BIODEGRADABILITY AND ANTIMICROBIAL ACTIVITY OF CHITIN NANOWHISKERS-CHITOSAN FILMS

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Abstract

Chitosan has a potential to become a key resources in the development of biodegradable film composites. Chitin Nanowhiskers (CNW) was prepared by hydrolyzing chitin powder with 3M HCl. The CNW/chitosan film was prepared at various CNW contents (1, 2, 3, 4, and 5 percent of weight). CNW was added to improve the strength of chitosan film. CNW/chitosan films were prepared by solution casting method and the biodegradability properties were evaluated in soil burial for 6 days. The biodegradability was determined by weight loss of the films that have been incubated in the soil. It showed that increasing the amount of CNW lead to greater weight loss of the films. The swelling test was done to study the effect of water absorption rate on the physical properties of the film. It showed that addition of CNW reduce the ability of the film to absorb water. Tensile properties were evaluated on the nanocomposite films. It showed that the tensile strength of the nanocomposite films increased as the CNW increased up to 3%. It was found that CNW/chitosan film have antimicrobial activity against food pathogenic bacteria such as *E.coli* and *S.aureus*. They can inhibit the growth of these bacteria since clear zone have been detected on the agar plate containing chitosan, 3% CNW/chitosan solution and 5% CNW/chitosan solution. Since the films obtained were transparent and displayed good mechanical properties, the nanocomposite film can be used in membrane and packaging applications.

Keywords: Biodegradable, chitin nanowhiskers, chitosan, water absorption, soil burial

INTRODUCTION

Recently, due to its non-toxicity, excellent film-forming property and biodegradable, the chitosan films have potential to be used as a packaging material for the quality preservation of a variety of food and agriculture product. Due to increasing usage of petroleum-based packaging materials in recent years, serious environment issue and food safety also increase rapidly and researchers nowadays have focused on the use of bio-based compounds [1].

Chitin is a natural, renewable and biodegradable polymer and the second most abundant natural polymer after cellulose with linear polymer structure of *N*-acetyl-D-glucosamine linked by (1,4) glycosidic bond. The fibrous material of cellular walls of mushrooms, algae and external skeletons in shellfish and insects are made of chitin. Chitin is disposed of as industrial waste in shellfish canning industries [2]. Chitins also have anti-microbial properties making them applicable for food processing, preservation and packaging. Chitin has mechanical properties that is found in crustacean shells comprises stacks of chitin nanocrystals [3].

Chitosan can be very useful in food packaging since its ability to be degraded. However, pure chitosan films have poor tensile strength and elasticity. Hence, development of high strength composites that are biocompatible can help in improvement of the chitosan film. This study was conducted to create a high strength biodegradable film composite which can be very useful in packaging application. Recently, CNW is finding its importance as fillers or reinforcement natural polymers to form biocomposites due to its highly crystalline nature. Therefore, the aim of this study is to improve the mechanical properties and biodegradability of chitosan film using CNW.

EXPERIMENTAL

Preparation of Chitin Nanowhiskers (CNW)

Commercial chitin (1g) was added to 3M HCl (30mL) and stirred at 100-105ºC for 90 minutes. Then, the solution was centrifuged at 3200rpm for 15 minutes. This step was repeated for three times using distilled water. The residue was collected after centrifugation and transferred into dialysis bag before dialyzed in running water for two hours. The dialysis bag was put into a beaker containing distilled water overnight. Finally, the pH of distilled water was measured so that it was higher than 4.

Preparation of chitin nanowhiskers/chitosan film

Commercial chitosan powder (1g) was dissolved in 2% v/v acetic acid (100mL). 1% of CNW was added in the solution. The solution was stirred until all the chitosan powder and CNW dissolved in the acetic acid. After that, the solution was spread on a stainless steel tray and dried in oven at 50ºC for 24 hours. The film was removed from the tray using 0.5M NaOH solution. The film must be kept in desiccator before characterization. This method was repeated using different weight percent of CNW which is 2%, 3%, 4% and 5%.

