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Antibacterial Properties of Hemp Hurd Powder Against *E. coli*

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ABSTRACT: Hemp (*Cannabis sativa L.*) is an eco-friendly and multifunctional plant. Hemp hurd is a by-product of hemp plant during hemp fiber separation. Although hemp hurd is repeatedly announced owing antibacterial activity, it has never been systematically investigated and reported. In this study, the antibacterial activity of hemp hurd powder against *Escherichia coli* is investigated. This article reveals antibacterial activity of hemp hurd where hemp hurd powder inhibits the growth of *E. coli*. Meanwhile, the self-contamination (forming during retting process) inside hemp hurd has dramatic impact on the antibacterial performance. To achieve better antibacterial activity, hemp hurd was heat treated to eliminate self-contaminations. The impact of the particle sizes and heat treatment on the antibacterial effectiveness was evaluated. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41588.

KEYWORDS: biomaterials; biomedical applications; morphology; properties and characterization; thermogravimetric analysis (TGA)

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INTRODUCTION

Botanically, hemp is a member of the most advanced plant family on earth. It is a dioecious woody, herbaceous annual plant that uses the sun more efficiently than any other plants and it can be grown in virtually any climate or soil condition. Hemp has a long history of cultivation for a variety of applications including textiles, medicine, recreational drugs, and food.[1,2] Hemp can be divided into fiber type (industrial hemp), intermediate type, and drug type (known as marijuana), with tetrahydrocannabinol (THC) content ranging from <0.3%, 0.3–1.0%, and 1–20%, respectively.[3,4]

Industrial hemp is a kind of yearly harvested plant where hemp fiber is the main product of its cultivation. Hemp hurd (also called hemp core) is a by-product and agricultural waste of hemp plant. It is a residue from the hemp stem after the bast fibers are removed for textile. Hemp hurd is mostly used for animal bedding because of its favorable properties: good absorbency, easy handling, and rapid composting after use.[5] Another application of hemp hurd is in construction sector. New utilizations of the hemp hurd are under development,[6] however, a great amount of hemp hurd (accounting for 70–80% of the hemp stem) is disposed by combustion or landfilling, which results in resource waste. To develop its utilizations, hemp hurd could be milled into powder. For instance, hemp hurd powder is a good filler in hemp-reinforced plastic composites.[7] Also, hemp hurd powder can be incorporated in 3D printing filament material in the emerging 3D printing technology.[8] Hemp hurd powder may also be applied to produce activated carbon with high specific surface areas, micro-porous structure, high adsorption capacity, and degree of surface reactivity.[9]

Recently, hemp as an antibacterial agent has been attracting more and more attention.[10,11] Hemp fiber has been confirmed with excellent antibacterial activity, which leads hemp fiber to be a good material for functional textiles.[12] However, antibacterial performance of hemp hurd has never been systematically investigated, although it is repeatedly announced that hemp hurd also has similar property.[13] The antibacterial properties in hemp hurd may come from cannabinoids, alkanoids, other bioactive compounds, or compounds of lignin.[11,14]

In this study, hemp hurd was milled into powder with different size. Then the antibacterial properties against *Escherichia coli* were investigated. The impact of retting process and particle size of hemp hurd powder on the antimicrobial performance was studied. Due to huge amount of hemp hurd waste every year, these observations may stimulate future inclusion of hemp hurd in antibacterial food package, hence utilize agricultural waste resource and reduce environmental pollution.[15]

MATERIALS AND METHODS

Material

Three types of hemp hurd (retted, semi-retted, and non-retted) were used in this study. Semi-retted and non-retted hemp hurd was obtained from Ecofibre Industries Operations Pty (Australia). Retted hemp hurd powder was received from Research

Table I. Retting Status and Particle Size of Hemp Hurd

Retting status	Mean particle size
Semi-retted	188.4 μm
	85.4 μm
	44.3 μm
	21.2 μm
Non-retted	204.0 μm
	99.8 μm
	47.2 μm
	20.5 μm
Retted	37 μm
	19.1 μm

Centre of China-Hemp Materials, Beijing, China. Retted hemp hurd was obtained at about 20°C for 8 days in dewing process. Semi-retted hemp hurd was achieved at about at 20°C for 3 days followed by mechanical separation. For the non-retted hemp hurd, it was separated through mechanical decortication.

