PREPARATION AND PROPERTIES OF CHITIN HYDROGEL APPLIED AS MOISTURE-SUPPORTER FOR SEED GERMINATION

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In this study, chitin was chemically extracted from shrimp shell, and then used for the development of a chitin hydrogel-based moisture supporter for hydroponic systems. Here, the chitin hydrogel was prepared at different concentrations of lithium chloride (LiCl), varying from 5 to 9%, in *N,N*-dimethylacetamide (DMAc) at room temperature. The results revealed that, while most characteristic functional groups of the chitin segment remained intact in all chitin hydrogels, higher levels of LiCl in DMAc led to a reduction in both crystalline index and equilibrium water content in the resulting chitin hydrogel. In a seed germination experiment, it was observed that the chitin hydrogel containing 5% LiCl concentration exhibited superior results in terms of root, stem, and leaf length, compared to other concentrations. Therefore, the chitin hydrogel was an effective moisture-supporter for seed germination and early plant development, compared conventional soil used as control. Moreover, SEM images illustrated that the chitin hydrogel possessed a pliable structure, indicating improved degradation when subjected to composting over a 15-day period.

***Keywords**:* chitin hydrogel, moisture support, shrimp shell, seafood processing by-product

INTRODUCTION

Agriculture confronts substantial challenges, including drought, salinity, and temperature fluctuations, which are anticipated to exacerbate because of factors such as land degradation, urbanization, and climate change.[1,2] Addressing these issues involves enhancing soil moisture retention and water-holding capacity, and one effective solution is the use of hydrogel-based superabsorbent materials. A hydrogel, which represents a network of hydrophilic polymer chains, possesses the ability to retain a substantial amount of water, resulting in considerable swelling.[3] This characteristic is very important for agricultural application, as it allows hydrogels to uphold a stable structure, while retaining water and nutrients in the proximity of plant roots, gradually releasing them to the root system. Consequently, hydrogels can be utilized as an addition to soil or as its substitute in soilless systems.

Research suggests that hydrogels can decrease irrigation requirements by up to 40% for lawns, golf courses, and various field crops, achieving even more substantial reductions – of up to 70% – for indoor crops.[4] Their use not only enhances soil bulk density, but also mitigates water content deficiencies in harvested vegetables, minimizing the impact of environmental stress on plant physiology and fostering growth, thereby increasing crop productivity.[5] By significantly boosting water storage capacity, hydrogels can reduce plant

stress or drought periods by up to 99%, resulting in a 22% increase in crop yields, compared to conventional practices.[6] Moreover, hydrogels play a role in minimizing leaching and can function as carriers for pesticides, fungicides, and herbicides.[7] Their inclusion in nutrient-poor soil enhances nutrient absorption and reduces nutrient loss, thus preventing groundwater pollution.[6] Hydrogels can also be utilized as seed growth media and for water and nutrient storage, aligning with the growing demand for materials with high moisture retention[8] and biodegradability in modern agriculture.[9] Given their biopolymer nature, hydrogels readily biodegrade in the soil, offering a source of minerals and nutrients for soil microorganisms, without the risk of bioaccumulation.[10] It is noteworthy that the hydrogel segments or molecules are too large to be absorbed into plant tissue, and are unlikely to bioaccumulate.

Hydrogels can be synthesized from various sources, ranging from natural to synthetic polymers.[8] Polysaccharide-based hydrogels, in particular, serve as soil conditioners and nutrient carriers for plants and soil.[11] Moreover, the utilization of reusable biopolymer-based hydrogels is crucial for fostering advancements in agricultural production,[12] combatting drought for crops,[7] stabilizing soil ecology,[13] and resisting climate change.[14] Hence, agro-waste-based biopolymers have been found attractive to develop sustainable materials for agricultural applications.[14] Notably, polysaccharides are primarily preferred for such applications due to their cost-effectiveness, abundance, renewability, and biodegradability.[10]

