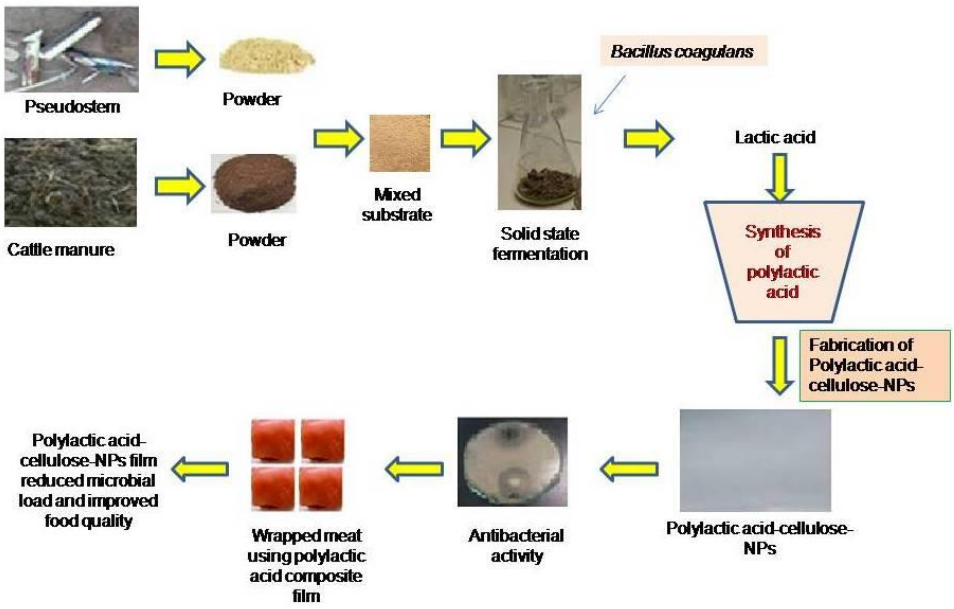
GRAPHICAL ABSTRACT



Banana Pseudo-Stem and Cattle Manure for Lactic Acid Production and the Application of Polylactic Acid-Cellulose Silver Nanoparticle-based Nanocomposite Films in Food Storage

Thankappan Sarasam Rejiniemon,[a] Hussain Rejula Raishy,[a] Rajamani Bhamadevi,[a] Manal Abdulaziz Binobead,[b] Reem M. Aljowaie,[c] and Rathi Muthaiyan Ahalliya [d,*]

Lactic acid is used in various industrial processes, including the production of emulsifiers, polymers, cosmetics, and pharmaceuticals. Fermentation of renewable biomass from natural sources has several advantages over costly chemical methods. Thermal and acidic pretreatments were used to improve the availability of sugars in the medium. *Bacillus coagulans* was isolated from the banana pseudostem; it was cultured with cattle manure-banana pseudostem for the improved production of lactic acid. Lactic acid production was high in the culture medium containing a 1:1 ratio of cow manure and banana pseudostem after 72 h of fermentation. After 24 h, lactic acid production was 19.4 ± 1.2 g/kg substrate, and it increased after 48 h (20.5 ± 0.1 g/kg substrate), and 72 h (26.3 ± 0.1 g/kg substrate). Lactic acid synthesized by *B. coagulans* was purified and used for the synthesis of polylactic acid. Polylactic acid was used for the fabrication of composite materials with cellulose and silver nanoparticles. The scanning electron microscopy image showed a smooth surface with uniform particle sizes. The fabricated nanoparticles showed antibacterial activity against food spoilage bacteria. The film was used to pack goat meat and chicken meat. The fabricated film reduced the bacterial load in the stored meat and improved food quality.

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Keywords: Cattle manure; Banana pseudostem; Low-cost substrate; Solid state fermentation; Lactic acid; Natural polymer

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INTRODUCTION

Lactic acid is used in pharmaceuticals, textiles, cosmetics, and as a preservative. It can be produced either by fermentation using carbohydrate-rich biomass or by chemical synthesis. The carbohydrate-rich agro-residues are used to produce lactic acid in solid-state fermentation, solid substrate fermentation, or submerged fermentation. Lactic acid is Generally Recognized As Safe (GRAS) for all organisms. The production cost of lactic acid is high due to the high cost of fermentation medium and downstream processing. Thus, it is recommended to use low-cost agro-residues to reduce the medium-cost culture. The agro-residues are rich in sugars, including glucose, maltose, and lactose, to produce lactic

acid. Lactic acid is used for the synthesis of poly(lactic acid) (PLA), which is one of the important biopolymers used for the preparation of packing material. To synthesize the high-grade PLA, high-pure lactic acid is required.

Various bacteria produce lactic acid, especially those from the genus *Bacillus*. These organisms utilize various lignocellulosic biomass, including agricultural straw, corn stover, sugar cane bagasse, cow dung, and other organic wastes (Abdel-Rahman *et al.* 2020). Recently, increasing attention has focused on the utilization of low-cost biomass to reduce production costs, environmental contamination, and the reduction of greenhouse gases. Banana pseudo-stems have a high content of cellulose (42.2 to 63.9%) and glucose (52.2 to 74%) (Bernstad *et al.* 2012). This is a low-cost lignocellulosic biomass, and this type of banana plantation is distributed throughout the world, especially in Europe and Asia. The cellulosic contents of pseudostem are high, and because of its high level of sugars, it is used in the preparation of manure, feed, the synthesis of sodium carboxymethylcellulose, chlorine-free formic acid pulping, and kraft pulping (Adinugraha and Marseno 2005). In agricultural fields, banana pseudo-stems affect the environment, including ammonia gas emissions and hydrogen sulfide production after microbial decay (Li *et al.* 2010). The availability of pseudo-stem is high and available throughout the year.

Cattle manure is one of the most prominent agro-wastes and is underutilized for industrial bioprocesses. Cow manure has been utilized to produce microbial amylases, proteases, cellulases, fibrinolytic enzymes, and growth hormones. Cattle manure contains lignocellulosic materials and can be used as a substrate to produce lactic acid by lactic acid-producing bacteria (Maas *et al.* 2008). Bacteria from the genus *Bacillus* are considered GRAS organisms to produce lactic acid using lignocellulose-rich agro-residues (Gonzales *et al.* 1986). The bioconversion efficacy was found to be >90%. *Bacillus* species can homoferment xylose and glucose and show high productivity. This organism can thrive under stress conditions, including moderate acidic conditions and moderate thermophilic conditions (van der Pol *et al.* 2016).