Swelling Test

The chitosan and nanocomposite films was cut into small pieces and weighed. All the samples were immersed into distilled water at room temperature. After 24 hours, the swollen film samples were removed from distilled water and quickly wipe to remove excess water on the surface. Finally, the weight of the swollen films was recorded.

$$\text{Degree of Swelling} = (W_w - W_d)/W_d$$

where W_w and W_d are weights of wet and dry film, respectively.

Biodegradability Test of CNW/chitosan Film

The pure chitosan and CNW/chitosan films was cut into 15mm X 15mm square pieces. The samples were dried in a desiccator until their weights became constant. The samples were then buried in the compost soil at a depth of 20-25cm from the surface for 6 days. The samples were taken out for testing every 3 days. Then, the samples were washed with distilled water to remove the sand from the surface of samples and dried at 55ºC until their weights became constant. The percentage of weight loss was calculated.

$$\text{Weight loss} = \frac{W_0 - W_t}{W_0} \times 100$$

where W_0 is the initial mass and W_t is the remaining mass at any given time. All results were the average of three replicates.

Antimicrobial Test by Well Diffusion Method

Antimicrobial activity of CNW/chitosan film was observed against *S.aureus* and *E.coli* using well diffusion method. Nutrient agar plate was spread evenly with 0.1ml microbial culture over the surface. The plates were allowed to dry for 15 minutes before four wells were made in the agar medium. A few drops of chitosan, 3% CNW/chitosan solution and 5% CNW/chitosan solution was placed into each well in a different plate. All the plates were then placed at 37ºC and incubated for 24 hours.

RESULTS AND DISCUSSION

Synthesis of chitin nanowhiskers (CNW)

The CNW have been synthesized by acid hydrolysis of chitin powder. Acid hydrolysis of chitin powder will remove amorphous structure from chitin so that it can be effective as reinforcement for chitosan film for example to increase the strength of the film. Figure 1 shows an AFM image of a dilute suspension of CNW from acid hydrolysis of commercial chitin. The suspension consisted of slender rods with sharp point. The average length of chitin whiskers are 100nm long. It has been reported that the normal CNW crystals ranged from 150nm to 800nm, while the width ranged from 5 to 70 nm [4].

Swelling Test of Nanocomposite Films

Swelling test was carried out to observe the influence of CNW on the degree of water absorption of the samples. Figure 2 shows water uptake of the pure chitosan and CNW reinforced chitosan films at various whiskers content up to 5%. Pure chitosan film showed the highest degree of swelling since it has hydrophilic properties that can absorb more water molecules. The higher water absorption by the pure chitosan film is as expected since the nature of the chitosan contains easy accessible hydroxyl group which gsve strong hydrophilic character to the chitosan film. However, the presence of CNW has decreased the affinity of chitosan films toward water. The reduction may be due to the highly crystalline CNW being more hydrophobic than chitosan and the strong interaction between the CNW and chitosan hence, decreasing the swelling properties [5].

