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RESEARCH ARTICLE

SOIL BURIAL ASSESSMENT OF CROSSLINKED FUNGAL CHITOSAN COMPOSITE FILMS REINFORCED WITH CELLULOSE NANOCRYSTALS EXTRACTED FROM SUGARCANE BAGASSE

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Abstract. An increase in non-biodegradable plastic waste has driven the hunt for sustainable packaging solutions. This study developed biodegradable composite films using fungal chitosan (FCH) reinforced with cellulose nanocrystals (CNC) from sugarcane bagasse (SCB) and crosslinked with glutaraldehyde (GA). The films were prepared by solution casting and characterised for biodegradability and structural changes. The formulations evaluated included non-crosslinked neat FCH (FCH0), non-crosslinked composites with 1, 3, 5, and 7 wt% CNC (FCH-CNC1 to FCH-CNC7), and glutaraldehyde-crosslinked variants (GA-FCH0 and GA-FCH-CNC1 to GA-FCH-CNC7). A 15-day soil burial test confirmed that all films were biodegradable, though they degraded at different rates. FCH0 exhibited the highest susceptibility to microbial attack, with approximately 72 % weight loss. Visual inspection further showed that FCH0 developed more voids and cavities compared to composite films, indicating weaker resistance to microbial activity. In contrast, the crosslinked FCH/CNC composite films exhibited a controlled, significantly slower degradation rate, attributed to the formation of a dense polymer network via covalent imine linkages that restricted microbial enzyme penetration. The incorporation of CNC and GA enhanced structural stability, resulting in fewer surface defects and reduced weight loss during soil burial. Field Emission Scanning Electron Microscopy (FESEM) analysis confirmed surface disintegration and void formation in degraded samples, while Fourier Transform Infrared (FTIR) spectroscopy evidenced the cleavage of glycosidic bonds. The results suggest that crosslinking effectively modulates the biodegradation rate without compromising the material's eco-friendliness. These findings establish crosslinked FCH/CNC composites as a promising, durable, and sustainable alternative to conventional plastics for food packaging applications.

Keywords: Fungal chitosan, cellulose nanocrystals, soil burial test, biodegradable composite films.

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1. INTRODUCTION

The growing volume of plastic waste from traditional food packaging poses a significant environmental issue due to its non-biodegradability and reliance on fossil fuels. The issue of non-biodegradable plastics stems from their fossil-fuel-based chemistry, which produces highly stable polymers capable of persisting for centuries. Global plastic waste, estimated at around 400 million tonnes in 2024, is projected to nearly triple by 2060, with approximately half ending up in landfills and less than 20 % being recycled [1]. This problem has led to intensified research into sustainable, biodegradable materials that can actively protect against microbial contamination [2]. Because of its broad-spectrum antibacterial qualities and biodegradability, chitosan, a deacetylated form of chitin, has become an attractive material for various uses. The non-toxic and biocompatible nature of chitosan further enhances its suitability for direct food-contact applications, addressing both safety and environmental concerns simultaneously, making it an attractive candidate for food packaging applications [3].

Traditionally, commercial chitosan is extracted from crustacean shell waste; however, this source poses challenges related to seasonal variability, supply chain instability, and potential allergenicity. On the other hand, fungal chitosan (FCH), derived from mushroom cell walls, offers a reliable, consistent, and non-allergenic alternative with comparable physicochemical properties, making it highly suitable for food-contact applications [4]. Despite its advantages, neat fungal chitosan films often suffer from high water sensitivity and insufficient mechanical strength for robust packaging applications. To overcome these limitations, the incorporation of nanofillers such as cellulose nanocrystals (CNC) has been widely adopted. CNC, particularly those extracted from agricultural residues like sugarcane bagasse (SCB), possess a high aspect ratio and specific surface area, which facilitates the formation of a rigid percolating network within the polymer matrix [5]. This reinforcement significantly enhances the tensile strength and barrier properties of the composite films.