Lactic acid bacteria are nonspore-forming, Gram-positive rods or cocci. They are distributed in a variety of habitats, including the animal gastrointestinal tract, the human gastrointestinal tract, meat, fermented dairy products, and vegetables (Ruiz Rodríguez *et al.* 2019). Lactic acid bacteria, including *P. pentosaceus* I13, *P. acidilactici* I8, *Pediococcus acidilactici* I5, *Lactobacillus reuteri* I2, *P. acidilactici* C3, and *Enterococcus faecium* C14, were isolated from broiler chickens (Reuben *et al.* 2019). Lactic acid bacteria are distributed in meat, fermented vegetables, and cereals (Mathur *et al.* 2020). LABs such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Lactobacillus delbrueckii*, were isolated from the fermented cassava (Ogunbanwo and Okanlawon 2009). *Bacillus* species, such as *Bacillus licheniformis*, *Bacillus coagulans*, and *Bacillus subtilis*, produce lactic acid (Zhang *et al.* 2017). *Bacillus coagulans* was used to produce L-lactic acid (L-LA) (Olszewska-Widdrat *et al.* 2020). *Bacillus* species are rod-shaped, Gram-positive, and aerobic endospore-forming organisms and are distributed in various environments, such as clay, soil, rocks, dust, vegetation, food, and aquatic environments (Zhao and Kuipers 2016). The present work was designed to analyse whether the prepared polylactic acid-cellulose-silver nanoparticle film reduces bacterial load and improves food quality at storage. Banana pseudostem and cattle manure were used to produce lactic acid in solid-state fermentation. Lactic acid was purified from the fermented medium, and polylactic acid polymer was produced. Then, polylactic acid-cellulose-silver nanoparticles were fabricated. The product was further evaluated for use as a food packing material.

EXPERIMENTAL

Substrates

Banana pseudostem and cattle manure were used as substrates to produce lactic acid. Banana pseudo-stem was collected from the agricultural field. The pseudo-stem was cut into small pieces (2 in length) and dried. Cattle manure was collected and dried for three days under the sun. These substrates were milled individually, and the powder (1.5 to 2.0 mm particle size) was used as the substrate to produce lactic acid. Equal amounts of cattle dung and pseudostem (500 g each) were mixed and used as substrates to produce lactic acid.

Isolation of Bacteria from Banana Pseudo Stem

About 10 g of pseudo-banana stem was surface sterilized to remove the epiphytes. Then, 50 mL of double-distilled water was added, and the mixture was ground with a glass mortar and pestle. The clear supernatant (10 mL) was added to 90 mL of double-distilled water, and the mixture was shaken at 125 rpm for 20 min. The clear supernatant was serially diluted up to 10^{-9} times the starting concentration and plated on Luria-Bertani-Agar plates. This step was repeated, and a total of 10 morphologically different colonies were isolated and maintained at 4 °C on Luria-Bertani-Agar slants (Al-Dhabi *et al.* 2020).

Screening of Acid-Production and Detection of Lactic Acid

The bacterial strains isolated from the pseudostem (n = 22) were cultured in MRS broth medium. Specimens were incubated for 24 h at 37 °C. The pH of the culture medium was tested after 24 h of incubation. The lactic acid production was analyzed and determined by the spectrophotometer method. Briefly, 0.3% $FeCl_3$ solutions (2 mL) were added into the test tubes, incubated with 100 µL of samples, and mixed. The absorbance of the sample was measured at 390 nm against the reagent blank. Lactic acid was prepared at various concentrations and used for the preparation of a standard curve (Karnaouri *et al.* 2020). In further experiments, only 10 lactic acid bacteria (KT1, KT2, KT3, KT4, KT5, KT6, KT7, KT8, KT9, and KT10) were used for solid-state fermentation.

Microorganism and Inoculums

The selected bacterial strains were cultivated in a liquid medium. The bacterial strains (KT1-KT10) were isolated from banana pseudostem and used for lactic acid production in solid-state fermentation. Briefly, the isolated strain was grown in MRS broth (Himedia, India) medium for 18 h at 32 °C in an orbital shaker incubator. The cell density was maintained between 1×10^6 and 1×10^6. About 100 µL of culture was spread on MRS agar plates, and the number of colonies was counted.

Characterization of Isolate KT-2

The lactic acid bacterial isolate KT-2 presented highest lactic acid production. Hence, it was selected for characterization studies. A single bacterial colony of strain KT-2 was picked and inoculated in an Erlenmeyer flask containing 25 mL of Luria-Bertani broth and cultured for 18 h at 37 °C. The culture was centrifuged at 6000 rpm for 10 min. Bacterial DNA was extracted using a DNA extraction kit (Qiagen, Germany), as described by the manufacturer's instructions. The 16S rDNA was amplified using universal forward and reverse primers by a thermocycler machine. It was loaded on Applied Biosystems, and the sequences were submitted into GenBank databases (Zhang *et al.* 2020).

Pre-treatment of Agro-Residues and Determination of Reducing Sugars

Cattle manure and banana pseudostem were pretreated individually, and the reducing sugar level was determined. To optimize the pretreatment conditions, the pseudostem (100 g) was mixed with 1% H_2SO_4 for two hours at selected temperatures (90, 100, 110, 120, and 130 °C). To analyze the effect of H_2SO_4 concentrations on reducing sugar yield, the pseudostem was treated with H_2SO_4 at different concentrations (0.8%, 1.0%, 1.2%, 1.4%, and 1.6%) and at optimum temperature (110 °C). The one-variable-at-a-time approach method was used to optimize the pretreatment condition. The amount of reducing sugar content in the sample after pretreatment was estimated using the dinitrosalicylic acid method. The reducing sugar yield (%) was calculated from the following formula:

$$\text{Reducing sugar yield (g/g, \%)} = \frac{\text{g of reducing sugar produced}}{\text{initial g of dry manure}} \times 100 \quad (1)$$

Valorization of Biomass for Lactic Acid Production

The isolated lactic acid bacteria were cultured in MRS broth medium, incubated for 18 h at 32 °C, and used as inoculum. To determine the effect of substrates on lactic acid production, substrates were taken at various compositions. Cattle manure and pseudo-stem were prepared at 1:0.25, 1:0.5, 1:0.75, 1:1, 0.25:1, 0.5:1, and 0.75:1 ratios, and lactic acid production was determined. The basal substrate (cattle manure and pseudo-stem mixture) was placed in a 250-mL Erlenmeyer flask, 70% moisture content was maintained, and the medium was mixed. It was sterilized for 30 min and cooled. The substrate was inoculated with inoculums (2%) and fermented for 48 h at 37 °C. After 48 h of anaerobic fermentation, the sample was collected and lactic acid was extracted. The pH of the fermentation medium was determined using a digital pH meter. The amount of lactic acid production was determined using high-performance liquid chromatography (DIONEX, USA), and an internal standard was applied (Singh *et al.* 2023).

