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Preparation and Application of a Xylan-Based Antibacterial Additive Agent against *Escherichia Coli* Bacteria

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Abstract: In this work, a xylan-based antimicrobial additive agent was prepared and aimed for uses in paper products against *Escherichia coli* bacteria. The derived Cationic-Xylan-grafted-PHGH (CX-g-PHGH) was successfully synthesized by graft copolymerization of cationic-xylan with guanidine polymer (PHGH) using ceric ammonium nitrate as initiator. The obtained CX-g-PHGH had maximum PHGH grafting ratio of 18.45% and efficiency of 58.45%, and showed good viscosity and thermal stability. Furthermore, the paper samples prepared in this work were reinforced obviously with the addition of CX-g-PHGH by improved mechanical properties. Compared to the reference paper without any of the xylan-derivatives, the index of tensile, tear, burst and folding endurance of the paper had increases up to 20.07%, 25.31%, 30.20% and 77.78%, respectively. Moreover, the prepared CX-g-PHGH paper exhibited an efficient antimicrobial activity against *E. coli* bacterial, by which a lot of

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applications based on the new xylan-derived additive agent obtained in this work could be found, especially in field of antimicrobial paper products against *E. Coli* bacteria from contaminated food.

Keywords: antimicrobial additive agent; cationic-xylan; *Escherichia coli*; mechanical properties; paper products; PHGH; thermal stability

1. Introduction

For a long term, the control of harmful bacterial infection has been receiving large amounts of concerns, especially with the increasing quality demand of living environment and working condition in current society [1]. Pathogenic *Escherichia coli* (abbreviated as *E. coli*) are naturally occurring bacteria found in environment, foods and mostly in intestines of people and animals, which are an important zoonotic bacterial pathogen and could cause a variety of intestinal and extra-intestinal diseases, such as diarrhea, urinary tract infections, septicemia, and neonatal meningitis, and other illnesses [2-6]. Contamination of food, specifically meat, with pathogenic *E. coli* can occur during evisceration and harvest of meat, making it one of the most common causes of food-borne diseases [7, 8]. Moreover, contact of pathogenic *E. coli* to human body via different kinds of residing platforms such as food-related paper products, e.g. packaging paper, baking paper, napkin paper, pulp molded tableware, etc. is also a common pathway caused human health problem. Therefore, using antimicrobial products for food-related applications is one of the most efficient and popular ways to

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reduce the infection from the daily eating.

The antimicrobial products based on various raw materials after modification has been extensively studied in the world [9-12]. Especially in recent years, antimicrobial products based on biological macromolecules from biomass including lignin, pectin, different kinds of polysaccharides (e.g. cellulose, starch and hemicellulose) have obtained great interests due to many superior properties of these kinds of macromolecules such as innocuousness to human health, biodegradability, good biocompatibility and bioactivity, wide range of resources, easy modification and so on [13-15]. For instance, composites of silver-nanoparticles/bacterial-cellulose exhibited significant antibacterial activities against *Escherichia coli* and other bacteria [16]. Antimicrobial nanostructured films based on starch has an antimicrobial activity against *Staphylococcus aureus* and other bacteria [17].

Food-related cellulosic products, such as food packaging paper, baking paper, napkin paper and so on, are important products in our daily life due to many superior properties of them such as light-weight, easy to modify and use, renewable, biodegradable and so on. However, cellulosic products originally do not have any antimicrobial activity. Bacteria can multiply rapidly in the products if proper conditions were fulfilled. Therefore, there have been many different kinds of natural and synthetic polymers or materials widely studied and used as antibacterial additive agents on these paper products [18-21]. Additive of antimicrobial agents especially the agents based on biodegradable biomass on the paper products is a promising way to introduce antimicrobial property to the required materials efficiently and sustainably.

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Xylan are the second dominating naturally occurring carbohydrate polymers in plant biomass world after cellulose. They are the most common hemicelluloses in angiosperms, grasses and cereals, where they exist in different composition and structures [22]. The content of xylan varies in different species, but it constitutes up to 35% in sugarcane bagasse and 50% in straws [23, 24]. Similar to cellulose, xylan are also poly-hydroxyl polymers with xylose as main sugar unit linked by glycosidic bonds and function as supporting material in the cell wall [25]. Xylan are readily available from pulp refining and cereal processing industries, and are at the same time environmentally friendly, renewable, sustainable, biodegradable and biocompatible. Xylan have been found with many potential applications in food, papermaking, textile, plastic industries and biomedical applications [26-28]. Moreover, xylan also have great potential to be applied as antimicrobial materials after modification and have received increasing attentions. Recently, the importance of xylan-based macromolecules [29-32] and materials [33-35] have obtained increasing focus, including antimicrobial modification of xylan used in the fields of packaging film, food preservative as well as in biomedical areas [36-39]. There have been many ways reported to prepare xylan-based antimicrobial materials. For example, a novel food preservative was prepared by co-heating xylan with chitosan which exhibited excellent antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* [28]. Chitosan-xylan/cellulose nanowhiskers (CNW) nanocomposite films with antibacterial and antioxidant properties were successfully prepared where CNW was used as nanofillers [40]. Carboxymethylated xylan was blended with Agar (Ag), Ammonium zirconium carbonate (AZC) and linoleic acid (LA)

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to produce edible films with antimicrobial activity [41]. Even though, the studies of xylan for high-value added applications are still insufficient. As one of the important subjects, the exploring of possibilities for xylan to be used as antimicrobial additive agent in paper products against *E. Coli* have being focused by researchers.

Same to cellulose, xylan originally do not have antimicrobial activity either, which also requires modification of it to endow the property. Polyhexamethylene guanidine hydrochloride (PHGH) is one kind of water-soluble polycations that has an antimicrobial activity against both Gram positive and Gram-negative bacteria, and low mammalian toxicity [15]. The introduction of PHGH could endow macromolecule with efficient antimicrobial activity [42, 43] which also offers a potential pathway of endowing xylan with antimicrobial ability against *E. Coli* bacteria.

Therefore, in this work, we prepared a new antimicrobial additive agent based on a xylan-derivative by graft polymerization of PHGH with xylan using ceric ammonium nitrate as initiator. The optimal condition for xylan-derivative preparation was firstly determined by evaluating the whole preparation process. The obtained xylan derivatives were characterized by its structure, thermal stability as well as the rheological behavior determined by Fourier Transform Infrared Spectroscopy (FTIR), Carbon Nuclear Magnetic Resonance Spectroscopy (^{13}C NMR), Thermogravimetric Analyser (TGA), Elemental Analyser (EA), and rheology meter. Moreover, mechanical property is also important aspect for the food-related paper products which needs to be concerned. Therefore, the antimicrobial activity against *E. coli* of the xylan-derivative obtained at the optimal condition as well as its ability as strengthening agent to improve mechanical

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properties of paper sheet were systematically investigated. This work found a new way to modify xylan, and investigated its applications as a new antimicrobial additive agent against *E. Coli* bacteria and mechanical enhancing agent for paper products. The obtained antimicrobial additive agent product would find great potential applications in many areas, especially for food-related paper products, such as packaging paper, baking paper, napkin paper and so on.