Furthermore, in addition to nanocrystalline reinforcement, chemical crosslinking using agents like glutaraldehyde (GA) offers an effective strategy for stabilizing the polymer network and enhancing its functional properties. The aldehyde groups in glutaraldehyde react with the primary amino groups of chitosan, resulting in the formation of covalent imine bonds (known as Schiff bases) between polymer chains. This crosslinking process significantly enhances water resistance, structural durability, and thermal stability by reducing the mobility within the polymer network and increasing the rigidity of the resulting films. Therefore, combining nanocrystalline reinforcement with chemical crosslinking provides a dual-mechanism approach to achieve the mechanical strength and barrier properties necessary for high-performance food packaging applications [6].

However, increasing the structural stability of biopolymers through reinforcement and crosslinking can inadvertently hinder their biodegradation, potentially prolonging their environmental persistence. It is therefore critical to evaluate how these modifications affect the material's end-of-life behavior. While the mechanical and barrier improvements of chitosan-CNC composites are well-documented, the biodegradation kinetics of crosslinked fungal chitosan films in soil environments remain underexplored [7]. Consequently, this study aims to develop non-crosslinked and GA-crosslinked FCH films reinforced with SCB-derived CNC and to comprehensively assess their biodegradability via soil burial tests. In this study, GA crosslinking is employed not only to enhance moisture resistance and microbial stability for extended shelf life, but also to elucidate its role in modulating biodegradation behavior under soil burial conditions. The degradation mechanism is systematically elucidated through weight loss measurements, morphological analysis (FESEM), and structural characterization (FTIR), providing essential insights into balancing functional performance with environmental sustainability.

2. MATERIALS AND METHODS

2.1 Materials

Sodium hydroxide, NaOH (≥ 99 %, CAS No: 1310-73-2) and sodium chlorite, $NaClO_2$ (80 %, CAS No: 7758-19-2) were acquired from Acros Organics (Belgium). Sulphuric acid, H_2SO_4 (98 %, CAS No: 7664-93-9) was supplied by Fisher Scientific (United States). Glycerol, $C_3H_8O_3$ (≥ 99 %, CAS No: 56-81-5); glacial acetic acid, CH_3COOH (99.9 %, CAS No: 64-19-7); and glutaraldehyde, $(CH_2)_3(CHO)_2$ (25 %, CAS No: 111-30-8) were sourced from Merck (Germany). FCH (*Pleurotus ostreatus*) with a degree of deacetylation ≥ 98 % was purchased from Qingdao Chibio Biotech Co., Ltd, China. SCB was collected from a local sugarcane juice vendor in Kuala Perlis, Malaysia. Nutrient agar and potato dextrose agar required were purchased from Merck (Germany).

2.2 Extraction of CNC

CNC was extracted from SCB following established pre-treatment and hydrolysis procedures. Initially, SCB was cut into ~2 cm pieces, sun-dried for 2–3 days and subjected to two pre-treatments to remove lignin and hemicelluloses. In the alkaline treatment, the fibres were ground, sieved to 125 µm, dispersed in 4 wt% NaOH, stirred at 80 °C for 2 h, washed to neutral pH, and the process repeated three times before drying at 50 °C. The resulting fibres were bleached with 1.7 wt% sodium chlorite in an acetate buffer at 80 °C for 2 h, washed to pH 7, and the process repeated three times, then dried at 50 °C [8]. CNCs were then isolated via sulphuric acid hydrolysis using 64 % H_2SO_4 at 50 °C for 1 h, quenched with distilled water, centrifuged at 8000 rpm until neutral, and finally ultrasonicated to obtain well-dispersed CNCs.

2.3 Fabrication of FCH/CNC Composite Films

CNC was added at 1, 3, 5, and 7 wt% (based on the dry weight of FCH) to create FCH/CNC composite films. After dissolving FCH in 2 v/v% acetic acid at room temperature, 10 % glycerol was gradually added. After adding the required quantity of CNC, the mixture was sonicated for 10 minutes to eliminate air bubbles. It was then cast into 90 mm plastic Petri dishes and dried for 48 hours at 50 °C in a non-air-circulating oven. The resulting non-crosslinked films (Table 1) were peeled and kept in a drying cabinet.