Chitin stands as the second most abundant organic substance in nature, derived from various sources, including crustaceans, mollusks, algae, insects, fungi, and yeasts.[15] Normally, the crustacean shell has been strongly suggested as a gold-mine for chitin extraction because the chitin content in these shells is in the range of 20-40%.[16,17] Chitin is known to contain 2-acetamido-2-deoxy-β-D-glucose via the β(1→4) linkage, which is considered to be similar structurally to cellulose, having the acetamide group (–$NHCOCH_3$) at the C-2 position instead of the hydroxyl (–OH) group in the case of cellulose (Fig. 1). Chitin exhibits robust internal molecular hydrogen bonding networks, imparting insoluble properties in common solvents, thereby limiting its applications.[18] Remarkably, the incorporation of lithium salt in an organic solvent, such as N,N-dimethylacetamide (DMAc), at room temperature, facilitated the dissolution of chitin, without inducing depolymerization of the resultant product.[19]

In agriculture, chitin can be employed to treat seeds, promoting growth. Its antibacterial properties make it effective in reducing worm populations around plant roots and in inhibiting fungal pathogens.[20] The hydrogels developed in the present work were conceptualized as prospective substrates to facilitate plant germination in hydroponic systems.

Hydroponics, a soilless cultivation method, involves placing plants on substrates and nourishing them with soluble nutrient solutions. In hydroponic systems, water circulates through the root zone via irrigation, characterized by a significantly higher rate, compared to diffusion processes. Consequently, hydroponic nutrient solutions can be more dilute than conventional irrigation solutions, while still providing plants ample opportunities for nutrient absorption.[23] In contemporary society, hydroponics is gaining attention as a forward-looking agricultural solution. This advanced cultivation technique offers substantial benefits for the environment, economy, and society at large. Key advantages encompass soilless cultivation, reduced reliance on pesticides and harmful chemicals, high productivity, and a minimal environmental footprint devoid of toxin accumulation or pollution.[24]

In previous literature, the development of crab-shell-based chitin hydrogels, prepared at different concentration of LiCl, while using water as coagulation agent, has been reported.[19,21,22] In this study, chitin was chemically extracted from another waste source, namely, shrimp shells, to prepare chitin hydrogels with varying LiCl concentrations in DMAc solvent during the phase inversion process under ethanol vapor. The prepared hydrogels were then characterized by Fourier-transform infrared spectroscopy, scanning electron microscopy, powder X-ray diffraction techniques and in terms of water content. Then, the hydrogel was assessed as a moisture supporter in a seed germination experiment and its biodegradability was also evaluated.

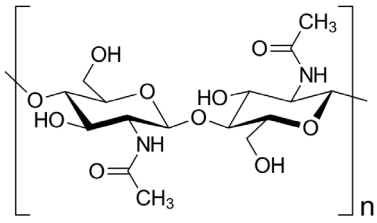


Figure 1: Chemical structure of chitin

EXPERIMENTAL

Materials

Shrimp shells were collected in District 8, Ho Chi Minh City. All chemicals used were of analytical grade. Hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium hydroxide (KOH), *N,N*-dimethylacetamide (DMAc), lithium chloride (LiCl) and ethanol were purchased from Nacalai Tesque, Inc. (Tokyo, Japan). Prior to using DMAc, KOH was placed in the solvent and stored over five days and LiCl was dried at 80 °C overnight to remove traces of moisture. Other chemicals were used without any further purification. Radish seeds were received from VietSeeds Company.

Methods

Extraction of chitin from shrimp shells

The extraction of chitin from shrimp shells (SS) was performed following the procedure from a previous study.[21] Shrimp shells (SS), upon collection, were subjected to washing with water to eliminate impurities, and then were finely ground to an average size of 5 mm. Subsequently, approximately 10 g of the crushed SS was introduced into 300 mL of 1M HCl aqueous solution and continuously stirred for 24 h at 300 rpm and room temperature, to eliminate mineral components from SS. Following demineralization, the treated SS underwent deproteinization with 300 mL of 10% NaOH aqueous solution for 5 hours at 90 °C and 300 rpm. After the protein-removal process, colored molecules in SS were eliminated with ethanol treatment for 6 hours at 60 °C and 300 rpm. Finally, the purified chitin (CT) was obtained and dried at 80 °C for 24 h (Fig. 2).