2. Materials and methods

2.1. Materials

Xylan (M_w 49000 g/mol, purity 85%) extracted from sugarcane bagasse was obtained from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Pretreated waste newspaper pulp (mainly American waste paper) was provided by local paper company (Guangzhou Paper Group Ltd., China). Ceric ammonium nitrate (CAN) (99.0%, AR) was purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). 2, 3-Epoxypropyltrimethylammonium chloride (ETA) (95%), hexamethylene diamine (98%, AR), guanidine hydrochloride (99%, AR), dimethyl sulfoxide (≥99%, AR) and glycidyl methacrylate (GMA) (97%, AR) were purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Acetone was purchased from Nanjing Chemical Reagent Co., Ltd (Nanjing, China). NaOH (95%, AR) and Ethanol (99%, AR) were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). Gram negative bacteria (*E. coli*, ATCC 25922) were purchased from Shanghai

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Beinuo Bio-Technology Co., Ltd (Shanghai, China). Chemicals used in this study were all analytical reagent grade and used without any purification. Deionized water was used in all experiments. Polyhexamethylene guanidine hydrochloride (PHGH) was prepared by condensation polymerization of hexamethylene diamine and guanidine hydrochloride as described in previous work [19].

2.2. Preparation of cationic-xylan

Cationic-xylan (CX) was prepared based on the procedure described in our previous work with minor changes [44]. Briefly, a solution of 3 g xylan in 90 mL deionized water was prepared in a 250 mL flask, and followed, 0.736 g NaOH (the molar ratio of NaOH and xylose unit in xylan was 0.8) was added for alkalization of xylan for 1 hour. Afterwards, the flask was placed in a microwave reactor (400W) (GAS-800, Beijing Xianghu Science and Technology Development Reagent Co., Ltd., Beijing, China) and 20.907 g of ETA (the molar ratio of ETA and xylose unit in xylan was 6) was added into the flask when the temperature of microwave reactor was reached to 70°C. After reaction of 40 min, the precipitate was formed in 100% ethanol and fractionated by filtration with three times the volume of ethanol. The precipitate was washed by filtration again with 70% ethanol for a few times until there was no white precipitate formed in the ethanol filtrates by titration with silver nitrate. The washed precipitate was dissolved in deionized water and dialyzed with membrane of molecular weight cut-off of 3500 in DI-water for 5 days until the pH of the dialyzed liquid reached to neutral. The CX was finally obtained after drying in a vacuum oven

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157 under 50°C for 24 h. The degree of substitution (DS) of the prepared CX was 0.38 which
158 was determined by elemental analysis method [45].

159

160 *2.3. Preparation of cationic-xylan-grafted-PHGH*

161

162 Unsaturated double bonds were introduced to PHGH by reacting with GMA
163 where the molar ratio of amino and epoxy groups was kept at 1.0 [42]. The reaction was
164 carried on at room temperature for 6 h. The obtained products were precipitated and
165 washed with acetone to remove unreacted GMA. The washed products were dissolved
166 in appropriate amount of methanol, followed by precipitation and wash with acetone
167 again. The same treatment was performed 3 times, and the final purified GMA-
168 modified PHGH product was obtained after drying in vacuum under 25°C for 12 h.

169 A solution of 0.33 g CX in 25 mL deionized water was prepared in a three-necked
170 round flask (250 mL) under stirring and purging with nitrogen for 20 min. Then 5 mL
171 CAN solution was added into the flask with purging of nitrogen continuously for
172 another 10 min. Subsequently, 10 mL GMA-modified PHGH was added into the CX
173 solution and kept stirring with a slow stream of nitrogen for 4 h. The solution was then
174 dialyzed for 3 days, and the CX-grafted-PHGH (CX-g-PHGH) was finally obtained
175 after drying in a vacuum oven at 50°C for 24 h. All conditions of preparing the CX-g-
176 PHGH were listed in Table 1.

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Table 1. Influence of the synthesis conditions on CX-g-PHGH

Sample number	Temperature (°C)	PHGH Concentration (mol/L)	Initiator Concentration (mmol/L)	Time (h)	Graft ratio (%)	Graft Efficiency (%)
1	60	0.039	3	4	15.54	52.54
2	60	0.039	4	4	18.45	58.45
3	60	0.039	5	4	16.9	56.69
4	60	0.039	6	4	15.93	53.35
5	60	0.020	4	4	7.27	56.65
6	60	0.078	4	4	16.01	53.21
7	60	0.117	4	4	15.26	40.73
8	60	0.156	4	4	15.03	36.58
9	50	0.039	4	4	15.66	49.60
10	70	0.039	4	4	16.82	53.28
11	80	0.039	4	4	16.24	51.44
12	60	0.039	4	2	13.56	50.26
13	60	0.039	4	3	14.18	53.47
14	60	0.039	4	5	17.21	57.56
15	60	0.039	4	6	16.52	55.48

Grafting ratio and efficiency of the as-prepared polymers were determined based on the equations of Eq. (1) and (2), and the mean value of the results obtained from three parallel samples under each condition was reported on Table 1:

$$\text{The grafting ratio} = (W_g - W_0)/W_0 \times 100\% \quad (1)$$

$$\text{The grafting efficiency} = (W_g - W_0)/W_p \times 100\% \quad (2)$$

where W_0 is the weight of CX; W_g is the weight of CX-g-PHGH; and W_p is the weight of functional PHGH.

2.4. Characterizations of the prepared products

Xylan, CX and CX-g-PHGH were dried in an infrared drying oven before characterization for their structures and properties. Fourier Transform Infrared (FT-IR) spectra was obtained with Fourier Transform Spectrophotometer (Nicolet 750, ThermoFisher Scientific, Waltham, FL, USA) appended with Attenuated Total

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Reflectance (ATR) technique. A total of 32 scans were accumulated in the transmission mode, with a resolution of 4 cm^{-1}. The spectrum was obtained from a range of 4000 cm^{-1} to 400 cm^{-1}.

The solution-state ^{13}C-NMR spectra was recorded on a Bruker DRX-400 spectrometer (Bruker, Karlsruhe, Germany) at 25°C after 15000 scans. The sample (80 mg) was dissolved in 1 mL D_2O. The running parameters were: 30° pulse flipping angle, 9.2 μs pulse width, 1.36 s acquisition time with 2 s relaxation delay.

Element analysis (EA) is for quantitative determination of specific elements of the samples. Specimens weighing approximately 3-5 mg were heated in a Vario EL Elemental Analyzer (Elementar, Germany) under oxygen atmosphere and elements of C, H and N in xylan, CX and CX-g-PHGH were determined.

The molecular weights of xylan, CX and CX-g-PHGH were determined by GPC on a PL aquagel-OH 60 column (300 mm × 7.5 mm, Agilent, USA) and calibrated with PL pullulan polysaccharide standard (average peak molecular weights of 783, 12200, 100000, 1600000 g/mol). A flow rate of 0.5 mL/min was maintained with ultrapure water as eluent. Samples were dissolved in ultrapure water to reach a concentration of 0.1% before characterization.

Dynamic rheological properties of CX and CX-g-PHGH were determined by a sandwich rheometer (AR2000, TA Instruments, New Castle, DE, USA). All samples were dissolved in water with a magnetic stirrer for 30 min to form stable solutions. The solutions were then dropped on Brookfield D VIII instrument panel. A software (Rheo 2000) provided by the manufacturer with the instrument was used to setup the

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parameter, perform rheometer control and collect data. The data of shear rate, frequency, viscosities, storage modulus (G') and the loss modulus (G'') of all samples were collected.