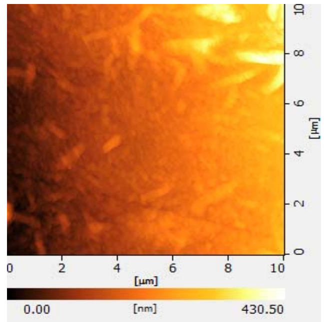


Figure 1. AFM image of CNW

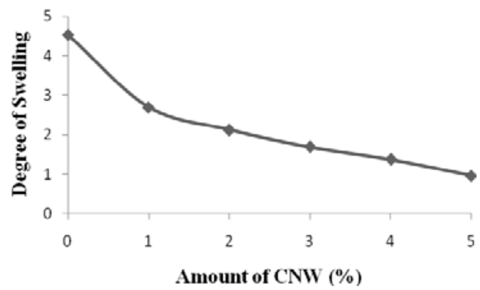
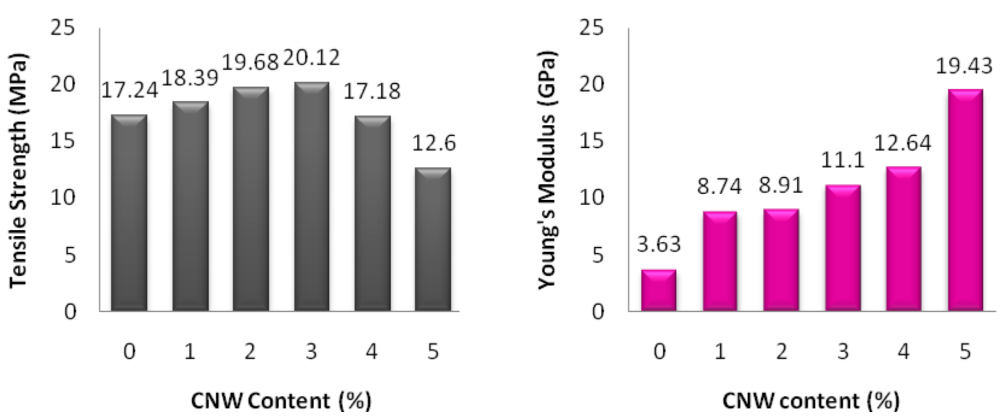


Figure 2. Water uptake of the pure chitosan and CNW reinforced chitosan films at various whiskers content up to 5%

Mechanical Properties of CNW/chitosan Films

Figure 3 (a) and (b) show the tensile strength and Young's Modulus of the pure chitosan and CNW/chitosan films. The tensile strength of the films initially increased from pure chitosan (17.24 ± 3.70 GPa) with increasing CNW content up to 3% (20.12 ± 9.08 GPa) and gradually decreased with further increase of CNW and the Young's Modulus are increased as the CNW increased. Increased in mechanical properties of the films were due to the strong hydrogen bonding between CNW and chitosan and making the matrix stronger [6].



(a) (b)

Figure 3. (a)Tensile strength and (b) Young's Modulus of pure chitosan and CNW/chitosan films at various CNW contents

Figure 4 shows the percentage of elongation at break of the nanocomposite films. The percentage of the elongation at break of the films was correlated with the tensile strength. The elongation of the nanocomposite films decreased as the CNW increased up to 3%. Interaction between chitosan molecules and CNW via hydrogen bonding increased the tensile strength of the nanocomposite films and caused the film to be more rigid thus reducing the percentage of elongation at break [4].

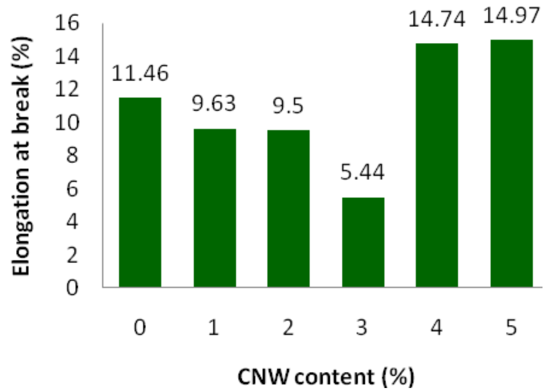


Figure 4. Percentage of elongation at break of pure chitosan and CNW/chitosan films at various CNW contents

Biodegradability Study of CNW/chitosan Films

Biodegradability study of CNW/chitosan film was done to determine the weight loss of prepared film samples incubated in soil for six days. The samples were taken out every day to record the weight loss. Triplicate samples were tested and presented in Figure 5.