The yield of the extracted CT from SS was calculated by the equation below:

$$\text{Yield (\%)} = m_0 - m_1 \times 100 / m_0 \quad (1)$$

where m_0 is the dry weight of the initial SS and m_1 is the weight of extracted CT.

Preparation of chitin hydrogel with different contents of LiCl in DMAc solvent

The chitin solution was created by dissolving 1 g of the extracted chitin (CT) in DMAc solvent, with the addition of LiCl contents ranging from 5 to 9%, over 5 days. Once homogeneously viscous solutions were achieved, these solutions underwent centrifugation at 6000 rpm for 30 minutes to eliminate insoluble matter. For the preparation of the chitin hydrogel, 20 g of 1% chitin solution, prepared with varying LiCl concentrations, was poured into a 50-mL beaker (6 cm × 4 cm), which was then placed into a container containing 50 mL of ethanol solution for the phase inversion process. After 24 hours, the chitin hydrogel was formed and washed with distilled water for 24 hours to remove any traces of solvent (Fig. 2c). The chitin hydrogels prepared with different LiCl concentrations were dried in an oven at room temperature for 24 hours, until reaching a constant weight, prior to measurements.

Characterization of extracted chitin and chitin hydrogel

Fourier-transform infrared spectroscopy (FT-IR) spectra were recorded using a JASCO FT-IR/4100 spectrometer (Model-8201; Shimadzu Corp., Japan). The spectra were obtained after grinding dried SC and CH with potassium bromide (KBr), in the absorbance mode, covering the wavelength range from 4500 to 500 cm^{-1} with a resolution of 2 cm^{-1}.

Scanning electron microscopy (SEM) was employed to analyze the morphology of the surface and cross-section of the hydrogel samples. For the measurement, the samples were fractured in liquid nitrogen and subjected to a 24-h freeze-drying process. Subsequently, gold sputtering was applied to create a conductive layer (S-4800, HI-9039-0006). SEM observations were conducted at various magnifications.

XRD (powder X-ray diffraction) serves as a valuable tool for analyzing crystalline and amorphous structures. XRD patterns were recorded in the 2θ range from 10° to 40° and the scanning speed was 0.01°/min. The crystalline index (CI) was calculated according to the following equation:

$$\text{CI (\%)} = (I_{110} - I_{am}) \times 100/I_{110} \quad (2)$$

where I_{110} is the maximum intensity at (110) – peak around $2\theta = 19°$ and I_{am} is the amorphous diffraction at $2\theta = 12.6°$.[25]

The water content (WC) of the hydrogels was measured at room temperature by immersing 20 mm × 20 mm samples of the dry samples in distilled water.

The value of WC after each given period was calculated for each sample using the equation:

$$WC = (m_1 - m_0)/m_0 \times 100 \quad (3)$$

where m_0 is the dry weight and m_1 is the weight of the samples after immersing in distilled water.

Water-supporter for seed germination

Firstly, radish seeds were soaked into warm water for about 5 h, and then drained. Next, the seeds were added into hydrogels and placed into the tray of a hydroponic machine. After this, the nutrient solution was poured into the container to initialize the hydroponic system, in which the watering frequency was set to 10 mL/time/day (Fig. 3).

a) b) c)

Figure 2: Images of shrimp shells (a), extracted chitin (b) and chitin hydrogel at 5% LiCl/DMAc (c)