Purification of Lactic Acid from the Fermented Medium

The fermented medium was filtered using a 100-µm pore size filter, and the filtrate was further microfiltered using a 0.2-µm pore size filter. The filtration step allows the removal of non-targeted compounds, including proteins and sugars. The sample was extracted with a mixture of n-butanol and ammonium sulfate (10 g of ammonium sulfate was mixed with 50 mL of n-butanol). Then, the organic phase was separated and dried.

Chemical Synthesis of Polylactic Acid

L-lactic acid (10 g) obtained by solid state fermentation was introduced in a reaction tube equipped with a Dean–Stark trap. Then, the reaction was initiated *via* addition of (w/v) 0.4% $SnCl_2$ and xylene (30 mL). The mixtures were refluxed for 24 h at 140 °C under agitation and water molecules were continuously removed. The water molecules were removed using molecular sieves, and the mixtures were distilled. Lactide was obtained, and it was stirred overnight and extracted with in ethyl acetate. The ethyl acetate phase was washed with 1M HCl three times and evaporated. It was recrystallized in acetone, then dried in an oven at 140 °C for 1 h with 0.4% $SnCl_2$. The final product was filtered, dried, recrystallized in methanol, and a pure polylactic acid was obtained (Zhang and Wang 2008). It was dried and heated at 140 °C for 1 h with 0.4% $SnCl_2$. The final product was mixed with ice cold methanol, and a white precipitate was obtained. It was filtered, dried,

recrystallized in methanol, and a pure polylactic acid was obtained (Zhang and Wang 2008).

Scanning Electron Microscopy Analysis

The morphology of polylactic acid was determined using scanning electron microscopy equipped with an EDX detector (Hitachi, Japan). The purified lactic acid samples were deposited on aluminum stubs, and morphology was determined.

Preparation of Polylactic Acid Film

A polylactic acid-nanocomposite film was prepared. Initially, the pure polylactic acid granules were dried for 10 h at 65 °C, ensuring the complete removal of moisture content. The granules were dissolved in chloroform (Himedia, India), and the solution was poured into a thin glass mold with the required thickness. It was stored at 28 to 30 °C, and chloroform was evaporated. Polylactic acid-based composites were prepared by mixing different concentrations of cellulose, and silver nanoparticles were added to chloroform and mixed. The suspension was stirred continuously for 12 h, and good dispersion was achieved after sonication for 20 min. The mixture was poured into glass molds, and the solvent was evaporated at room temperature (28 to 30 °C). The dried polylactic acid-nanocomposite material was removed from the mold and dried. The moisture content of the material was removed before any characterization studies were performed (Roy and Rhim 2020).

Properties of Polylactic Acid-Cellulose-Silver Nanoparticle Composite Materials

The tensile strength of the film was tested using a stretching device. The film was placed between two jaws of the device, and tensile strength was calculated after being elongated at 2 mm/min. The thermal stability of the sample was tested using 50 mg of film at various temperatures (50 to 500 °C). To test the water vapor permeability, the film was cut and placed in a vial. It was placed in a desiccator containing water and incubated at 28 to 30 °C. The weight was measured, and thermal stability was determined. The film was cut, placed in a vial, and incubated at 28 to 30 °C in a desiccator. Then, the weight of the vial was analyzed at regular intervals for 3 days, and the water vapor permeability was determined. The destruction of film in deionized water was determined by immersing film in water for 10 days. After 10 days, it was heated in an oven at 60 °C for 4 h, and the initial and final weights were determined (Wang *et al.* 2020).

Antibacterial Activity of the Film

The antibacterial activity of the film was tested against two bacterial pathogens (*Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes*, and *Staphylococcus aureus*) by the disc diffusion method. Briefly, Mueller-Hinton agar medium was prepared and sterilized. After sterilization, 0.1 mL of culture was spread on the agar plates. Then, the film was cut into 6-mm pieces and placed on the plates. It was incubated, and the zone of inhibition was measured. Chloramphenicol (10 μg) was used as the positive control (Wang *et al.* 2020).

Application of Film in Meat Packing and Physical Properties Analysis

Goat meat and chicken meat were cut (100±5 g) into small pieces. The meat was firmly packed with the developed film and sealed using sealing equipment. The physical

changes, such as appearance and odor, of the goat and chicken meat were analyzed for three weeks to determine the potential of the polylactic acid composite films for preserving meat samples. The prepared polylactic acid composite films were more effective at controlling microbial contaminations in chicken meat than goat meat. Hence, chicken meat was further subjected to microbial analysis.

Application of Film in Chicken Meat Packing and Microbial Analysis

Chicken meat was purchased from the meat stall and packed in film, and the physical nature and microbial load were determined. Briefly, chicken meat was cut into small pieces (50 g) and wrapped in a rectangular bag made of film. Then, it was stored at 4 °C (de Ch Minsal 2010). Next, changes in physical properties were observed every five days up to 25 days. The viable aerobic mesophilic bacteria, psychrophilic bacteria, enterobacteria, and *Salmonella* spp. were determined. About 1 g of chicken meat was homogenized for 3 min, and the clear supernatant was obtained after centrifugation. It was spread on nutrient medium, and the log colony-forming unit/mL (CFU/mL) was determined. To determine the mesophilic and psychrophilic bacterial populations, 0.1 mL of a serially diluted sample (10 to 7 dilution) was spread on plate count agar and incubated at 30 °C for mesophilic bacteria for three days and at 10 °C for seven days. The selective agar medium (EMB Levine agar) was used for the determination of enterobacteria (Al-Dhabi *et al*. 2020). In the control set of the experiment, chicken meat (50 g) was not wrapped in film and was stored at the same temperature (4 °C).