Thermal stability analysis was used to determine thermodynamic properties of xylan, CX and CX-g-PHGH. The analysis was carried out using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermalgravimetric analyzer (TGA Q500, TA Instruments, New Castle, USA). About 5 mg samples were heated to 700°C from room temperature with the heating rate of 10°C/min in a nitrogen atmosphere.

2.5. Preparation and mechanical properties tests of CX-g-PHGH paper sheets

Five sheets with grammage (weight per unit area) of ~55 g/m^2 were prepared based on norms GB2828-81 (Chinese Technical Association of Pulp and Paper). The mass ratio of CX-g-PHGH and the waste newspaper pulp was 3~15:1000. Briefly, CX-g-PHGH and the waste newspaper pulp were homogenized under stirring for 10 min before forming paper sheets. The sheets were formed via a fast papermaking machine (MESSMER 255, USA) and dried. The obtained sheets were cut to a certain shape and placed in humidity room at 25°C for 24 h before mechanical tests. As comparison, CX paper sheets were also produced via the same preparation process. A Chinese Standard (Chinese Technical Association of Pulp and Paper) was applied for mechanical tests of the sheets. The tests were carried out 10 times for each sample, and the mean value of the mechanical tests was reported in this work.

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241 *2.6. Antimicrobial tests of the xylan derivatives*

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243 The antimicrobial activity was tested against Gram negative bacteria (*E. coli*,
244 ATCC 25922). The *E. coli* bacteria were cultured and grown in Luria Bertani (LB)
245 liquid medium (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, and at pH 7.0) for 12
246 h at 37°C. The bacteria were further diluted with NaCl solution (0.85%, w/v) to get
247 concentration of about 10^5 CFU/mL. The diluted suspensions (0.1 mL) of *E. coli* were
248 then distributed homogeneously onto the LB agar medium (10 g/L peptone, 5 g/L yeast
249 extract, 10 g/L NaCl, and 15 g/lagar). The obtained paper sheets with xylan-derivatives
250 in diameter of 6-mm was prepared and placed in the plate culture medium that was
251 coated with the bacteria suspension. The antimicrobial ability of the xylan-derivatives
252 was evaluated by measuring the diameter of the inhibition zones to *E. coli* on the paper
253 sheets.

254

255 **3. Results and Discussion**

256

257 *3.1. Synthesis determination of CX-g-PHGH*

258

259 The Ce^{4+} in ceric ammonium nitrate could attack and convert the hydroxyl groups
260 of CX to free radicals, which could have activity to further react with modified PHGH
261 and form CX-g-PHGH [46]. The synthesis procedure was proposed in Scheme I.

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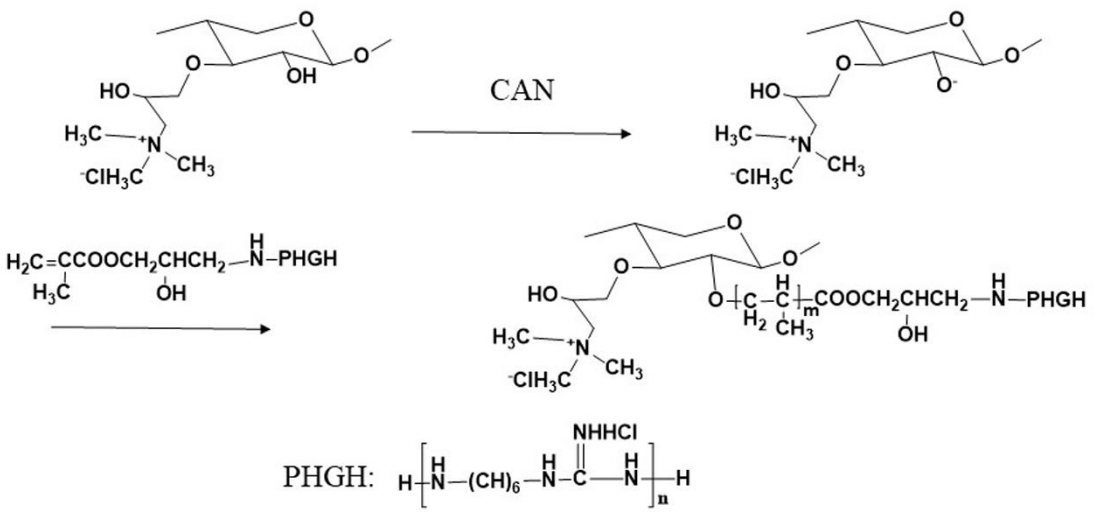

Scheme I. Copolymerization of PHGH onto CX.

There are many factors in the reaction such as the concentration of initiator, amounts of PHGH addition, reaction temperature and time that could affect clearly the grafting ratio and grafting efficiency of the final products. The influences of initiator concentration on grafting ratio and grafting efficiency of the CX-g-PHGH are shown in Figure 1a and detailed values were shown in Table 1.

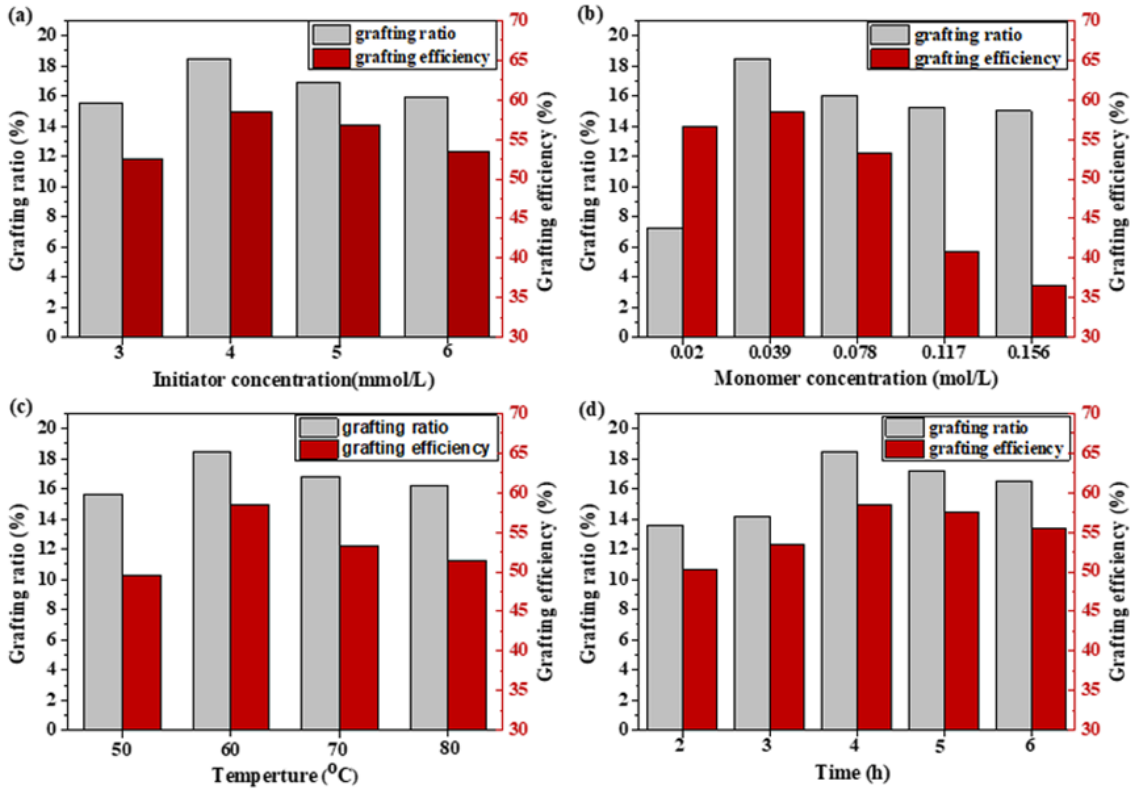

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Figure 1. Influences of conditions on grafting ratio and efficiency of CX-g-PHGH, a) initiator concentration; b) PHGH concentration; c) temperature; and d) time.