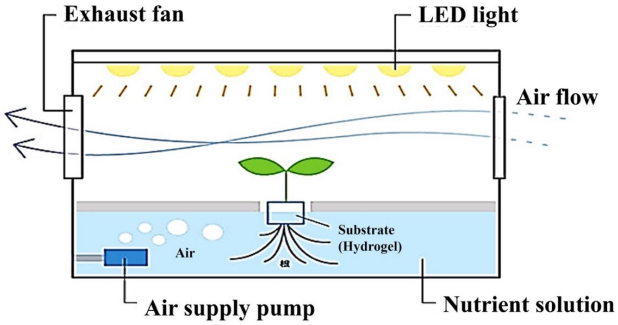


Figure 3: Hydroponic system for chitin hydrogel used as water-supporter

The hydroponic solution used in the experiment was a Hydro Umat F hydroponic solution for fruits and vegetables, and included 2 parts: A and B. About 10 mL of nutrient A and 10 mL of nutrient B were mixed into 4 L of distilled water to form a hydroponic solution (1:200 vol%). The nutrient composition of solution A was the following: nitrate (21.17 g/L), calcium (32.13 g/L), potassium oxide (43.98 g/L) and Fe-EDTA (0.6 g/L). The solution B was composed of phosphorus pentoxide (15.10 g/L), sulfur (13.31 g/L), magnesium (10.00 g/L), manganium (190 mg/L), borum (90 mg/L), zinc (29 mg/L), copper (21 mg/L) and molybdenium (18 mg/L).

The temperature and light were controlled, and the hydroponic solution was automatically aerated to ensure the amount of dissolved oxygen needed for plant growth. For the control, the prepared seeds were placed in humus soil, in order to compare the difference between the plant growth in the presence and in the absence of the CT hydrogels.

After 7 days, the plants were removed from the hydrogel, and their stem, root, and leaf lengths were measured. Standardized methods were employed for accurate measurements, involving rulers for plant, stem, and root lengths. Leaf length was determined by measuring the largest leaf's size. Biomass and moisture content were assessed by recording the fresh weight of the plants.

To investigate the biodegradability of the chitin hydrogel material, experiments were carried out by the procedure described in a previous study.[26] The CT hydrogel samples prepared with different LiCl/DMAc solvent systems were placed in soil and the conditions of 70% relative humidity (RH) and 25 °C were ensured to allow for the composting process to take place. The biodegradability percentage (BP, %) was measured by the following equation after the given period of time, namely, 5, 10 and 15 days:

$$BP = (m_0 - m_1)/m_0 \times 100 \quad (4)$$

where m_0 and m_1 are the initial weight and the weight after 5, 10 and 15 days.

RESULTS AND DISCUSSION

Appearance of extracted chitin and chitin hydrogel

Demineralization of shrimp shells is a necessary step for efficient chitin extraction, targeting the removal of minerals. Hydrochloric acid (HCl) was chosen considering its high treatment efficacy in this experimental procedure. During the proteinization phase, sodium hydroxide (NaOH) solution was applied to hydrolyze proteins, with the process conducted at a temperature of 90 °C. Subsequently, chemicals, such as sodium hypochlorite (NaOCl), were utilized to eliminate any remaining color molecules in the shrimp shell. To promote environmental sustainability, decolorization was achieved using an ethanol solution. Following decolorization, the product underwent drying at 60 °C for 24 h, resulting in the production of chitin (CT), with a yield of approximately 19.22% (Fig. 2).