Statistical Analysis

All experiments were performed in triplicate, and the mean value was subjected to data analysis. A one-way analysis of variance was used to test the significance, and a p-value <0.05 was considered statistically significant. Duncan's multiple range analysis was performed to test the significance level between the experimental groups at $p<0.001$.

RESULTS AND DISCUSSION

Production of Lactic Acid in Solid State Fermentation

The banana pseudo-stem and cow dung mixture were used to produce lactic acid in solid-state fermentation. The collected solid substrates and processing methods are illustrated in Fig. 1. The isolated lactic acid-producing bacteria were inoculated, and lactic acid production was observed for 72 h. Lactic acid production was high in the medium fermented with stain KT-2. Lactic acid production was high in the culture medium inoculated with the bacterial strain KT-2. After 24 h, lactic acid production was 19.4 ± 1.2 g/kg substrate, and it increased after 48 h (20.5 ± 0.1 g/kg substrate) and 72 h (26.3 ± 0.1 g/kg substrate) (Table 1). The low pH value of the medium did not affect the synthesis of lactic acid in the KT-2 strain. Lactic acid production was affected after 72 h in the strains KT1, KT3, KT7, and KT8. The amount of lactic acid production varied based on the types of bacteria, the types of residues, and the pretreatment of the substrate. Xavier *et al.* (2024) used low-cost agro-residues to produce lactic acids. The selected sugarcane bagasse served as a nutrient source and supported the production of lactic acid by *Limosilactobacillus fermentum*. Kaur *et al.* (2022) used agro-industrial wastes to produce organic acids, and the agro-residues improved organic acids in the medium. Bacteria from the genus *Bacillus*

produce lactic acid. Ouyang *et al.* (2013) used low-cost glucose derived from corn stover as a cheap substrate to produce l-lactic acid.

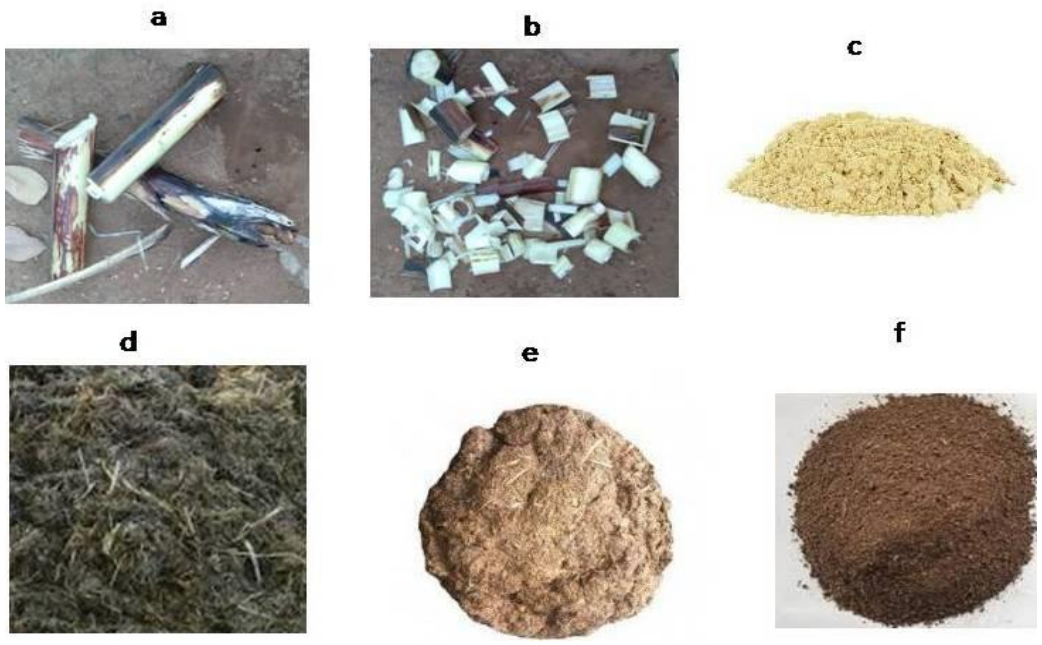

Fig. 1. Banana pseudo stem (a), pseudo stem pieces (b), pseudo stem powder (c), wet cow dung (d), dried cow dung (e), and cow dung powder(f). The pseudo-stem was cut into small pieces (2-cm in length) and dried. Cattle manure was dried and powdered for solid-state fermentation.

Table 1. Production of Lactic Acid in Solid State Fermentation Using Banana Pseudo Stem-Cow Manure Substrate

Bacteria	Lactic Acid Production (g/kg Substrate)		
	24 h	48 h	72 h
KT1	10.3 ± 1.6	9.2 ± 0.04	8.5 ± 0.2
KT2	19.4 ± 1.2	20.5 ± 0.1	26.3 ± 0.1
KT3	18.4 ± 0.3	19.4 ± 1.1	10.5 ± 0.3
KT4	1.4 ± 0.0	4.8 ± 0.2	5.7 ± 0.1
KT5	0.3 ± 0.1	1.5 ± 0.1	0.52 ± 0.14
KT6	15.2 ± 0.04	18.6 ± 0.1	19.9 ± 0.4
KT7	18.2 ± 0.2	20.1 ± 0.03	13.8 ± 0.1
KT8	8.6 ± 0.1	4.9 ± 0.1	2.5 ± 0.2
KT9	9.4 ± 0.2	10.5 ± 0.2	11.2 ± 0.2
KT10	11.4 ± 0.2	16.3 ± 0.3	18.5 ± 0.21

Pre-treatment of Agro-Residues and Reducing Sugar Concentration

The amount of reduced sugar concentration varied widely based on the pretreatment condition. The variations observed in reducing sugar concentration for the present experiments revealed that temperature is one of the main factors in reducing sugar percentage in the substrate. At 110 °C, reducing sugar concentration was high in the cattle manure (69.2 ± 2.4%), and 120 °C was optimum for pretreatment of pseudo stem (70.4 ±

1.8%). The reducing sugar level decreased at higher temperatures. The sulfuric acid-treated agro-residues showed improved reducing sugar yield and were concentration-dependent. In our study, 1.2 to 1.4% sulfuric acid was optimum for maximum sugar yield from cattle manure and pseudostem, respectively (Fig. 2). Pretreatment of agro-residues is a useful approach to improving the availability of reducing sugars for microbial fermentation. Acid and heat treatment were preferred to improve the amount of reduced sugar. Pretreatment strategies were used to improve reducing sugar in agro-residues (Woiciechowski *et al.* 2014). Diluted sulfuric acid was used for the preparation of cardoon, and the produced sugars improved ethanol production (Ballesteros *et al.* 2008). Diluted acid and enzymatic hydrolysis were preferred for reducing sugar production from sugarcane bagasse (Timung *et al.* 2016). In another study, soaking-assisted thermal pretreatment was used to produce sugar from cassava peels (Aruwajoye *et al.* 2017).