Table 1: Nomenclature of non-crosslinked FCH/CNC composite films

Abbreviation	Samples
FCH0	Non-crosslinked neat fungal chitosan composite film
FCH-CNC1	Non- crosslinked 1 wt% CNC-fungal chitosan composite film
FCH-CNC3	Non-crosslinked 3 wt% CNC-fungal chitosan composite film
FCH-CNC5	Non-crosslinked 5 wt% CNC-fungal chitosan composite film
FCH-CNC7	Non-crosslinked 7 wt% CNC-fungal chitosan composite film

Before sonication, 1 wt% GA was added to the chitosan–CNC mixture for the crosslinked films. The mixture was then cast and dried under the same circumstances. The dried films were then subjected to microwave curing using a Sharp R-202 commercial microwave oven (2450 MHz) to achieve crosslinking, and the final films (Table 2) were stored at room temperature for at least one week before characterization.

Table 2: Nomenclature of crosslinked FCH/CNC composite films

Abbreviation	Samples
GA-FCH0	Glutaraldehyde crosslinked neat fungal chitosan composite film
GA-FCH-CNC1	Glutaraldehyde crosslinked 1 wt% CNC-fungal chitosan composite film
GA-FCH-CNC3	Glutaraldehyde crosslinked 3 wt% CNC-fungal chitosan composite film
GA-FCH-CNC5	Glutaraldehyde crosslinked 5 wt% CNC-fungal chitosan composite film
GA-FCH-CNC	Glutaraldehyde crosslinked 7 wt% CNC-fungal chitosan composite film

2.4 Characterization Techniques

2.4.1 Biodegradation test

A soil burial test was conducted in accordance with ASTM D5988 to evaluate biodegradability. At the Faculty of Chemical Engineering & Technology, Universiti Malaysia Perlis (UniMAP), Malaysia (latitude 6°27′0′N, longitude 100°15′E), neat fungal chitosan and FCH/CNC composite films, both crosslinked and non-crosslinked, were buried at a depth of 10 cm in natural soil. Every three days, the samples were collected, carefully cleaned with distilled water to remove any remaining soil, and then dried for 24 hours at 50 °C in an oven before weighing again. The proportion of weight loss over time was used to calculate the biodegradation rate. Equation 1 was used to calculate the weight loss of composite films over time to measure the rate of biodegradation [9].

$$\text{Weight loss (\%)} = \frac{W_a - W_b}{W_b} \text{ x } 100 \quad (1)$$

where W_a is the final weight of the specimens following soil burial and W_b is the initial weight of the specimens before soil burial. Each formulation underwent three replicates of the biodegradation test.

2.4.2 Physical and Morphological Characterization

The physical appearance of films was systematically monitored at successive retrieval intervals, with particular attention to surface degradation characteristics, including the formation of visible cracks, voids, and areas of microbial erosion. A field emission scanning electron microscope (FESEM, NOVA NANOSEM 450, FEI, USA) was used to analyze the morphological characteristics of the crosslinked and non-crosslinked FCH/NCC composite films. Carbon adhesive tape was used to adhere the composite films to aluminum stubs after they had been cryo-fractured in liquid nitrogen and sputter-coated with gold. To examine surface morphological changes induced by microbial colonisation and enzymatic attack, imaging was performed at accelerating voltage of 3–5 kV.

2.4.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were recorded using a Spectrum RX spectrophotometer (PerkinElmer, USA) over the wavenumber range of 4000–450 cm^{-1} with a resolution of 4 cm^{-1}. The attenuated total reflectance (ATR) mode was used for composite films before and after soil burial test. Each spectrum was collected from 16 scans, and the resulting bands were analyzed to identify hydrogen bonding interactions, amine and hydroxyl vibrations, and other chemical modifications in the composites.

3. RESULTS AND DISCUSSION

3.1 Weight Loss and Physical Appearance

Figure 1 shows the weight loss percentages of FCH/CNC composite films and non-crosslinked and crosslinked neat fungal chitosan after 15 days of soil burial. The non-crosslinked neat fungal chitosan film demonstrated the greatest reduction in weight, reaching approximately 72 %. It is well known that chitosan, including fungal-derived chitosan, is highly biodegradable in soil and composting conditions. When exposed to microbiological activity, pure chitosan films quickly deteriorate [10].