Characterization of chitin hydrogels

Figure 4 presents the FT-IR spectra of SS, CT, commercial CT, and chitosan. In comparison with SS, the absence of the peak at 861 cm^{-1}, associated with the calcite compound, indicates the effectiveness of the demineralization process. The specific functional groups in the original chitin include the O−H stretching band at 3434 cm^{-1}, the amide II group at 1553 cm^{-1}, C−H bonding at 2885 cm^{-1}, the ring C−O−C at 1071 cm^{-1}, and amide I bands at 1648 cm^{-1} and 1625 cm^{-1}, which are characteristics of the α-chitin structure commonly found in shrimp shells (Fig. 4a). In the FT-IR spectrum of chitosan, the absence of the amide I peak at 1625 cm^{-1} and the presence of the $-NH_2$ peak at 1590 cm^{-1} indicate that the extracted CT in this study did not undergo transformation into chitosan.[25] Figure 4b displays the FT-IR spectrum of the resulting CT hydrogel prepared using various LiCl/DMAc solutions. Notably, peaks representing the amide II group appeared at 1651 cm^{-1} and 1618 cm^{-1}, indicating the presence of the α-structure of CT. The O−H group peak emerged at 3471 cm^{-1}, and the C−H bonding was observed at 2889 cm^{-1}. Likewise, the C−O−C ring maintained its presence at 1075 cm^{-1}. It is evident that, following the preparation through the phase inversion process under ethanol vapor, most of the initial and functional groups remained virtually unchanged in the hydrogel structure.

As illustrated in Figure 5, the incorporation of varying amounts of LiCl into the DMAc solvent during the preparation of CT hydrogels resulted in distinctive morphological changes in the cross-sectional area. Notably, the CT hydrogel created with 5% LiCl exhibited a less densely packed structure, leading to a more uniform distribution of internal pores, compared to other variations. Conversely, higher concentrations of LiCl resulted in a compacted structure, making it challenging to discern pores, as evident in the CT hydrogel with 9% LiCl. It has been documented that an increased LiCl concentration in the DMAc solvent can facilitate the formation of macro-cations between Li^+ ions and DMAc molecules, effectively serving as crosslinkers within the chemical structure of CT segments.[21] Therefore, a reduction in water absorption was expected in the case of the CT hydrogels obtained with 7 and 9% LiCl content.

As depicted in Figure 6, a crystalline region remained evident in the hydrogel sample containing 9% LiCl, showcasing the highest CT content at 76.3%. This was followed by the CT with 7% LiCl – with a CI of 33.1%, and the lowest CI was observed for the 5% LiCl hydrogel – at 25.2%. These findings strongly indicate that the 5% LiCl/DMAc solvent system was more efficient at dissolving CT at room temperature, compared to concentrations of 7% and 9%. This observation aligns with previously published reports.[21] The aggregation of CT segments within the hydrogel structure resulted from the cross-linking process, leading to reduced CT dissolution in the LiCl/DMAc solvent. Consequently, a higher CI was obtained due to the scarcity of dissolved CT in the solution.

The water content (WC) of the CT hydrogels at different LiCl content, after being immersed into distilled water for a given period of time, is presented in Figure 7. It can be clearly observed that all the hydrogel samples were rapidly saturated after 12 h of immersion into distilled water at room temperature. For instance, in the case of the CT having 5% LiCl, the WC increased from 81.1% recorded after 0.5 h soaking time to 106.1% after 12 h. However, the value seemed to remain constant after 24 h – at 108%. SEM images suggested that higher LiCl content in CT hydrogel might contribute to a reduction in the

WC because of the compact structure formed by the macro-cation-based cross-linker and CT segment. Hence, the WC of the CT hydrogel with 7% and 9% LiCl content decreased to 94.5% and 85.6%, respectively, after 12 h of immersion. The denser structure of the hydrogel sample may impede the diffusion of water molecules into the polymeric network, leading to a lower water-absorption capacity.[19]

Chitin hydrogel as moisture-supporter for seed germination

Figure 8 shows the results of seed germination in the presence of chitin hydrogels with varying LiCl/DMAc concentrations. Chitin, acting as a growth regulator for plants, plays a crucial role in promoting seed germination. Remarkably, significant differences were observed in the roots and stems, when the seeds were exposed to hydrogel samples with 5%, 7%, and 9% LiCl/DMAc content. The hydrogel prepared with 5% LiCl/DMAc solvent demonstrated the most notable results, particularly in terms of plant root length. This notable variation in root length can be attributed to the physical properties of the hydrogel itself. It appears that, at lower concentrations of LiCl/DMAc, the hydrogel adopts a softer texture, facilitating unhindered root growth that readily attaches to the substrate. This unique feature of the 5% LiCl/DMAc hydrogel underscores its potential as a superior medium for seed germination and root development. Moreover, the comparison between seedlings grown on hydrogels with 5% and 7% LiCl/DMAc content and those in soil yielded intriguing results.