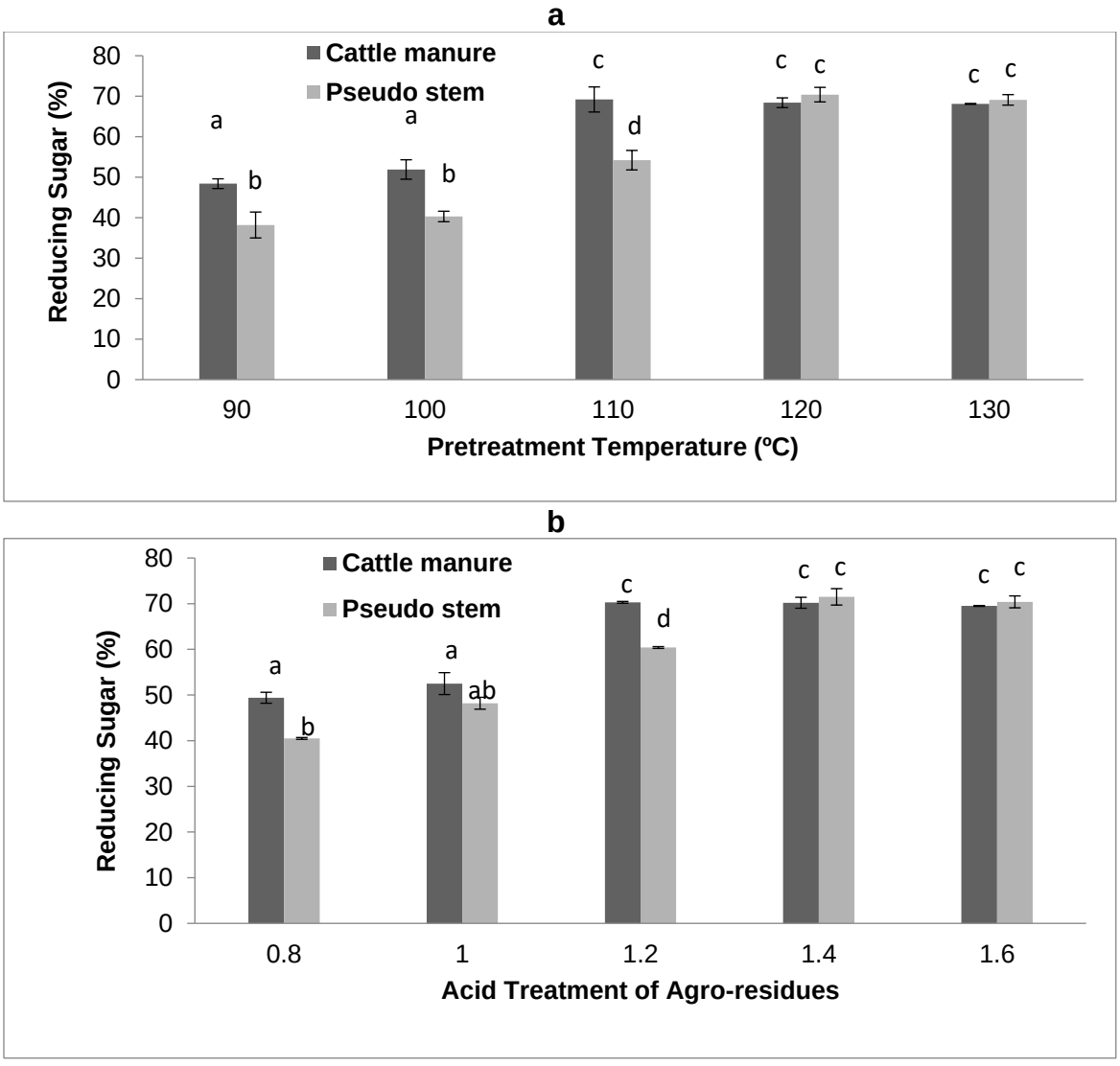

Fig. 2. Effect of pretreatment on agro-residues and reducing sugar concentration. Cattle manure and pseudostem were powdered, and the reducing sugar content was estimated after pretreatment at various temperatures (a) and different concentrations of sulfuric acid (b). Results in the bar followed by different lowercase letters means significantly different according to Duncan's multiple range test at $p<0.001$.

Valorization of Biomass for Lactic Acid Production

To improve lactic acid production using low-cost substrates, cattle manure and banana pseudo-stem were fermented in various combinations using *Bacillus coagulans* KT2. The yield of lactic acid varied, depending on the composition of nutrients, especially sugars in the substrate. Lactic acid production was high in the culture medium containing a 1:1 ratio of cow manure and banana pseudostem after 72 h of fermentation (Fig. 3). Azaizeh *et al.* (2020) used banana, carob, and sugarcane lignocellulose biomass for lactic acid production. Lactic acid bacteria utilized corn crop residues and improved lactic acid production (Malacara-Becerra *et al.* 2022). The lactic acid bacteria *Lactobacillus rhamnosus* IMC501 converted lignocellulosic biomass from grape stalks into lactic acid (D'ambrosio *et al.* 2023). The mixed hexose-pentose sugars improved the production of lactic acid by co-cultivating *Lactobacillus rhamnosus* SCJ9 and *Enterococcus mundtii* WX1 (Klongklaew *et al.* 2021). *Bacillus amyloliquefaciens* was screened to produce lactic acid, and improved lactic acid production was achieved after optimization steps (Vignesh Kumar *et al.* 2022). *Bacillus coagulans* utilized corn stalks and produced L-lactic acid in anaerobic fermentation (Yang *et al.* 2023). *Bacillus coagulans* DSM2314 used sugars from pretreated sugarcane bagasse and produced lactic acid (Alves *et al.* 2023).