A cutter mill (Pulverisette 19 from Fritsch Gmbh, Germany) was used for chopping hemp hurd chips into approximately 1 mm snippets. Hemp hurd snippets were passed through a rotary mill (Pulverisette 14 from Fritsch) to be milled repeatedly up to 55 times to obtain desired particle size. Different size of sieve was applied to separate the hemp hurd powder into different particle sizes.

Particle size distribution was measured using a Mastersizer 2000 (Malvern Instruments, UK) fitted with Hydro 2000S. The dispersion medium was deionized water.[16] Table I shows the particle size of the different retted samples with various time of milling. All results were presented according to a volume-based particle size distribution.

Antibacterial Test

The hemp hurd powder was tested for its antibacterial performance against *E. coli* (ATCC25922). The bacterial cultures were maintained on nutrient agar slopes. They were grown in sterile Tryptic Soy Broth and incubated at 37°C for 18 h. Working buffer solution (0.3 m*M* KH_2PO_4) was adjusted pH to 7.2 ± 0.1 with a dilute solution of NaOH, and then capped, sterilized, and stored at room temperature.

To prepare the working bacterial dilution, the culture was diluted with the sterile buffer solution until the solution has an absorbance of 0.28 ± 0.02 at 475 nm (as measured spectrophotometrically), which corresponds to a concentration of 1.5–3.0 $\times 10^8$ colony forming units per milliliter (CFU/mL). The Atherton cyber series autoclave was used for sterilization and media preparation at 121°C for 20 min.

Antibacterial performance of the hemp hurd powder was investigated according to ASTM E2149-10, described as the following: (1) 1.0 g of hemp hurd powder was placed into a 250-mL flasks with 50 ml working bacterial dilution; (2) the flask was shaken on an agitation shaker at a speed of 300 rpm at 25°C for 1 h $\pm$ 5 min; (3) 1 mL of the solution before or after shaking was inoculated on a plate containing 15 mL tryptic soya agars; (4) the inoculated plate were cultivated at 37°C for 24 h; and (5) active bacteria were counted and antibacterial effect was calculated. Percent reduction of the organisms resulting from treated sample directly compared to "inoculum only" sample after specified contact time is calculated. Results are presented in percent reduction by counting CFU/mL of bacteria.

Heat Treatment and Thermogravimetric Analysis

To study whether the antibacterial property in the hemp hurd can be preserved after hemp hurd powder being processed at different processing temperature, hemp hurd powder was heated at 80, 120, 140, and 160°C for 0.5–3 h in a dry oven. Hemp hurd powder was kept open in a ceramic beaker to allow the evaporation of moisture from the material.

Thermogravimetric analysis (TGA) was used to determine the thermal stability of the hemp hurd powder. The constituents of hemp hurd powder have different thermal reactivity and decomposition at different temperatures. TGA analysis on hemp hurd powder was carried out by a thermal gravimetric analyzer (TGA Q500). In ramp method, the samples were heated from 10°C to 400°C at a rate of 20 °C/min. In ramp and isothermal method, the sample was heated up to 100°C at a rate of 20 °C/min and held for 20 min, then it was heated up to 160°C at a rate of 20 °C/min and held for another 20 min. Experiments were carried out in a nitrogen medium (60 mL/min) and the weight was recorded as a function of increasing temperature.

Morphologies

The morphologies of milled hemp hurd powder were studied by scanning electron microscopy (SEM) images. The samples were sputter gold-coated for 120 s and were examined in a JEOL JCM 6000 SEM operated at 10 kV with use of the secondary electron signal.