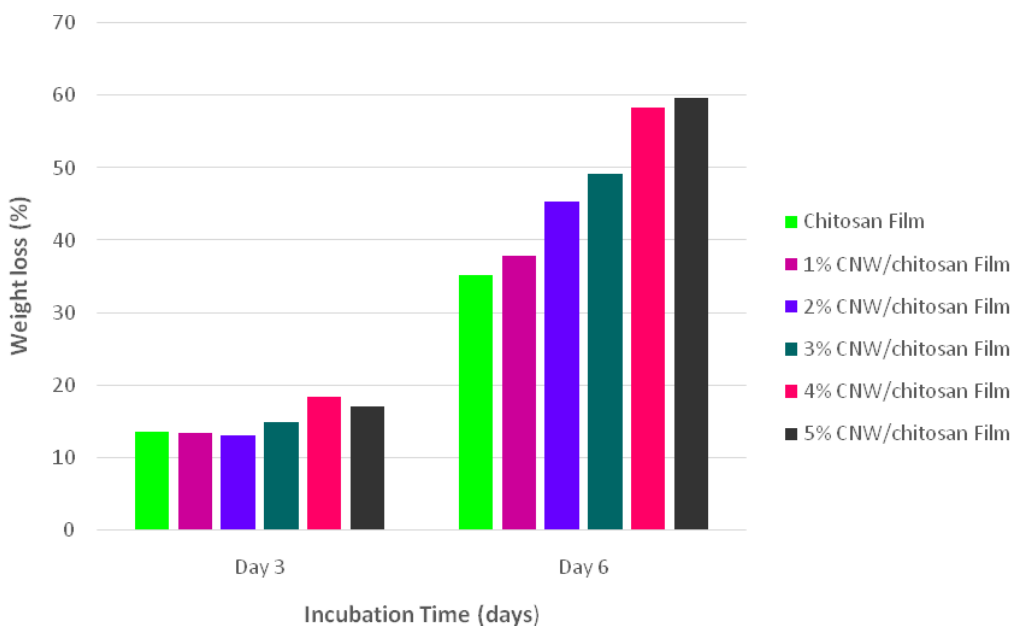


Figure 5. The weight loss of the film samples

The rate of weight loss is independent on the amount of CNW for the first three days. This result shows that the addition of CNW as a fillers to chitosan film did not affect the biodegradability properties of chitosan. However, after six days of incubation the weight loss of the chitosan films increases with increasing amounts of CNW. This is due to the large distribution of chitin hydrolyzing enzyme-producing organism in the soil. Soil usually contains different types of microorganism depending on the growth temperature [7]. This study demonstrates the positive and practical application of CNW on chitosan film as fillers to manufacture chitosan-based biodegradable packaging materials which are readily degrade and broken down in the soil environment.

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Antimicrobial Activity of CNW/chitosan Films

E.coli and *S.aureus* were used in this study to observe the antimicrobial properties of CNW/chitosan films against food pathogen using well diffusion method. As presented in Figure 5 and Figure 6, pure chitosan and CNW/chitosan solution with CNW content 3% and 5% shows the formation of inhibition zone toward both tested pathogenic bacteria.