As it is shown that grafting ratio and efficiency increased drastically with the increase of initiator concentration until 4 mmol/L, valued up to about 18.45% and 58.45% for grafting ratio and efficiency, respectively. However, when higher initiator concentration than 4 mmol/L was applied, both the grafting ratio and efficiency decreased. This phenomenon was in accordance to previous finding by Qian, which was probably because the excess initiator started to participate in the termination step of the growing chains and subsequentially initiated the homopolymerization of PHGH [18].

The influence of PHGH concentration on the grafting ratio and grafting efficiency was shown in Figure 1b. It is very clear to see that the grafting ratio and efficiency increased sharply up to 18.45% and 58.45%, respectively, when the PHGH concentration was lower than 0.04 mol/L. However afterwards, the grafting ratio and efficiency decreased rapidly which was probably caused by the self-polymerization resulted from high PHGH concentration [15].

The increase of reaction temperature may lead to multiple effects, including increasing the diffusion of CX and PHGH; facilitating redox initiator system and enhancing the chain propagation, but likely increasing the rate of termination and homopolymerization in bulk phase [47, 48]. Therefore, when the temperature was lower than 60°C, there was a sharp increase for both grafting ratio and efficiency reached to maximum value of 18.45% and 58.45%, respectively (Figure 1c). However, the grafting

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ratio and efficiency decreased when the reaction temperature was higher than 60°C which was due to the domination of homopolymerization caused by high temperature.

Furthermore, the reaction time also had important impact on the grafting ratio and efficiency of CX-g-PHGH as it is shown in Figure 1d. When the reaction time increased, the grafting ratio and the efficiency firstly increased and then decreased. Reaction time of 4 h was the optimal time for the grafting ratio and efficiency with maximum value of 18.45% and 58.45%, respectively. This can be explained by the decrease of PHGH concentration and free radicals in the system as the increase of reaction time, which resulted in the leveling off of the grafting ratio and efficiency [49].

It can be concluded from above that the optimal condition for preparing CX-g-PHGH were: initiator concentration 4 mmol/L; PHGH concentration 0.039 mol/L; reaction temperature 60°C and reaction time 4 hours. Therefore, in the following studies, the CX-g-PHGH (sample number 2, as CX-g-PHGH-1) obtained at the optimal condition with maximal grafting ratio of 18.45% was used for characterizations and tests.

3.2. FTIR Spectra

In order to confirm the successful grafting of PHGH on CX, several analyses, including spectra of FTIR and ^{13}C-*NMR*, element analysis and average molecular weight, were performed.

The FTIR spectra of xylan, CX and CX-g-PHGH-1 are illustrated in Figure 2. The signals at 3433 cm^{-1}, 2918 cm^{-1}, 1637 cm^{-1}, 1464 cm^{-1}, 1033 cm^{-1} and 897 cm^{-1} in

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the spectrum represent the transmittance peaks of molecular bonds for xylan. The transmittance band at 2918 cm^{-1} is assigned to the C-H stretching vibration of alkane in xylan. The transmittance peak of 1044 cm^{-1} is ascribed to the C-O-C stretching of the ether groups [44].

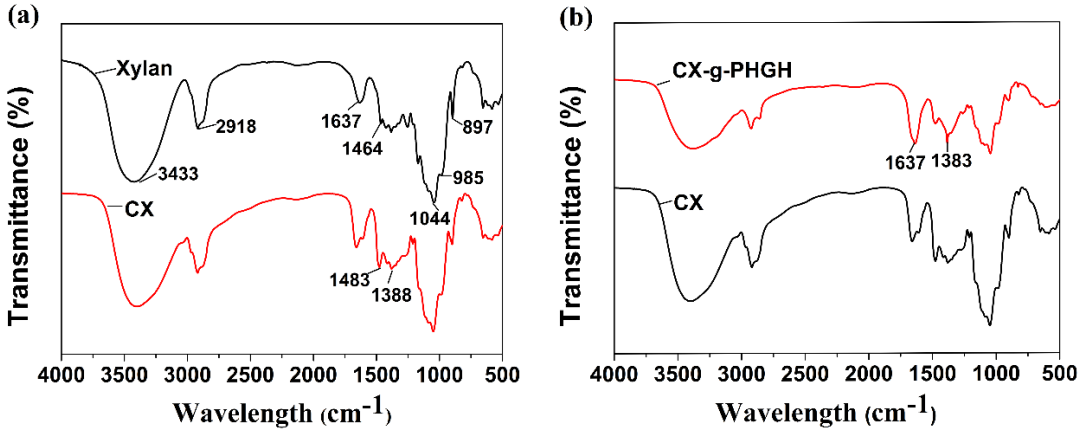

Figure 2. FTIR spectra of (a) xylan and CX; (b) CX and CX-g-PHGH-1.

As we can see from Figure 2a, in the FTIR spectra of CX, an enhancement in the transmittance intensity of ether bond at 1044 cm^{-1} was found compared with the spectrum of xylan, indicating that more ether bonds were introduced to xylan. The transmittance intensity around 1388 cm^{-1} increased due to stretching vibration of C-N. The stretching vibration of $-CH_2$ and $-CH_3$ on the quaternary ammonium group enhanced the intensity of the transmittance at 1483 cm^{-1}. All these changes in transmittance peaks indicated that cationic groups were introduced to xylan successfully [50].

In Figure 2b, compared with the spectrum of CX, enhancement in the intensities of transmittance at 1383 cm^{-1} and 1637 cm^{-1} was discovered in the FTIR spectra of CX-g-PHGH, corresponding for the stretching vibration transmittance peaks of C-N, C=N, respectively which confirmed the successful introduction of PHGH to CX [42].

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3.3. ^{13}C-NMR spectra

The molecular structures of xylan, CX and CX-g-PHGH-1 were further characterized by ^{13}C-NMR (Fig. 3). In the spectrum of xylan (Fig. 3a), the main (1→4)-linked *β*-D-xyl*p* units are obviously characterized by the signals at *δ* of 102 ppm, 72.9 ppm, 4.3 ppm, 75.9 ppm and 63.1 ppm, which are attributed to C-1, C-4, C-3, C-2 and C-5 of *β*-D-xylopyranosyl units, respectively [50].