Surprisingly, the seedlings developed on the hydrogels outperformed their counterparts that grew in soil. This finding suggests that the hydrogel medium enhances nutrient transfer to the seeds, promoting more robust seedling growth. Notably, the sample with 5% LiCl/DMAc content stood out, with its root length being double that of the seedlings grown in soil. Figure 9 presents the fresh weight of the plants grown on chitin hydrogels prepared with 5%, 7%, and 9% LiCl/DMAc, as well as that of the control grown in soil. The data reveal interesting trends in water content among these samples. Seedlings on the 5% and 7% LiCl hydrogels displayed similar weight, indicating that the hydrogels prepared with these LiCl concentrations offer comparable hydration levels for plant growth. However, when compared to the 9% hydrogel and the soil-based growth, the fresh weight of the plants grown in the presence of the 5% hydrogel sample was significantly higher.

This observation strengthens the notion that lower concentrations of LiCl/DMAc during the preparation of the hydrogel yield better moisture support hydrogels, leading to superior root development and higher water and nutrient uptake by the plant. In essence, the use of hydrogels, particularly in the case of 5% LiCl/DMAc content, not only fosters optimal root growth, but also ensures a more efficient supply of water and nutrients to the seeds, resulting in superior seedling development, compared to conventional soil cultivation methods.

These findings open exciting possibilities for harnessing chitin hydrogels in agricultural practices to enhance plant growth and productivity.

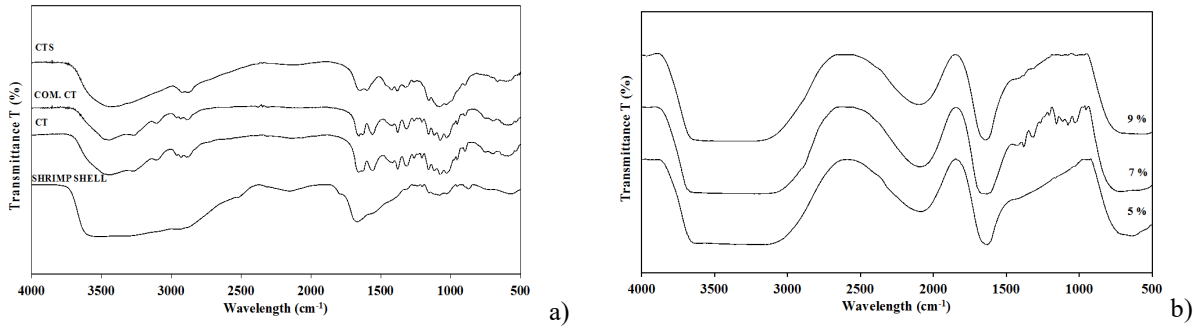


Figure 4: FT-IR spectrum of (a) extracted CT, in comparison with those of commercial CT (COM. CT), chitosan (CTS) and shrimp shells; and (b) FT-IR spectra of CT hydrogels prepared with different LiCl content