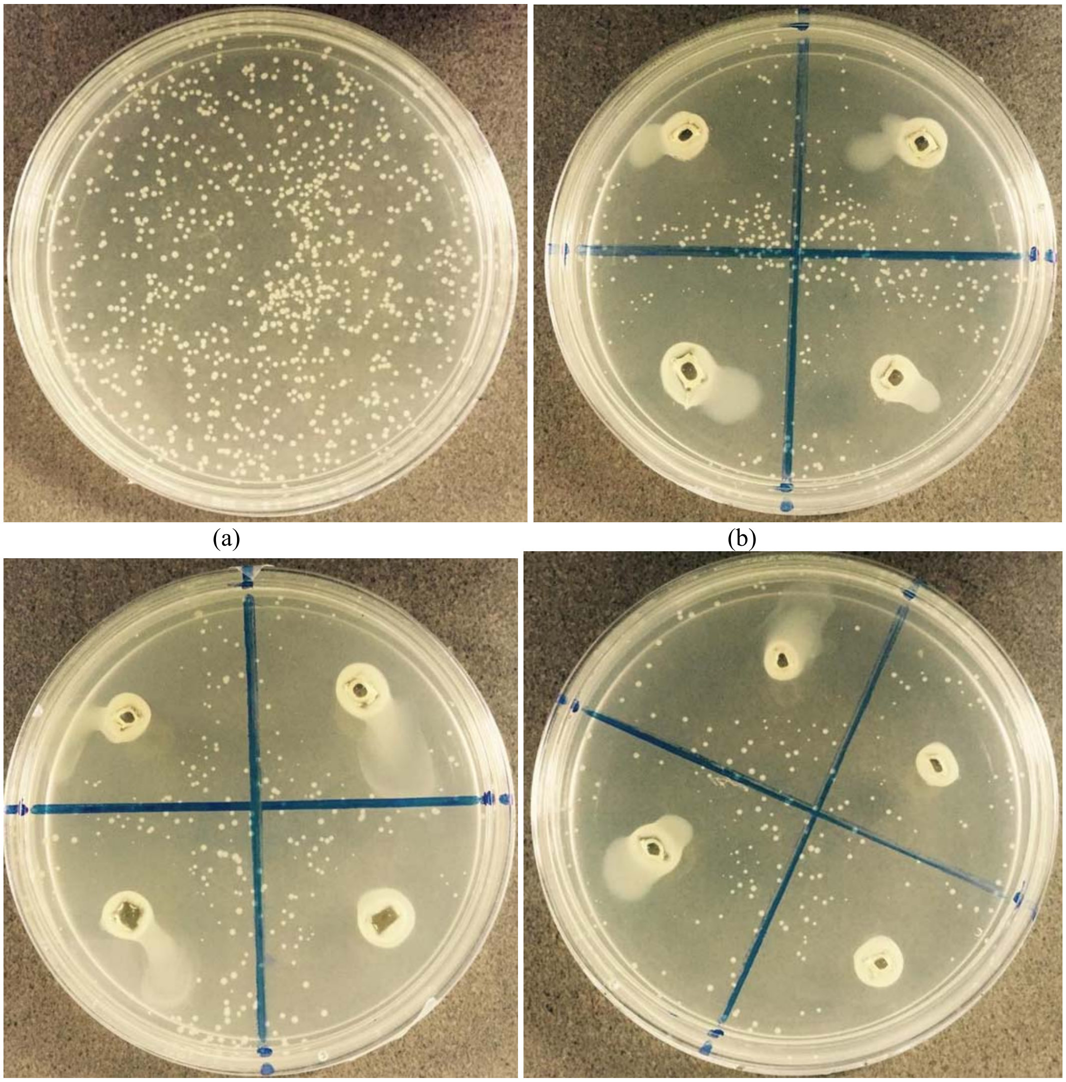
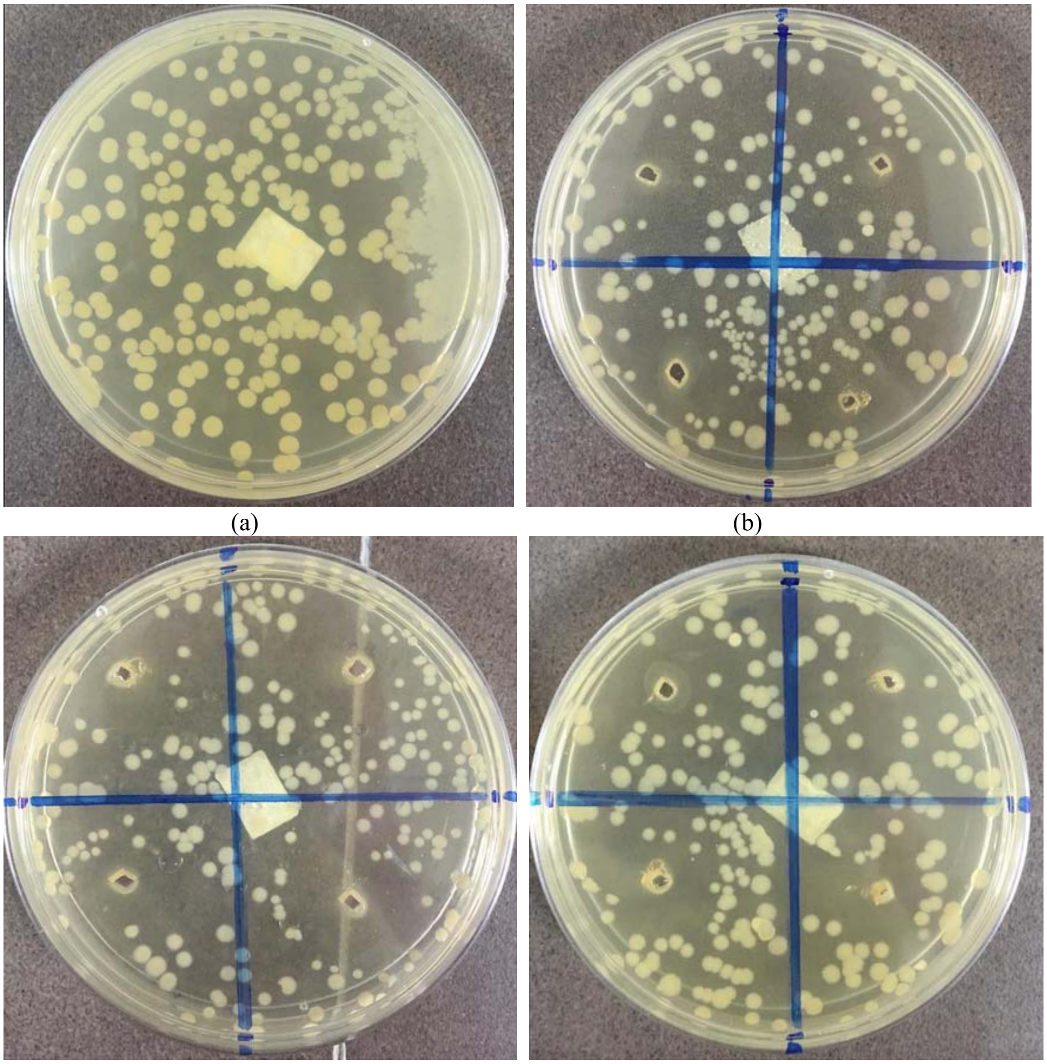