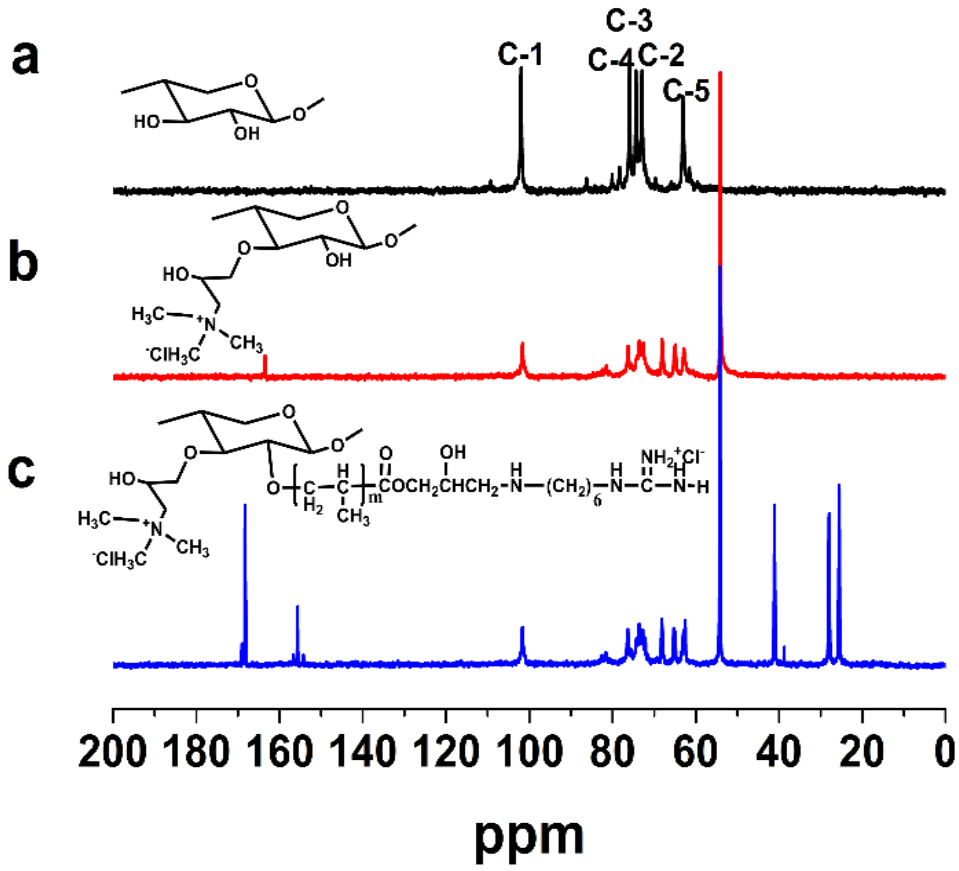

Figure 3. ^{13}C-NMR spectra of a) xylan, b) CX and c) CX-g-PHGH-1.

Compared with the spectrum of xylan, the spectrum of the CX (Fig. 3b) had great changes in both amounts and positions of the strong signals. The most intensive signal was appeared at 54.1 ppm which is assigned to carbons of the quaternary ammonium

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moiety and the signal at 68.1 ppm is attributed to the carbon C (CH_2) [51]. This proved that the cationic groups were introduced successfully onto xylan. In ^{13}C-NMR spectrum of CX-g-PHGH-1 (Fig. 3c), some new peaks appeared in comparison with CX. The peaks at 28.0 ppm, 41.1 ppm and 168 ppm are attributed to the signal peaks of -CH_2-, C-N and ester group carbon [52], respectively, which indicated that PHGH was grafted onto CX successfully.

3.4. Element analysis

The element analysis of xylan, CX and CX-g-PHGH-1 are showed in Table 2. The contents C, H and N in xylan were 40.36%, 6.91% and 0.00%, respectively, while they became 40.36%, 7.43% and 2.16% after the cationic modification of xylan, indicating that cationic groups were grafted on xylan.

Table 2. The element analysis of xylan, CX and CX-g-PHGH-1.

Sample	C (%)	H (%)	N (%)
Xylan	40.36	6.91	0
CX	40.36	7.43	2.16
CX-g-PHGH	40.88	7.79	9.46

Furthermore, the contents of C, H and N in CX-g-PHGH-1 were 40.88%, 7.79% and 9.46%, respectively. The increase in the N content confirmed the presence of nitrogenous compounds (PHGH) in CX-g-PHGH-1.

3.5. Average Molecular Weight

Average molecular weight is also a promising factor that can confirm the

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367 successful conversion of xylan to its derivatives. The molecular weight and molecular
368 weight distribution of xylan, CX and CX-g-PHGH-1 are shown in Table 3.

369 **Table 3.** The molecular weight and molecular weight distribution of xylan, CX and
370 CX-g-PHGH-1.

Sample	M_w (g/mol)	M_w/M_m
xylan	49000	4.59
CX	37500	1.99
CX-g-PHGH-1	1477129	1.15

371 As it is shown obviously, the molecular weight of CX was lower than that of
372 xylan, which was probably caused by the degradation of xylan during chemical reaction
373 under alkaline conditions. As expected, the molecular weight of CX-g-PHGH-1 was
374 higher, being about 30-40 folds than that of xylan and CX, which indicated the
375 successful copolymerization of xylan and CX with PHGH. In addition, CX and CX-g-
376 PHGH-1 had a relatively low index of polydispersity (1.14-1.99) than xylan (4.59),
377 which indicated that the molecular chain length distribution of CX and CX-g-PHGH-1
378 was more uniform than the one of xylan.

379

380 *3.6. Rheological properties*

381

382 Properties of xylan, CX, CX-g-PHGH-1 as well as their influences to paper
383 products were tested in order to fulfill the requirements used as additive agent for
384 antimicrobial paper products.

385 The tests of rheological behavior for xylan, CX and CX-g-PHGH-1 would
386 provide better understandings of the physico-chemical properties of the polymers and
387 consequently discover their potential applications, e.g. as coating additive agent to

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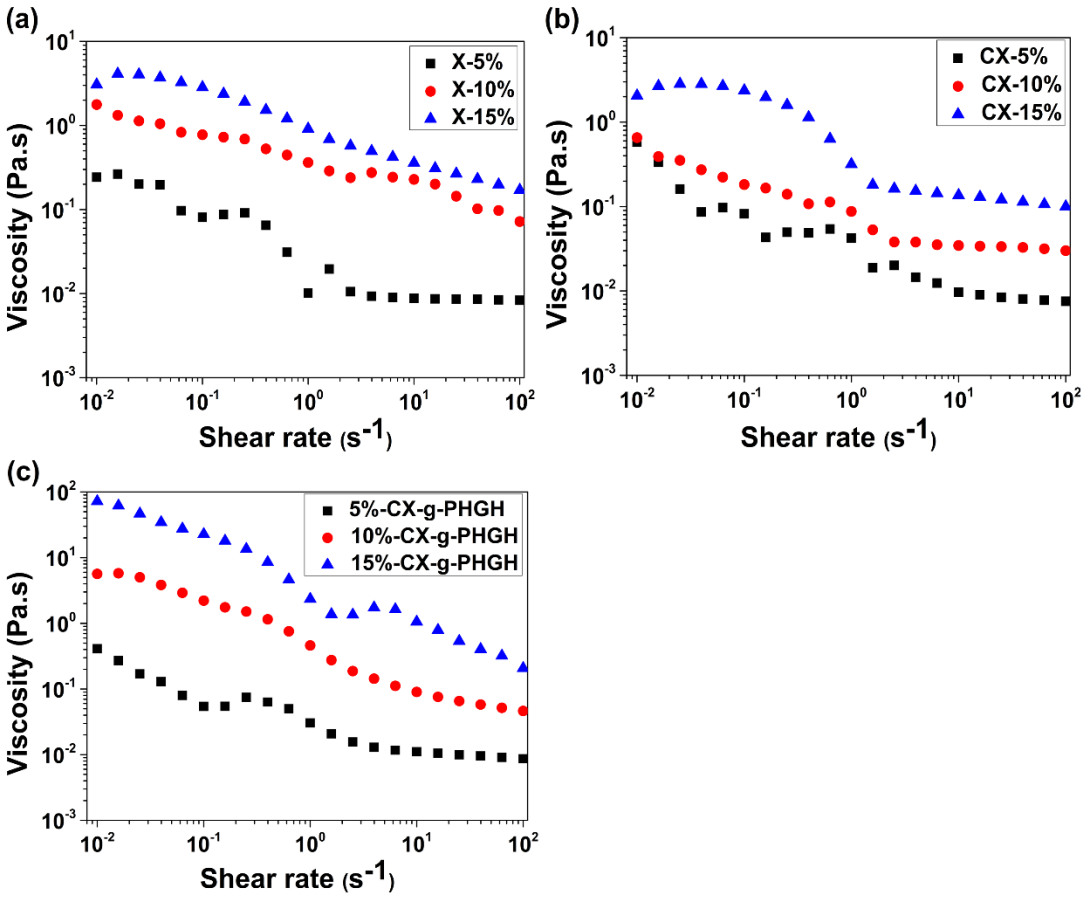
388 paper products. The rheological behavior of xylan, CX and CX-g-PHGH-1 are shown
389 in Figures 4 and 5.