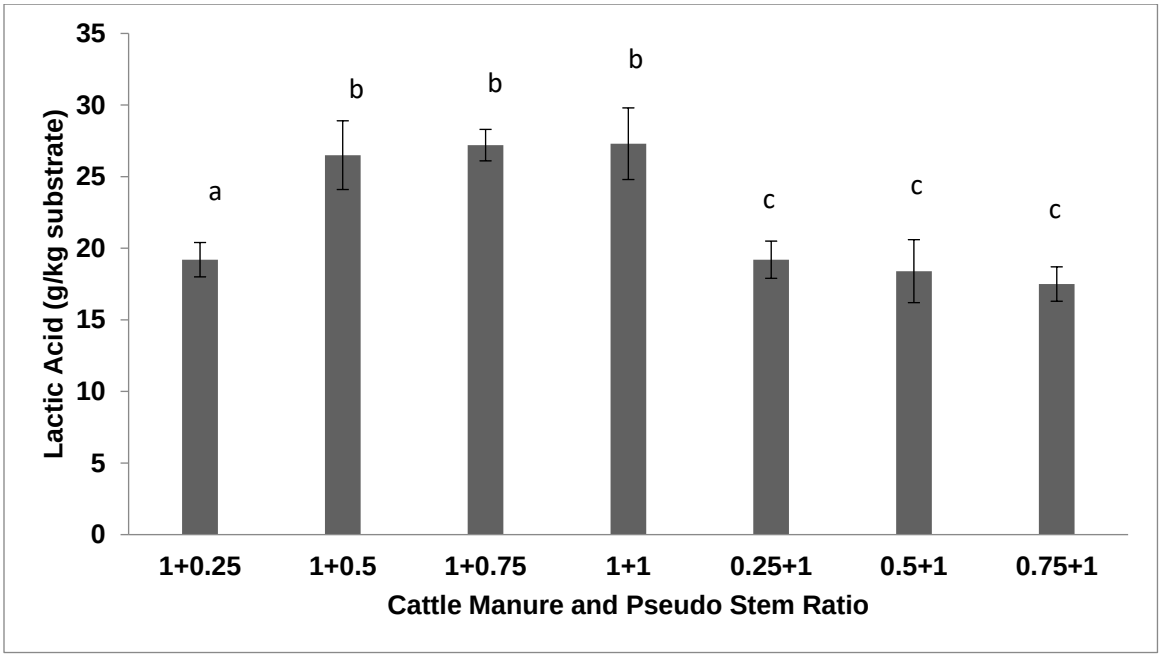

Fig. 3. Effect of substrate cattle manure and pseudo-stem concentration (1:0.25, 1:0.5, 1:0.75, 1:1, 0.25:1, 0.5:1, and 0.75:1 ratio) on lactic acid production in solid-state fermentation. *Bacillus coagulans* was inoculated at 1% in the solid substrate and incubated for 72 h, and lactic acid production was tested. The error bar represents the standard deviation. Results in the bar followed by different lowercase letters means significantly different according to Duncan's multiple range test at $p<0.001$.

Morphology of Polylactic Acid and Polylactic Acid-Cellulose-Silver Nanoparticle Composite Materials

The microstructures of the polymer and the bioactive composite materials were observed, and the results are depicted in Fig. 4. As shown, the morphology of the polymer, and the cellulose-silver nanoparticles added to the to the polymer show the microstructure of the nanoparticles (Fig. 4b). The nanocomposite materials were observed in the film and

were uniformly distributed. No incomplete dispersion of the nanoparticles was observed. The SEM analysis of polylactic acid showed a smooth surface with uniform particle sizes. The composite materials showed some micro-cracks in certain places and large voids. Moreover, the surfaces did not become rougher, and cellulose particles were observed in the composite materials. Lactic acid-cellulose composite materials were prepared to improve the functional properties of polylactic acid (Khoo *et al.* 2016; Yin *et al.* 2017; Wang *et al.* 2020). The morphological characteristics of polylactic acid-cellulose-silver nanoparticles were similar to those of previous studies (Sullivan *et al.* 2015). The addition of cellulose nanomaterials altered the crystallization behavior of polylactic acid, and the supplemented cellulose promoted crystallization. The supplemented cellulose alters the fracture characteristics of the composite materials (Todo *et al.* 2007; Pei *et al.* 2010).

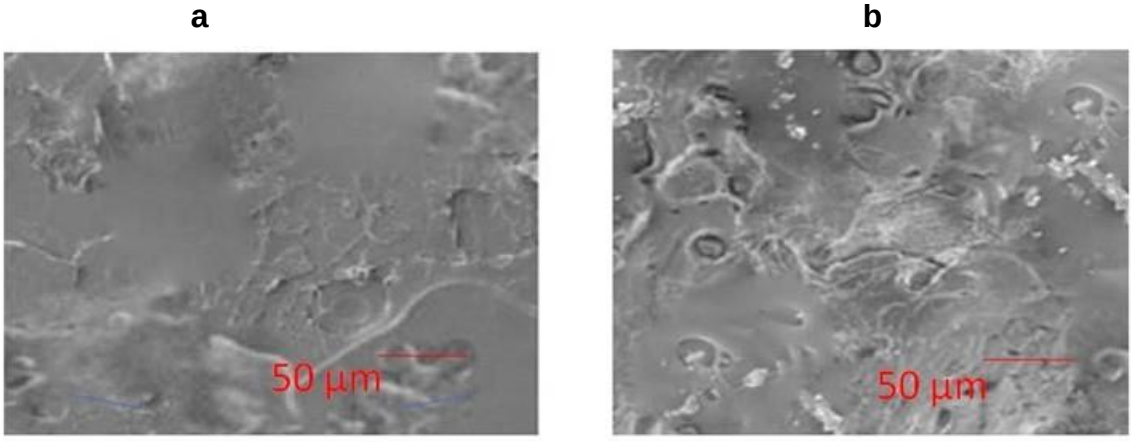

Fig. 4. Scanning electron microscopy images of polylactic acid (a) polylactic acid-cellulose-silver nanoparticle composite materials (b)

Antibacterial Activity of Polylactic Acid-Cellulose-Silver Nanoparticle Composite Materials

The fabricated nanoparticles showed antibacterial activity against bacterial pathogens. The zone of inhibition ranged from 10 ± 1.9 mm to 14 ± 0.9 mm (Fig. 5, Fig. 6).