Figure 5. Growth of *S.aureus* on (a) nutrient agar plate, (b) nutrient agar plate with chitosan solution, (c) nutrient agar plate with 3% CNW/chitosan solution and (d) nutrient agar plate with 5% CNW/chitosan slution.

The result shows that the inhibition zone for Gram-positive bacteria (*S.aureus*) were greater than the inhibition zone for Gram-negative bacteria (*E.coli*). This is due to the outer wall of the Gram-positive bacteria charges predominate in the N-terminal ends with dispersed hydrophobic domains thus results in defined sites suitable for polymer incorporation onto the surface. This can inhibit or destroy the cellular functions of the bacteria [8]. From this result, it shows that the addition of CNW as a fillers to the chitosan film did not affect the antimicrobial properties of chitosan.



(a) (b) (c) (d)

Figure 4.11: Growth of *E.coli* on (a) nutrient agar plate, (b) nutrient agar plate with chitosan solution, (c) nutrient agar plate with 3% CNW/chitosan solution and (d) nutrient agar plate with 5% CNW/chitosan slution

CONCLUSION

CNW/chitosan films were prepared by solution casting method. CNW prepared by acid hydrolysis consisted of crystalline structure which can enchance the properties of the chitosan film. The presence of CNW in chitosan film lowers the ability of the chitosan film to absorb water due to hydrophobicity of the CNW. The tensile strength of the nanocomposite films increase as the CNW increased up to 3% and decreased gradually with the increased of the CNW. Biodegradability of the films also increased as the amount of CNW increased due to large distribution of chitin hydrolyzing enzyme-producing organisms. Finally, CNW/chitosan films also show antimicrobial activity against food pathogen such as *E.coli* and *S.aureus* due to the presence of clear zone around the solution. Therefore, these properties suggest that the CNW/chitosan films can be used in packaging industry.

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