There was considerably less biodegradation when CNC was added to the chitosan matrix. Strong intermolecular interactions between CNC and chitosan, which improve the structural integrity of the composite films and prevent microbial accessibility, are mainly responsible for this drop. By restricting water intake and decreasing enzymatic diffusion, the homogeneous distribution of CNC further reinforces the polymer network. Furthermore, the composite matrix is more resistant to microbial attack due to CNC's high crystallinity, which further delays biodegradation [11].

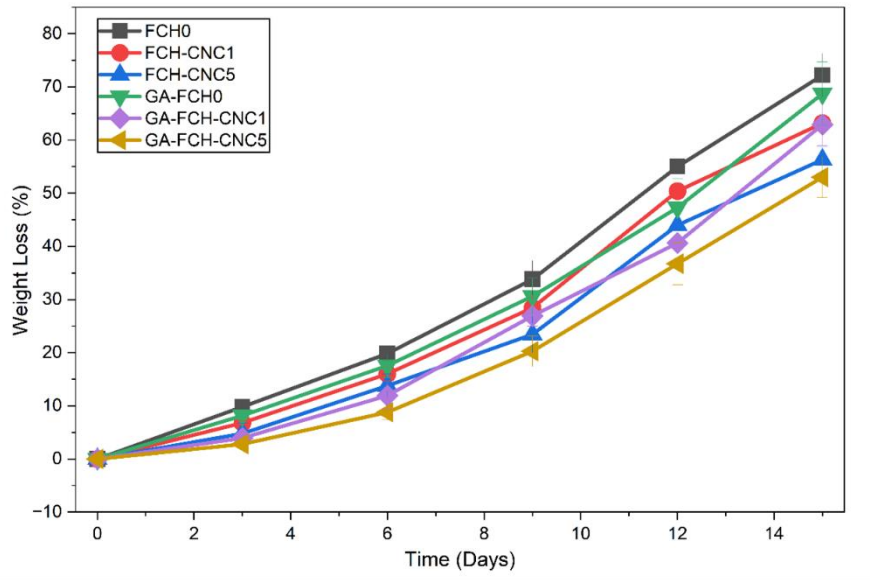

Figure 1: Weight loss of non-crosslinked and crosslinked neat chitosan and FCH/CNC composite films after 15 days of soil burial

The physical changes of crosslinked and non-crosslinked FCH/CNC composite films after 15 days of soil burial are depicted in Figure 2. Compared to FCH-CNC5, specimen FCH0 has more voids and cavities in its physical appearance. The incorporation of CNC into chitosan composite films has strengthened the resistance of films against the attack of microorganisms [12]. Weight loss was significantly reduced when GA was added to the chitosan composite films, suggesting improved structural stability. The development of imine (C=N) connections between the aldehyde groups of GA and the amino group of chitosan is mainly responsible for this enhancement. These links create a dense crosslinked network that prevents microbial destruction. The resulting rigid matrix minimizes void spaces and internal micro-cracks, thereby limiting water diffusion throughout the film and reducing microbial penetration, as illustrated in Figure 3. Furthermore, the crosslinking process increases the crystallinity of the composite films, which contributes to their enhanced resistance against enzymatic and microbial attack [13].

Figure 2: Physical appearance of non-crosslinked and crosslinked neat chitosan and FCH/CNC composite films after 15 days of soil burial

The crosslinked FCH/CNC composite films demonstrated the highest structural stability and strongest resistance to biodegradation. Among these, GA-FCH-CNC5 showed the lowest weight loss during soil burial. The dense imine-crosslinked network formed by glutaraldehyde limits water uptake, reduces void spaces, and prevents microbial access, which slows down both enzymatic and microbial breakdown [14]. In contrast, non-crosslinked films, particularly neat FCH0, degraded rapidly, experiencing a 72 % weight loss in just 15 days due to high water sensitivity and easy enzymatic penetration. While GA crosslinking reduces antimicrobial activity, it offers a significant advantage in long-term durability. Therefore, GA-FCH-CNC5 is the most suitable formulation for soil-contact or agricultural applications that require controlled, delayed biodegradation.