390

391 **Figure 4.** Shear rate dependence of viscosity for (a) xylan, (b) CX and (c) CX-g-PHGH-
392 1 at different concentrations.

393 As it can be seen from Figure 4, viscosities of xylan, CX and CX-g-PHGH-1 all
394 decreased with the increase of shear rate, exhibited pseudoplastic or shear-thinning
395 behavior of these solutions in the range of shear rates tested, which was due to the
396 damage of network structure of xylan and its derivatives [45]. Accordingly, with a certain
397 additive speed, xylan, CX and CX-g-PHGH-1 would be easily applied as coating agent
398 on the surface of materials such as paper materials. Furthermore, the viscosity of CX-
399 g-PHGH-1 solution was higher than that of xylan and CX solutions in the whole shear

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rates range when the concentrations were 5% and 10% as well as for higher shear rate than 10 s^{-1} when the concentration was 15%. This can be suggested that CX-g-PHGH-1 solution had stronger intermolecular interactions than other two solutions [53].

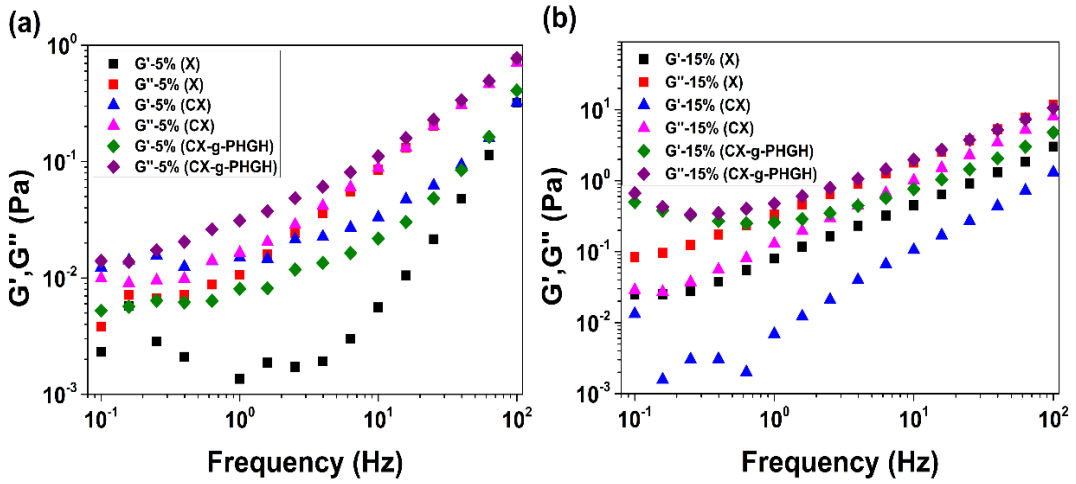

Figure 5. Frequency dependent modulus of the solutions of xylan (X), CX and CX-g-PHGH-1 (a) at 5% concentration; (b) at 15% concentration.

In Figure 5, the rheological properties (storage modulus G' and loss modulus G") of xylan, CX and CX-g-PHGH-1 are illustrated. When the concentration was 5%, the storage modulus in the whole frequency region of xylan and CX-g-PHGH-1 solutions was lower than the loss modulus, exhibiting a viscous behavior. For CX solution with concentration of 5% in the range of 10^{-1} to 10^{0} Hz, the storage modulus was higher than the loss modulus, showing stronger elastic properties due to stronger molecule entanglement of CX than xylan and CX-g-PHGH-1 [45]. When the concentration of solution was 15%, all storage modulus of xylan, CX and CX-g-PHGH-1 in the whole frequency region were lower than that of loss modulus, showing a viscous behavior. In addition, when the concentration of solution was 15%, the storage and loss modulus of CX-g-PHGH-1 was higher than the modulus of xylan and CX, indicating a greater

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viscosity behavior for CX-g-PHGH-1 than xylan and CX.

These results can be explained by the influence of molecular weight and functional groups of macromolecule chains [45]. Higher molecular weight and guanidine group caused more chain segments needed for the movement of the viscous flow, thus increased the frictional resistance and showed a greater viscosity behavior. On the other hand, lower molecular weight as well as cationic groups on the polymer chain may reduce or prevent the associative interactions among themselves in solutions, and thus changed the dynamic shear rheological properties of CX [54]. The studies above not only further confirmed the successful grafting of PHGH to xylan, but also indicated that the xylan-derivative obtained in this work is a promising candidate as coating additive agent which can be applied for paper products.

3.7. Thermal stability analysis

The thermal stability analysis of the samples could facilitate the application of xylan-derivative in more scopes. The typical TGA/DTA curves of xylan, CX and CX-g-PHGH-1 are displayed in Figure 6. There was a weight loss starting from 200°C which corresponded to the evaporation of water from the samples.

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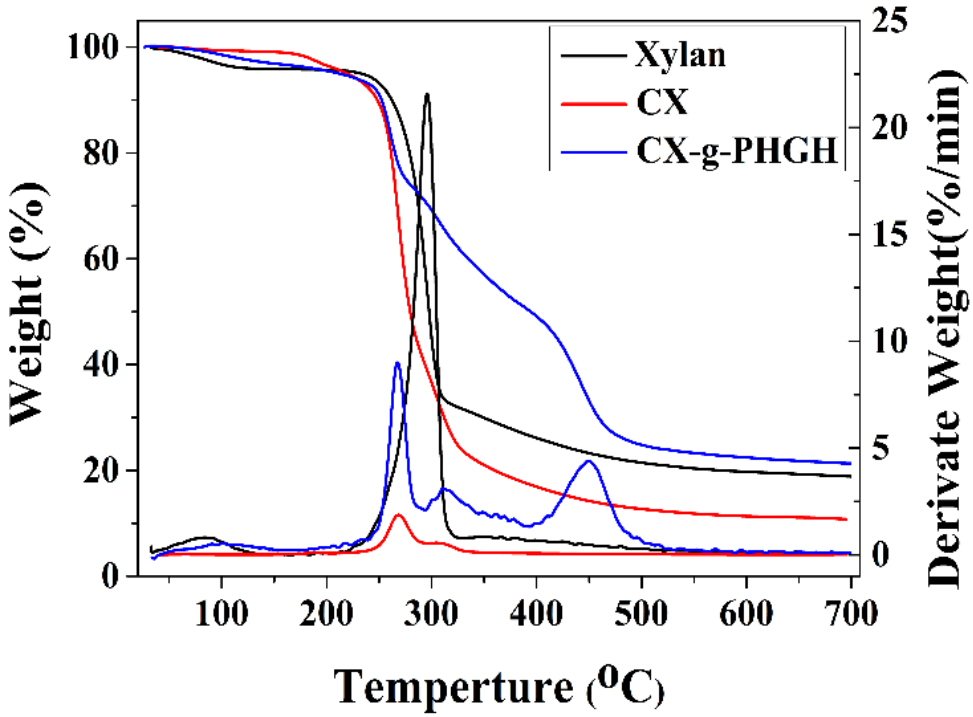

435

436 **Figure 6.** The TGA/DTA curves of xylan, CX and CX-g-PHGH-1.