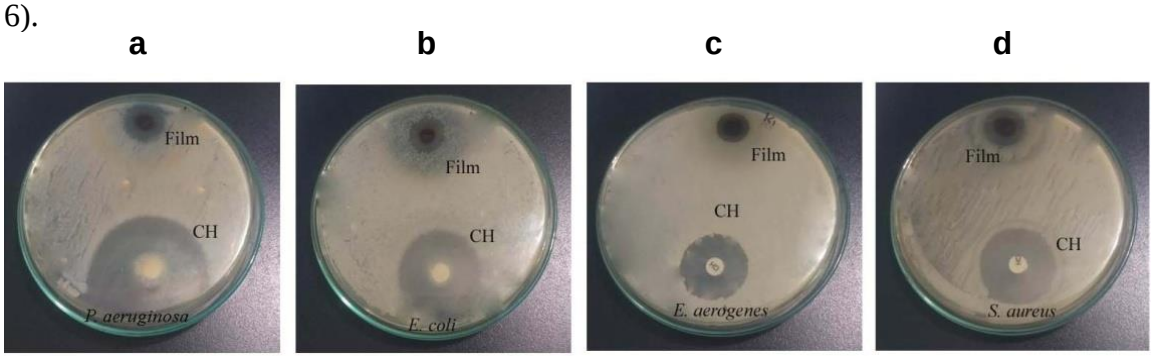

Fig. 5. Antibacterial activity of polylactic acid-cellulose-silver nanoparticle composite materials; The film was cut into 6-mm diameter and placed on Mueller Hinton Agar medium.
a: *Pseudomonas aeruginosa*; b: *Escherichia coli*, c: *Enterobacter aerogenes*, and d: *Staphylococcus aureus*; CH- Chloramphenicol

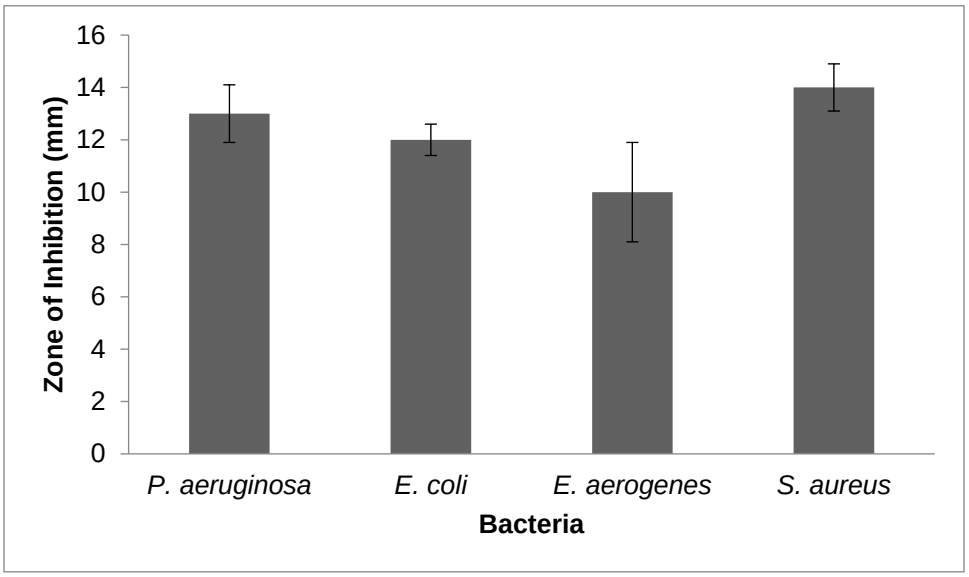

Fig. 6. Antibacterial activity of polylactic acid-cellulose-silver nanoparticle composite materials; the result was expressed as zone of inhibition (mm).

The nanocomposite film exhibited high activity against S. aureus (Gram-positive bacteria) and showed the least activity against *E. coli* and *E. aerogenes*. Cellulose is a non-toxic material, and the supplemented silver nanoparticles improved their antibacterial activity. Antibacterial polylactic acid biofilm is preferred for food packaging applications. *In vitro* analysis is a preliminary study to determine antibacterial efficacy. The polylactic acid-based composite materials were prepared using chitosan and essential oil (Rihayat *et al.* 2021) and surface-modified polylactic acid nanospheres and chitosan (Yao et al. 2021).

Application of Polylactic Acid-Cellulose-Silver Nanoparticle Composite Material in the Store Goat Meat

The physical changes of the meat after three weeks of goat meat storage were tested in the laboratory. The appearance was not changed after three weeks of experimentation. However, odor was smelled after three weeks of storage and was comparatively less than the control. These findings revealed that polylactic acid composite films are effective for storing meat.

Mesophilic and Psychrophilic Bacterial Population in Chicken Meat

The mesophilic, psychrophilic, and enterobacteria bacterial populations were observed in the bag containing chicken meat (Fig. 7). The total bacterial counts of mesophilic aerobes, psychrophiles, and Enterobacteriaceae are the major indicators of food quality, and these populations reflect the shelf life of food. Analysis of these microbial populations is critical from an industry point of view. A bacterial load of over 7 CFU/mL Log_{10} is not suitable for human consumption. The mesophilic bacterial population increased continuously during storage. However, less bacterial growth was observed in the chicken meat covered with nanocomposite film. In the control sample, the population of psychrophilic bacteria increased during storage due to their growth at low temperatures (4 °C). The biocomposite film decreased the population of all three studied groups. Among the enterobacterial group, bacterial growth was minimal in the first 10 days of the treatment and further increased.