3.2 Morphological Study

FESEM was used to analyse surface changes in the chitosan composite films before and after soil burial, as shown in Figure 3. Before soil burial (0 day), all specimens had uniformly smooth

surfaces. Non-crosslinked chitosan composites developed holes and cracks due to microbial attack after 3 days of biodegradation. More holes and fractures were seen after 15 days of soil contact, particularly in sample FCH0. The formation of holes and fractures between the composite film interfaces was lessened by the even distribution of CNC in the chitosan matrices and the strong interfacial adhesion between filler and polymer [15].

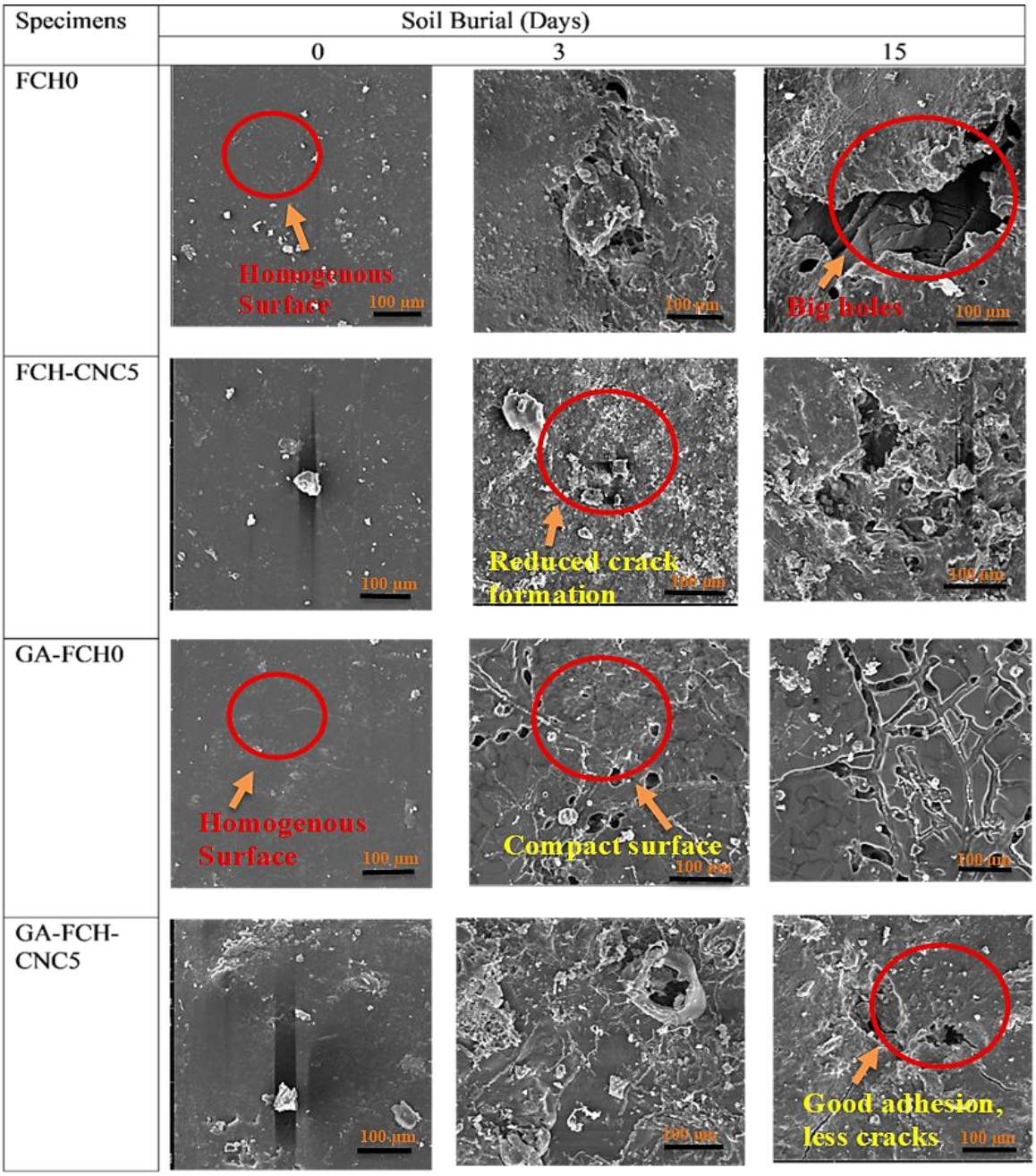

Figure 3: Surface morphology of non-crosslinked and crosslinked neat chitosan and FCH/CNC composite films after 15 days of soil burial (500X Magnification)

Compared to non-crosslinked fungal chitosan composite films, the crosslinked FCH/CNC composite films' FESEM micrographs showed fewer and less noticeable holes and fractures. The surface of the film composites showed visible gaps and fissures after 15 days of soil burial. The development of a denser and more rigid polymer network by covalent imine connections, which successfully prevents the flow of water and microbial enzymes into the matrix, explains why the crosslinked films have less severe surface damage than the non-crosslinked ones [16].