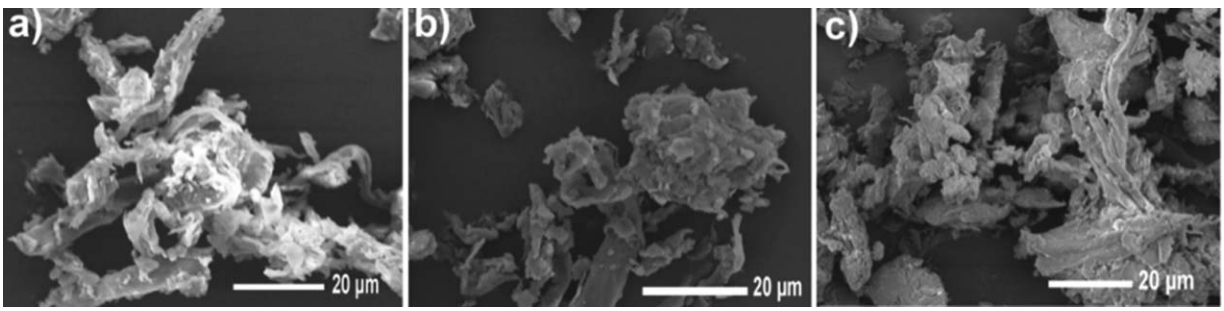

Figure 1. SEM images of hemp hurd powder: (a) retted; (b) semi-retted; (c) non-retted.

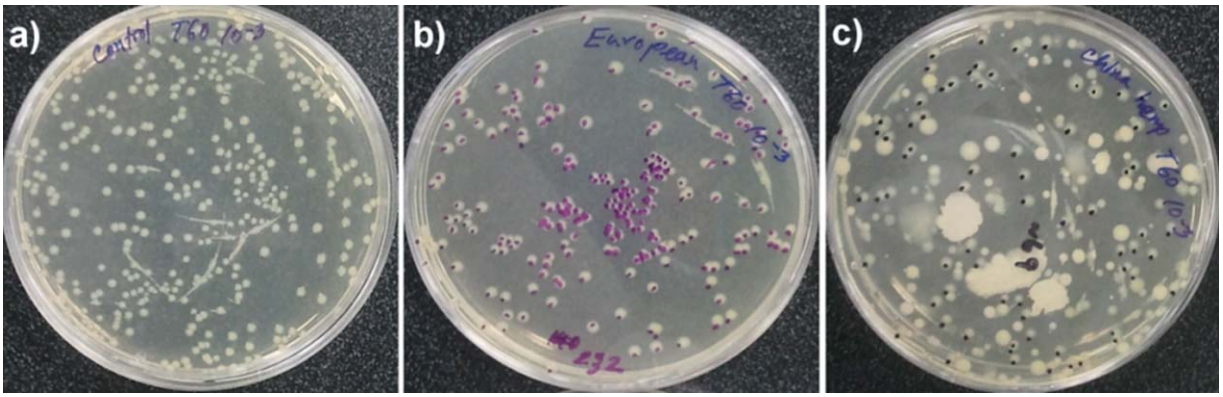

Figure 2. Inhibition of bacterial growth of hemp hurd powder: (a) control; (b) non-retted hemp hurd powder; (c) retted hemp hurd powder. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS AND DISCUSSION

Morphologies

Figure 1 shows the SEM image (High-vac, 10 kV) of hemp hurd powder. A number of thin hemp hurd slices and irregular-shaped aggregation were clearly observed. In addition, some short fibers still existed because of insufficient pulverization.[17] In general, it shows an irregular rough surface with sharp edges and grooves. The hemp hurd powder shows the porous structure, indicating low bulk density and high water absorption capacity. The high surface roughness of the hemp hurd powder ensures a good bond with the polymer matrix, so that the hemp hurd powder will be able to be applied to reinforced plastic and other composites.