437 It can be seen that the substantial weight loss of xylan occurred at 250-320°C,
438 which could be attributed to backbone scission and following fragmentation of xylan
439 [52]. The CX exhibited a similar thermal stability pattern to that of xylan, but its
440 decomposition temperature was lower, indicating that CX was more unstable than xylan.
441 This is the fact that the hydrogen bonds and molecular structure were destroyed to a
442 certain extent after cationization, and the grafted cationic groups were not stable [44. 49].
443 Similar to the TGA curves of xylan and CX, there was also a slight loss below 200°C
444 for the CX-g-PHGH-1 which was attributed to surface water evaporation. A substantial
445 weight loss of CX-g-PHGH-1 was found in the range of 230-320°C which was due to
446 the thermal decomposition of xylan. A substantial weight loss of CX-g-PHGH-1 was
447 also occurred in the range of 320-470°C which was attributed to the thermal
448 decomposition of PHGH. Above 470°C, the polymer began to carbonize and the weight

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of matter tends to be constant gradually [55]. As a whole, the derivatization of xylan with PHGH endowed the new xylan-derivative with sufficient thermal stability which is beneficial for it to be used under high temperature, such as producing paper and container products, baking foods, packaging hot foods and so on.

3.8. Influence of xylan-derivatives addition on mechanical properties of paper sheets

The mechanical properties of the paper products are important in the aspect of applications in packaging, baking, cleaning and so on. The influences of xylan, CX and CX-g-PHGH-1 addition on mechanical properties of the paper sheets are shown in Table 4, 5 and 6, respectively.

Table 4. Mechanical properties of paper sheets after addition of xylan.

Amount of xylan (wt %)	Tear index ($mN \cdot m^2/g$)	Burst index ($kPa \cdot m^2/g$)	Tensile index (Nm/g)	Folding endurance (times)
0	6.36	2.02	35.67	9
0.3	6.39	2.10	36.83	9
0.5	6.42	2.18	37.26	10
1.0	6.45	2.33	39.17	12
1.5	6.40	2.24	38.23	11

Table Footnote: Quantitative of each sheet was approximately 55 g/m^2, the stirring time of pulp after addition of xylan was 10 min.

It is obviously to see from Table 4, that addition of xylan had only a little effect on all mechanical properties of the paper sheets listed in the table. When the amount of xylan was 1.0 wt% in paper sheet, compared to the mechanical properties of reference paper without xylan addition, the index of tensile, tear and burst were increased by 9.81%, 1.42% and 15.35%, respectively. Meanwhile, there was also a minor increase for the folding endurance after addition of xylan.

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Table 5. Mechanical properties of paper samples after addition of CX.

Amount of Cationic-xylan (wt %)	Tear index ($mN \cdot m^2/g$)	Burst index ($kPa \cdot m^2/g$)	Tensile index (Nm/g)	Folding endurance (times)
0	6.36	2.02	35.67	9
0.3%	6.44	2.26	37.45	9
0.5%	6.65	2.38	38.89	10
1.0%	7.12	2.51	40.48	14
1.5%	6.89	2.31	39.06	12

Table Footnote: Quantitative of each paper was approximately 55 g/m^2, the stirring time of pulp after addition of CX was10 min.

The addition of CX also improved the mechanical properties of the paper sheets but to a larger extent than addition of xylan which can be seen from Table 5. This was probably because the cationic groups in CX could absorb the anionic groups (-OH) on the fibers, which could improve the combined forces among fibers, thereby increased the mechanical properties of sheets [56]. Compared to the reference paper sheet, when the amount of CX was 1.0 wt %, the index of tensile, tear and burst were increased by 13.48%, 11.95% and 24.26%, respectively, and folding endurance was increased by 55.55%. The mechanical properties of paper sheets became lower when the addition of CX was over 1.0 wt%. This was probably caused by excessive addition of cationic group in CX which prevented hydrogen bonding between cellulose fibers, correspondingly resulted in the decrease of the paper strength [43].

When CX-g-PHGH was added into the paper sheets, there were obvious improvements to the mechanical properties of the paper sheets which was affected not only by the amount of addition but also the grafting ratios of PHGH (Table 6).

Table 6. Mechanical properties of paper samples after addition of CX-g-PHGH.

Grafting Ratio (%)	Amount of CX-g-	Tear index ($mN \cdot m^2/g$)	Burst index ($kPa \cdot m^2/g$)	Tensile index	Folding endurance

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	PHGH (wt %)			(Nm/g)	(time)
0	0	6.36	2.02	35.67	9
14.27	1	7.35	2.48	40.96	12
15.54	1	7.84	2.59	41.97	15
16.90	1	7.73	2.60	42.00	15
18.45	1	7.97	2.63	42.83	16
18.45	0.3	6.71	2.22	40.35	13
18.45	0.5	7.35	2.57	40.97	14
18.45	1.5	7.26	2.55	41.23	12

Table Footnote: Quantitative of each paper was approximately 55 g/m^2, the stirring time of pulp after addition of CX-g-PHGH-1 was 10 min.

As it is shown clearly in Table 6, CX-g-PHGH with higher grafting ratio led to higher mechanical properties of paper. When CX-g-PHGH-1 (the highest grafting ratio 18.45% obtained at optimal condition) was added, compared with the reference paper sheet, the index of tensile, tear, burst and folding endurance increased by 20.07%, 25.31%, 30.20% and 77.78%, respectively. It is also well-known that paper strength is dependent on the fiber strength as well as primarily the hydrogen bonding force among fibers. PHGH contains guanidine group with positive charge which endows PHGH ability to adsorb onto fibers with anionic group [15]. Therefore, the improvements of mechanical properties were explained probably by such: when CX-g-PHGH was added into the paper pulp, CX-g-PHGH filled or adhered to the space between fibers which resulted in the increase of the bonding point and bonding area between fibers, correspondingly improved the mechanical properties of sheets [57]. Moreover, the guanidine group and cationic groups of CX-g-PHGH adsorbed negative charge of fibers, which thereby resulted in the increase of fiber retention, improvement of the bond between fibers and correspondingly improved the mechanical properties of paper sheets [56]. The mechanical properties of the paper samples were improved as the increase of

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CX-g-PHGH-1 addition. Furthermore, the amount of added CX-g-PHGH-1 also affected the mechanical properties of the paper samples. However, the mechanical properties of paper began to decrease when the amount of added CX-g-PHGH-1 was over 1.0 wt%. This can be explained that the excessive NH_2 brought from CX-g-PHGH-1 prevented the hydrogen bonding between cellulose fibers, which resulted in the decrease of the paper strength [43].