The biodegradation of chitosan-based composite films primarily occurs through microbial assimilation and enzymatic depolymerization of the polymer matrix, where microorganisms utilize chitosan as a carbon and energy source. Notably, absorbed water enhances microbial colonization and enzymatic activity, leading to cleavage of glycosidic bonds; thus, greater water uptake accelerates degradation, establishing a direct link between moisture diffusion and biodegradation [17]. In addition, the incorporation of CNCs, due to their high crystallinity, increases matrix complexity, reduces microbial accessibility, and retards degradation. Because crystalline regions are more resistant than amorphous domains, CNC–chitosan composites therefore exhibit greater biodegradation resistance than neat chitosan films [18]. Moreover, GA crosslinking further enhances resistance by forming covalent imine linkages that generate a rigid network. This structure decreases free volume, limits crack formation, and restricts water diffusion, thereby hindering microbial penetration and enzymatic activity [19].

3.2 Fourier Transform Infrared (FTIR) Analysis

Figure 4 illustrates the FTIR spectra of non-crosslinked and crosslinked FCH/CNC composite films before and after the soil burial test. In the non-crosslinked samples (a–b), a broad absorption band was observed at 3364 cm^{-1}, attributed to O–H and N–H stretching vibrations, indicating intermolecular hydrogen bonding within the chitosan matrix. The characteristic C–H stretching band was detected at 2925 cm^{-1}. The peaks at 1654 cm^{-1} and 1564 cm^{-1} in pure FCH (a) and the non-crosslinked composite (b) represent Amide I (C=O stretching) and Amide II (N–H bending) vibrations, respectively, confirming the native structure of chitosan [20]. The intense peak at 1066 cm^{-1} is associated with C–O–C stretching vibrations originating from the glycosidic linkages in both CNC and chitosan. With the incorporation of CNC (sample b), the peak at 3399 cm^{-1} became more prominent, reflecting an increase in –OH content due to CNC's hydroxyl groups. Moreover, the peaks at 1066 cm^{-1} and 1037 cm^{-1} increased in intensity, which supports the interaction and integration of CNC into the chitosan matrix [21].