Inhibition of Bacterial Growth

The antibacterial activity of hemp hurd powder is presented in Figure 2. It is evident that the hemp hurd powder inhibited the growth of *E.* coli as the plates of control and the hemp hurd powder have shown different appearance in the bacterial lawn. The controlled sample has the CFU 340; while the non-retted hemp hurd powder CFU reduces 30% to 235; and the retted hemp hurd powder CFU decreases 78% to 75. These results demonstrate that retted hemp hurd is more effective for the inhabitation of *E. coli* bacteria. However, in the petri dish, there are still some other types of microorganisms remaining in both retted and non-retted hemp hurd powder (although only *E. coli* was expected). These contaminations are from hemp hurd powder itself, and they might have occurred from the fields where they grow up and existed during fiber processing (retted or non-retted). It is also notable that retted hemp hurd embraced more severe contamination than the non-retted hemp hurd.

The retting process is a controlled degradation of plant stems to allow the fiber to be separated from the woody core (hurd). Dew, water, enzymatic, mechanical, and chemical retting processes are common for hemp.[18,19] Therefore, the retting process of hemp provides more opportunity for hemp hurd to be contaminated and promulgated.

Self-Contamination of Hemp Hurd

To remove self-contaminations, retted hemp hurd powder was treated in the air oven at 180°C for 20 min. Figure 3 shows the antibacterial performance of retted hemp hurd powder before and after heat treatment. It is clearly observed that self-contaminations are eliminated after heat treatment. Compared with the non-treated hemp hurd powder having around 78%

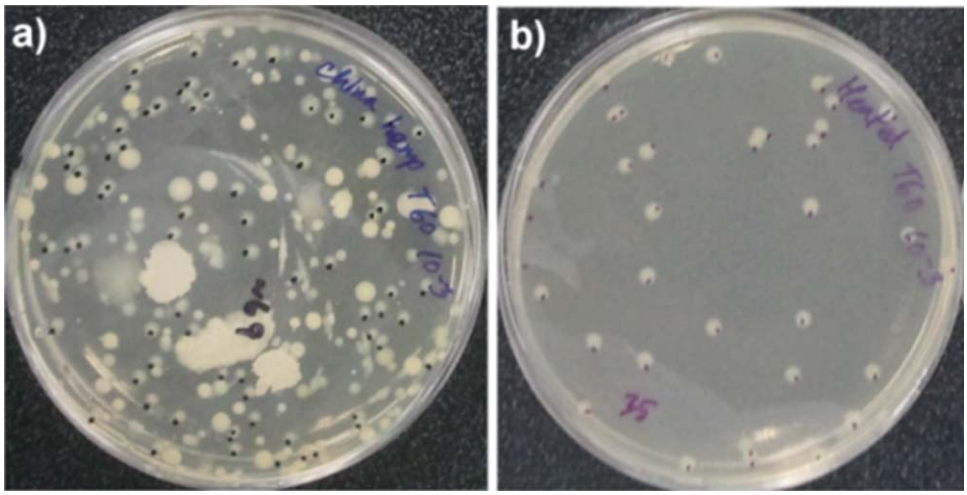

Figure 3. The antibacterial activity of retted hemp hurd: (a) before and (b) after heat treatment. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