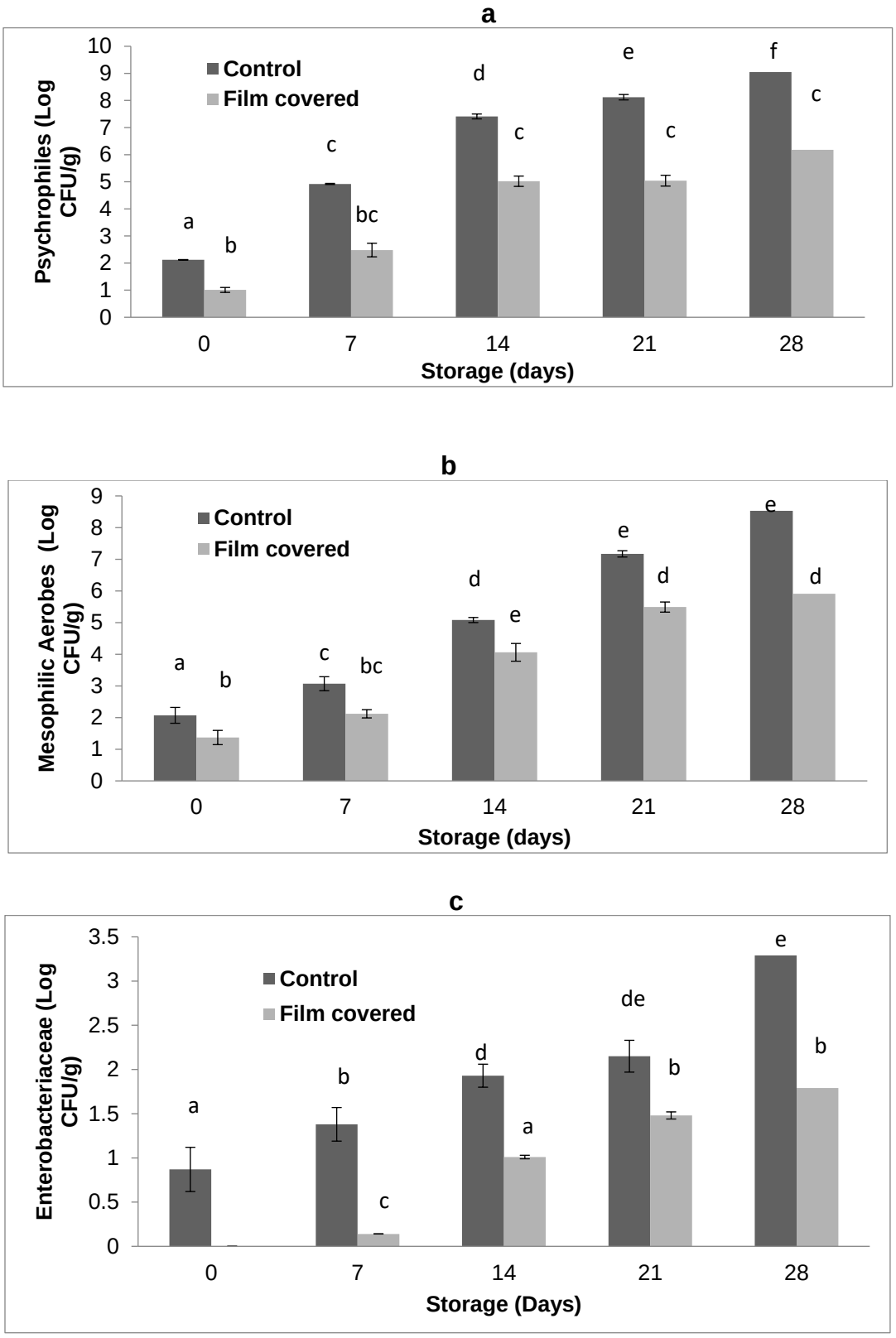

Fig. 7. Bacterial count for chicken meat stored for four weeks at 4 °C in contact with polylactic acid-cellulose silver nitrate film: (a) Psychrophiles (Log CFU/g), (b) mesophilic aerobes (Log CFU/g), and (c) Enterobacteriaceae (Log CFU/g). Results in the bar followed by different lowercase letters means significantly different according to Duncan's multiple range test at $p<0.001$.

The present finding of this work revealed that polylactic acid films with fabricated cellulose-silver nanoparticles showed potential control of common human pathogens. Machado de Melo *et al.* (2012) used antibacterial substances in the film, and sampling was made after six days from the chicken to determine psychrophile bacteria and showed a high level of psychrophile, which was higher than the current study. The biometallic-based polylactic acid film used to store chicken reduced the bacterial load (Ahmed *et al.* 2018). The bactericidal activity was attributed mainly to the interaction of metal ions with bacteria. Therefore, the prepared film can be used to store chicken for up to two weeks at 4 °C.

Analysis of Yield and Cost Effectiveness of Agro-residues for Lactic Acid Production and Biodegradable Film in Environmental Management

The agro-residues such as cattle manure and banana pseudostem are considered waste, and the utilisation of these agro-residues reduced the production cost of lactic acid. The thermal (68.1 to 69.1%) and acidic treatments (69.5 to 70.4%) increased the sugar level in the substrate material. Chemical, physical, and biological methods were used to convert lignocellulosic waste into monomers and polymers (Periyasamy *et al.* 2022). Chemical synthesis of lactic acid was costly, and biological synthesis of lactic acid using bacteria was cost-effective. The isolated bacterial strain *Bacillus coagulans* converted low-cost agro-wastes into lactic acid in solid-state fermentation. The cellulose content of the pseudostem used in this study was 44.8%, which was consistent with the previous report (Bernstad *et al.* 2012). Lactic acid production was maximum (27.3±2.5 g/kg substrate) in mixed substrate (cattle manure and pseudostem (1:1 ratio). Recently, Garrido *et al.* (2023) used cow manure as a low-cost medium for lactic acid production. The optimised simple pretreatment method using heat treatment and acid hydrolysis and the cost-effective substrate could reduce the production cost of lactic acid. The microbial lactic acid was used for the preparation of polylactic acid, and the mechanical strength was improved by the addition of cellulose, and the biological properties were enhanced by the addition of silver nanoparticles (Sanchez-Garcia and Lagaron 2010; Li *et al.* 2017). The multifunctional silver nanoparticle polylactic acid food packing film exhibited excellent antimicrobial activities, and the inhibition rate was high against foodborne bacterial pathogens and extended the shelf life of chicken meat. The polylactic acid composite film improved the chemical and sensory properties of chicken fillets during refrigerated storage (Javaherzadeh *et al.* 2020; Scaffaro *et al*. 2020). Polylactic acid films are biodegradable polyesters that are degraded under specific environmental conditions by thermophilic microorganisms. The use of polylactic acid films is an alternative solution for conventional toxic polymers and the avoidance of non-degradable microplastics (Sun *et al*. 2012).

CONCLUSIONS

1. Banana pseudostem and cattle manure were used as cheap substrates for the production of lactic acid in solid-state fermentation. The purified lactic acid was utilised for the preparation of polylactic acid.
2. The physical properties and antibacterial mechanisms were improved by fabricating polylactic acid-cellulose-silver nanoparticles.

3. The fabricated polylactic acid may improve the shelf life of chicken meat during storage.
4. The antimicrobial properties of film could control the growth of food-borne bacterial pathogens.

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