3.9. *Antimicrobial test of the paper sheets with addition of CX and CX-g-PHGH*

In this work, the antimicrobial activity of the paper sheets with addition of the xylan-derivatives was evaluated against *E. coli* bacteria by inhibition zone method and shown in Figure 7. As comparison, the antimicrobial activity tests for CX-g-PHGH (sample number 1 in Table 1, as CX-g-PHGH-2) obtained with lower initiator concentration and a relative lower grafting ratio of 15.54% was also studied.

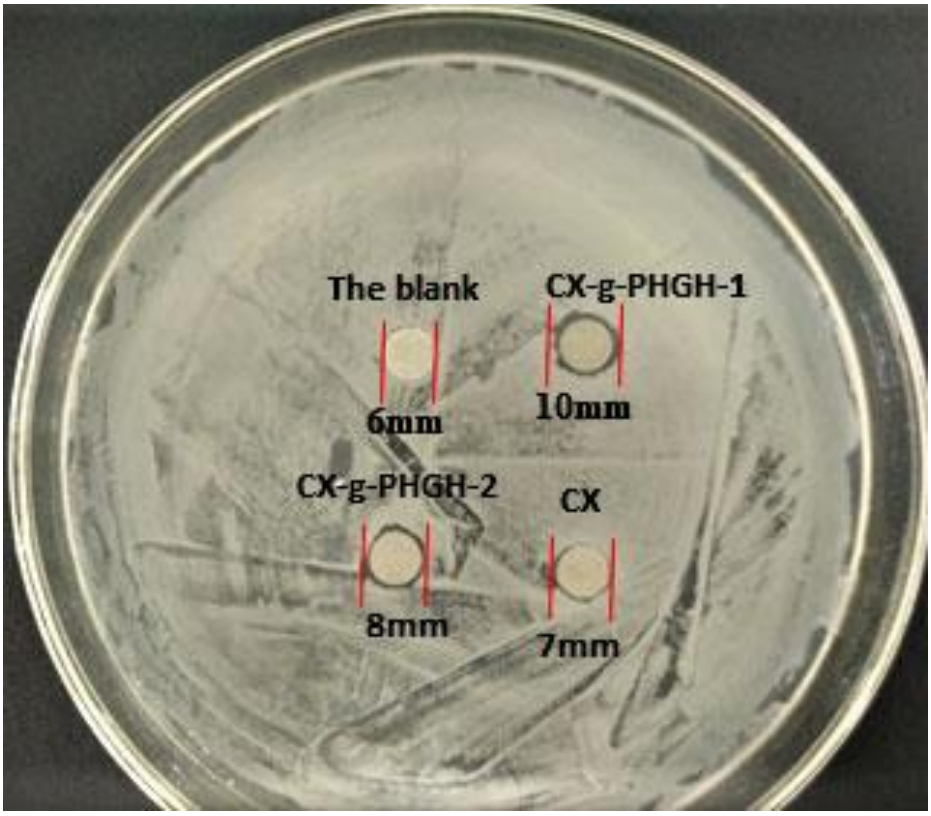

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Figure 7. The antimicrobial activity of the paper samples with the addition of CX, CX-g-PHGH-1, CX-g-PHGH-2 against *E. coli* bacteria.

Inhibition zone method is the most used method for antimicrobial activity tests of materials. By using this method, the growth of bacterial was inhibited in the formation of transparent circles by diffusion of antimicrobial agents in agar plates. The antimicrobial ability of the agents was evaluated by the size of the inhibition circle. Guanidino groups of guanidine polymers adsorbed the anions on the cell surface of *E. Coli* bacterial by electrostatic action which following destroyed normal metabolism and surface structure of the living bacterial cells, sequentially inhibited the growth of bacteria effectively [18].

It can be seen that addition of CX and CX-g-PHGH endowed paper sheet antimicrobial property. CX paper sample showed weaker antimicrobial ability than CX-g-PHGH samples with smaller inhibition zone diameter of 7 mm. The diameter of inhibition zone for the paper of CX-g-PHGH-1 (grafting ratio 18.45%) and CX-g-PHGH-2 (grafting ratio 15.54%) against *E. coli* enlarged from 6 mm to 10 and 8 mm, respectively, which suggested that the CX-g-PHGH paper had excellent antimicrobial activity against *E. coli* and the antimicrobial activity was improved by the increase of PHGH contents grafted on the CX.

4. Conclusions

In present study, a novel xylan-based antimicrobial additive agent was

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542 successfully prepared and applied in cellulosic paper sheets with improved mechanical
543 properties and antimicrobial activity against *E. Coli* bacteria. The derived xylan,
544 cationic xylan-grafted-PHGH (CX-g-PHGH), was successfully synthesized by graft
545 copolymerization of cationic xylan (CX) with guanidine polymer (PHGH) using ceric
546 ammonium nitrate (CAN) as initiator. The optimal reaction parameters for obtain
547 efficiently the CX-g-PHGH were 4 h at 60°C with PHGH concentration of 0.039 mol/L
548 and initiator concentration of 4 mmol/L, by which the maximum grafting ratio and
549 efficiency of 18.45% and 58.45% were reached, respectively. Furthermore, the
550 mechanical properties of the paper sheets with addition of CX-g-PHGH had obvious
551 improvements. Compared to the mechanical properties of paper sheet without any
552 addition of the xylan derivatives, the addition of CX-g-PHGH improved the mechanical
553 properties of the sheets by up to 20.07% (tensile index), 25.31% (tear index), 30.20%
554 (burst index) and 77.78% (folding endurance). Meanwhile, the paper sheet with
555 addition of CX-g-PHGH exhibited improved viscosity, thermal stability as well as
556 excellent antimicrobial activity against *E. Coli* bacterial which was inherited from the
557 antimicrobial activity of guanidine in CX-g-PHGH.

558 The present work found a new way of synthesizing xylan-derivative and used it
559 as antimicrobial additive agent against *E. Coli* bacteria in paper product. The obtained
560 paper product with highly improved mechanical strength, antimicrobial property as
561 well as biodegradable and renewable properties would find great potential applications
562 especially in food-related areas, e.g. packaging, baking and napkin paper, as well as
563 other areas such as pharmaceuticals, cosmetics and so on, which expanded the

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564 applications of xylan in more high value-added areas.

565

566 **Author Contributions:** Guibin Xu, Bei He and Minmin Chang performed the
567 laboratory work; Guibin Xu wrote the main manuscript text; Tao Song and Junli Ren
568 supervised and revised the manuscript.

569 **Funding:** This work was financially supported by State Key Laboratory of Pulp and
570 Paper Engineering, grants number 2017C02 and 201714, Science and Technology
571 Planning Project of Guangdong Province, grant number 2017A010103032, The
572 Guangdong Program for Support of Top-notch Young Professionals, grant number
573 2016TQ03Z585, the Fundamental Research Funds for the Central Universities of
574 SCUT, grants number 2017ZD081 and 2017MS080, Guangdong Provincial Natural
575 Science Foundation Project, grant number 2018A030313211 and Guangdong Province
576 Science Foundation for Cultivating National Engineering Research Center for Efficient
577 Utilization of Plant Fibers, grant number 2017B090903003.

578 **Conflicts of Interest:** The authors declare no conflict of interest.

579

580 **References**

581

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