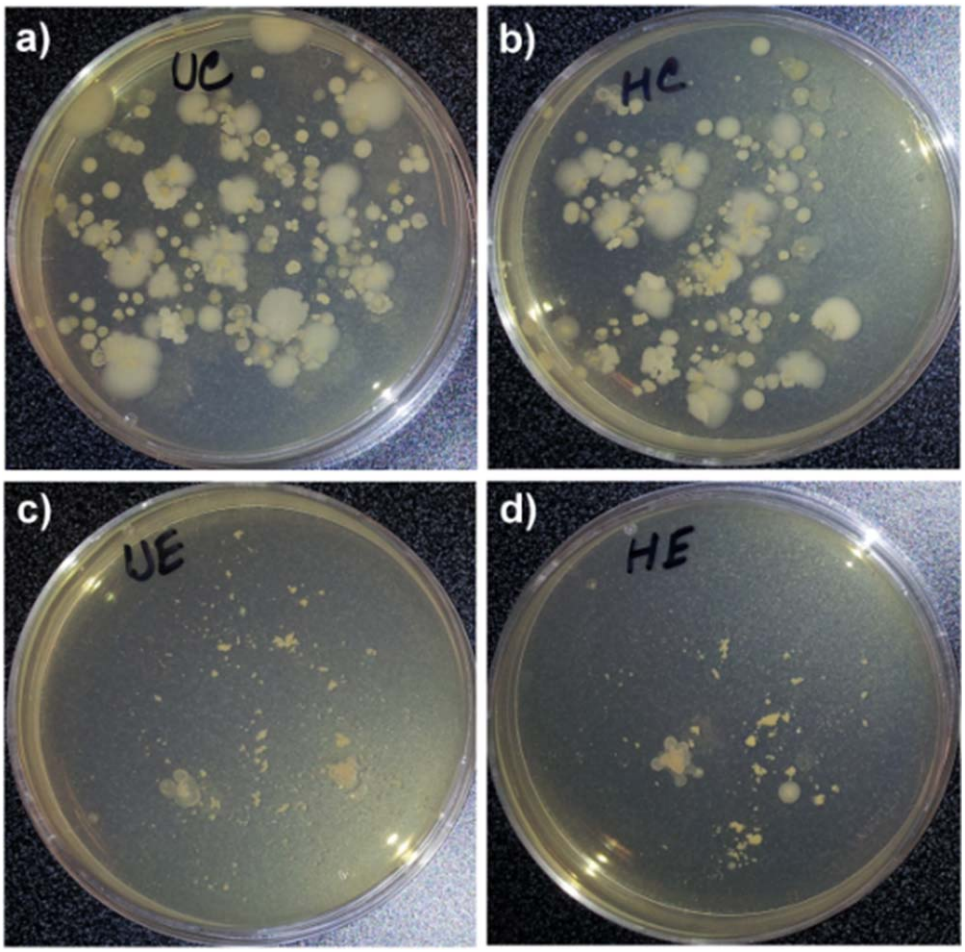

Figure 4. Status of self-contaminations: (a) retted hemp hurd powder without heat treatment; (b) retted hemp hurd powder treated at 80°C; (c) un-retted hemp hurd powder without heat treatment; and (d) un-retted hemp hurd powder treated at 80°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

reduction in CFU, the heat treated hemp hurd powder had more efficient antibacterial activity with 90% reduction in CFU. Therefore, to achieve the best performance, the elimination of self-contamination is certainly important. Inactivation of microorganisms (such as bacteria) can be achieved by chemical and/or physical means, such as heat, chemical solutions, gases, and radiation.[20–22] It has been confirmed that heat has detrimental effects on living cells,[20] and the heat-based sterilization techniques were developed and commercially used to dispel undesired medium preservation.[23]

Table II. Observation on Elimination of Self-Contaminations After Heat Treatment

	120°C	140°C	160°C
30 min	×	×	×
60 min	×	×	√
90 min	×	√	√
120 min	×	√	√
150 min	×	√	√
180 min	√	√	√

(×) = contamination remaining; (√) = contamination completely eliminated.

To obtain appropriate and effective contamination elimination, different heat treatment was investigated in this study. Hemp hurd powder was kept in the oven at 80°C for more than 3 h until all the moisture was completely removed. Then, the powder was spread on agar containing petri dishes and incubated at 35 ± 2°C for 24 h. Figure 4 shows the effect of low temperature treatment to eliminate the self-contaminations, presenting that

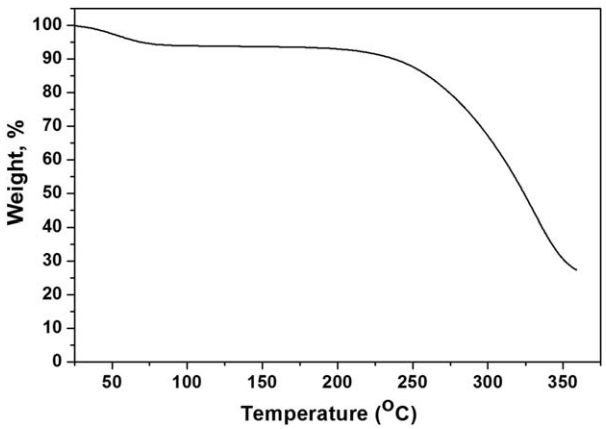

Figure 5. TGA curves of retted hemp hurd.