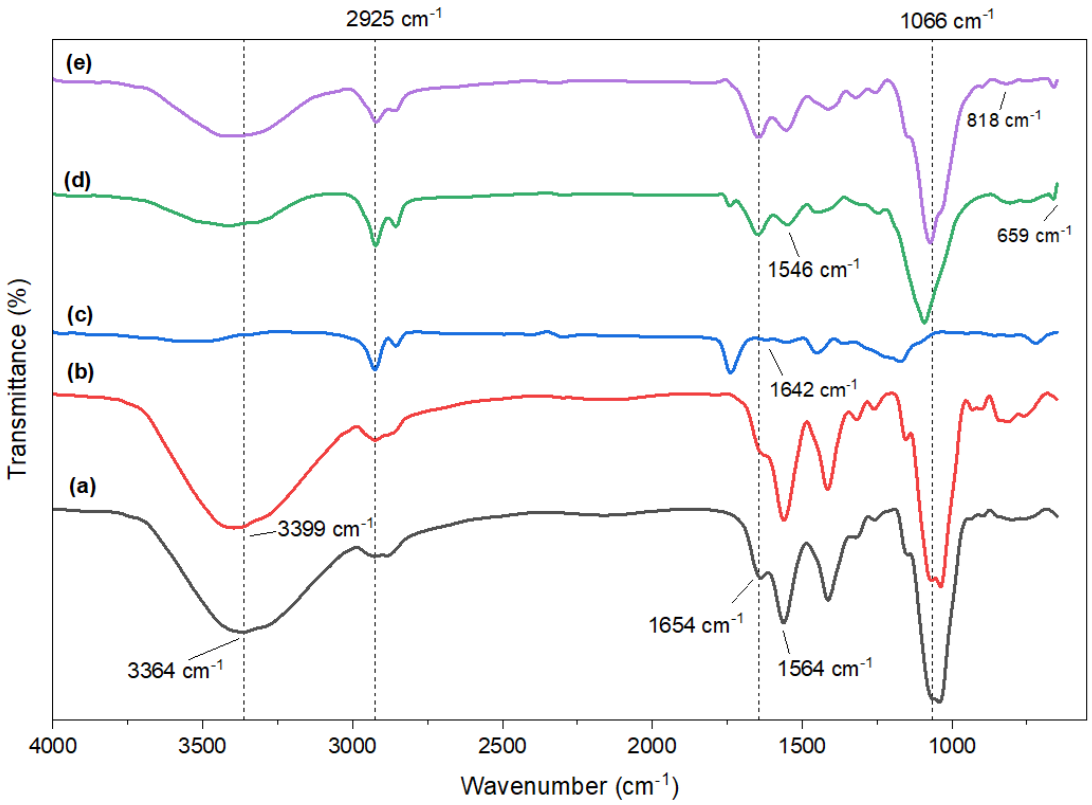

Figure 4: FTIR spectra of FCH/CNC composite films before soil burial (a) FCH0 (b) FCH-CNC5 (c) GA-FCH-CNC5 and after soil burial (d) FCH-CN5 and (e) GA-FCH-CNC5

Crosslinking with GA induced noticeable spectral shifts in the composite (c). The Amide I peak shifted from 1654 cm^{-1} to 1642 cm^{-1}, which is indicative of Schiff base formation (C=N stretching) between the aldehyde groups of the crosslinker and the amine groups of chitosan [22]. This shift confirms the successful formation of a crosslinked network structure, which consumes free amino groups and alters the vibrational environment of the polymer backbone.

Following the soil burial test, the retrieved films (d and e) exhibited distinct spectral changes confirming biodegradation. In the degraded non-crosslinked sample (d), the Amide II band shifted significantly from 1564 cm^{-1} to 1546 cm^{-1}, accompanied by a broadening of the peak. This shift suggests the hydrolytic cleavage of the polymer chains and interactions with soil microbial biomass [10]. Notably, new absorption peaks appeared at 818 cm^{-1} and 659 cm^{-1} in the degraded crosslinked film (e). These bands are attributed to the accumulation of soil minerals (such as silicates) and microbial biofilm byproducts adhering to the film surface during the degradation process. Furthermore, the intensity of the glycosidic bond peak at 1066 cm^{-1} was reduced in the buried samples relative to the pristine films, providing evidence of enzymatic depolymerization of the polysaccharide backbone by soil microorganisms [23].

4. CONCLUSION

In conclusion, non-crosslinked neat FCH degraded most rapidly, losing approximately 72 % of its weight after 15 days of soil burial due to its high water sensitivity and ease of microbial penetration. The incorporation of CNC improved the structural integrity of the films, reducing void formation and slowing enzymatic attack. GA-crosslinked composites exhibited the highest resistance to biodegradation, with GA-FCH-CNC5 showing the lowest weight loss, attributed to the formation of a dense imine-crosslinked network that limited water uptake and microbial accessibility. FESEM analysis confirmed reduced surface erosion in CNC-reinforced and crosslinked films, while FTIR spectra provided evidence of glycosidic bond cleavage and matrix degradation after burial. However, while the 15-day soil burial test confirms initial biodegradation, it is insufficient to predict full mineralization of GA-crosslinked films, which typically require 12–24 weeks or up to 180 days (per ASTM standards) for complete degradation due to their dense crosslinked structure and limited enzymatic accessibility. Overall, the findings show that controlled crosslinking and CNC reinforcement can strategically modulate the degradation rate of fungal chitosan composites, enabling a balance between durability during use and biodegradability at end-of-life. These characteristics position GA-FCH-CNC films as promising sustainable candidates for food packaging and other applications requiring adjustable environmental persistence.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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