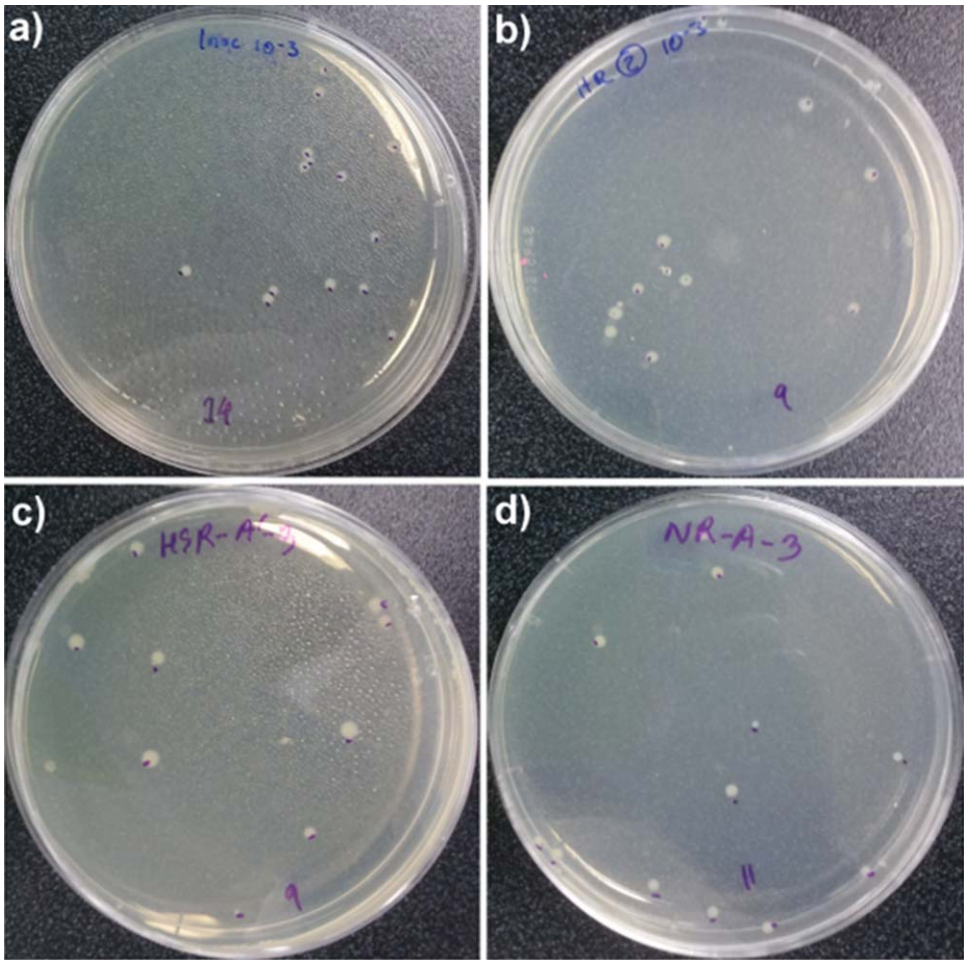

Figure 6. Antibacterial activities of different types of hemp hurd powder (20 μm): (a) control; (b) retted hemp hurd powder; (c) semi-retted hemp hurd powder; and (d) non-retted hemp hurd powder. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

heat treatment at 80°C for 3 h was not sufficient to eliminate the existing contaminations of hemp hurd powder. This is due to the higher decomposing temperature of some contaminations.[24] Dew, water, enzymatic, mechanical, and chemical retting processes are common for hemp. Therefore, the retting process of hemp provides more opportunity (along with the favorable condition for bacterial growth) to hemp hurd to be contaminated and promulgated. It is necessary to remove those contaminations for further antibacterial application.

To investigate heat-based conventional sterilization methods, both moist heat and dry heat were studied. In the case of moist heat in an autoclave, a temperature of 120°C at a pressure of 15 psi was applied for 20 min, while dry heat sterilization was proceeded at 170°C for 1 h.[23] To study the impact of temperature and holding time on the self-contamination elimination in hemp hurd powder, a series of experiments were carried out, and the status of self-contaminations was observed by simply spreading out heat-treated hemp hurd powder on the agar containing petri dish, followed by incubating at 35 ± 2°C for 24 h. Table II summarizes the contamination status at different heat-curing conditions, indicating that the higher the curing temperature, the shorter the curing time to eliminate self-contaminations.

Thermal Degradation

TGA was applied to study the weight loss of hemp hurd powder with the increase of temperature. The degradation process was divided into four stages: moisture evaporation, hemicellulose degradation, cellulose degradation, and lignin decomposition. Moisture is present in the material in two forms: free water and linked water. Free water is attached on the fiber surface and evaporates at lower temperature (25–150°C). The linked water with the hydroxyl groups is bonded in hemicellulose and lignin, and decomposes at higher temperatures.[25] After the removal of free water, the degradation process begins in the cellulose, hemicellulose, lignin constituents, and the associated linked water.[26,27] TGA curves in Figure 5 suggest that at 160°C, no thermal degradation will occur to the hemp hurd powder itself.

Effect of Particle Size and Temperature

Retted (37 μm, 19.1 μm), semi-retted (188.4 μm, 85.4 μm, 44.3 μm, 21.2 μm), and non-retted (204 μm, 99.8 μm, 47.2 μm, 20.5 μm) hemp hurd powder of different particle sizes were investigated for antibacterial activity. Heat treatment (160°C for 2 h) was carried out to remove self-contaminations before testing. Particle size did not show any effect on antibacterial activity, and 2-h contact time was sufficient for both fine and coarse particle to present

maximum antibacterial activity. Figure 6 shows antibacterial result of selected hemp hurd powder, where both retted and semi-retted hemp hurd were heat-treated, and non-retted hemp hurd was applied without further treatment due to non-contaminations. Figure 6(a) is the control without any hemp hurd powder, and Figure 6(b–d) contain hemp hurd powder. All the three types of hemp hurd powder show similar antibacterial activity, which further confirmed that self-contaminations in hemp hurd are the main impact factor on the antibacterial performance.

Potential Applications and Future Work

There is a great concern of contaminations by microorganisms in a variety of areas, for example, medical devices, healthcare products, water purification systems, hospitals, dental office equipment, food packaging, food storage, and household sanitation.[28] One possible way to address microbial contamination is to develop materials with antimicrobial properties.[29]

Polymers such as polyethylene, polyurethane, polytetrafluoroethylene, polyacetal, polymethylmethacrylate, polyethylene terepthalate, silicone rubber, polysulfone, polyetheretherketone, poly(lactic acid), poly(glycolic acid), and so on, are used in various biomedical fields.[30] Incorporating antibacterial hemp hurd powder as filler in the polymer composites could provide not only lighter weight but also protection against bacterial attachment. Hemp hurd reinforced polymer composites could be an excellent choice for prostheses. Similarly, hemp hurd powder can be incorporated in food packaging composites, and be considered as eco-friendly. Hemp hurd powder incorporated polymer composites formed by injection molding can provide packaging materials with a wide range of shape and sizes for containing a range of foods including meat, salads, and ready-made food products.

CONCLUSION

In this study, the antibacterial activity of hemp hurd powder against *E. coli* was systematically studied by applying different hemp hurd powder (retted, semi-retted, and non-retted) with different size. To apply the retted and semi-retted hemp hurd powder as antibacterial materials, it is necessary to eliminate contaminations that come from external surroundings (such as humid and temperature) during retting process. The heat-treated hemp hurd powder at 160°C for 2 h showed efficient antibacterial activity up to 90% reduction in CFU. It was also explored that the particle size of hurd powder has no obvious impact on the antibacterial performance. It is proposed that the hemp hurd powder has many potential applications in biomedical, food packaging, polymer composites, and other value added diversified products.

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