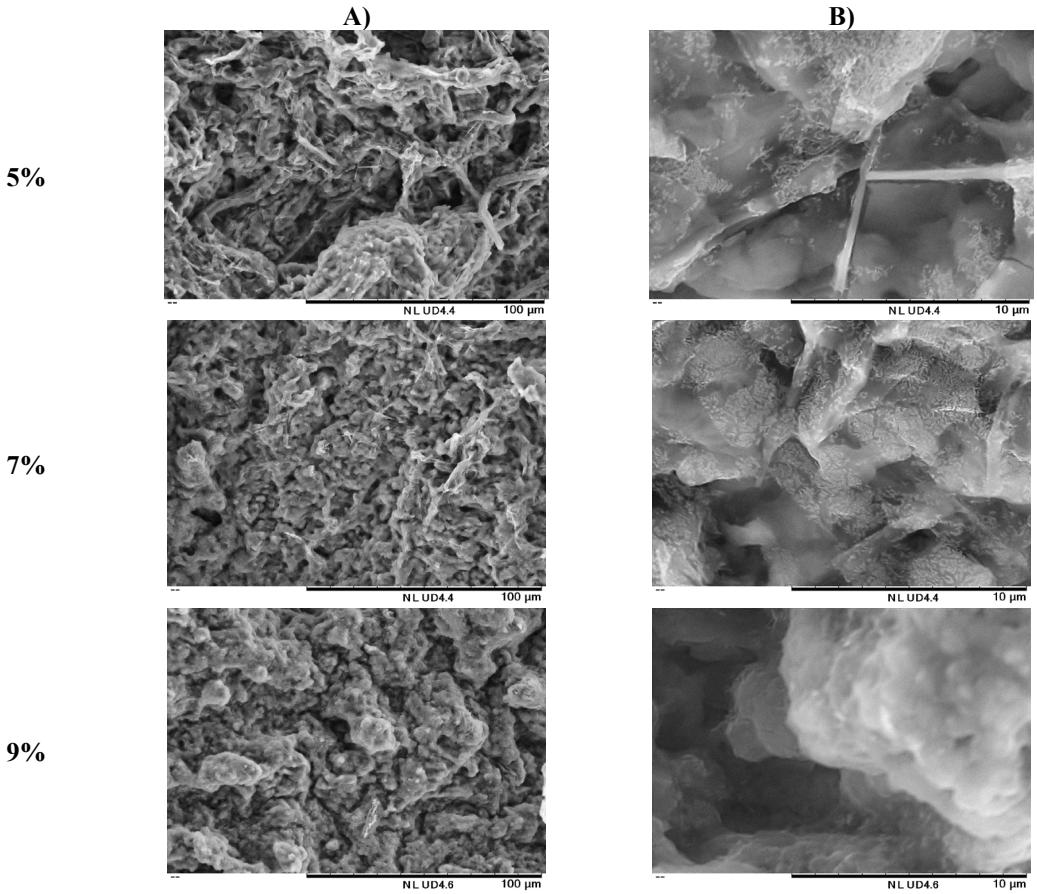


Figure 5: Cross-sectional SEM images of chitin hydrogels with different LiCl contents at 1000× (A) and 10000× (B)

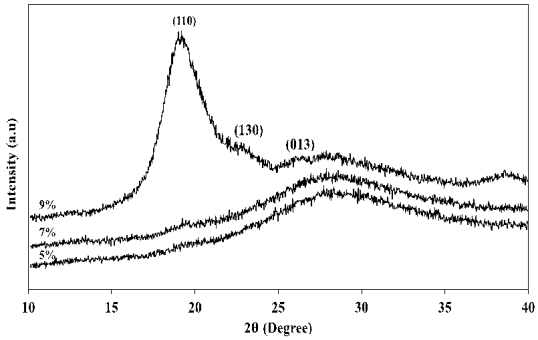


Figure 6: XRD patterns of chitin hydrogels prepared with different LiCl content

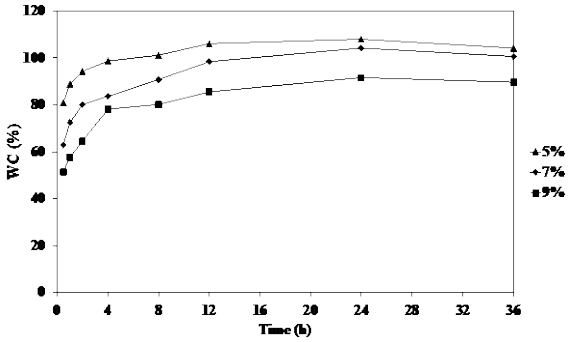


Figure 7: Water content of chitin hydrogels prepared with different LiCl contents

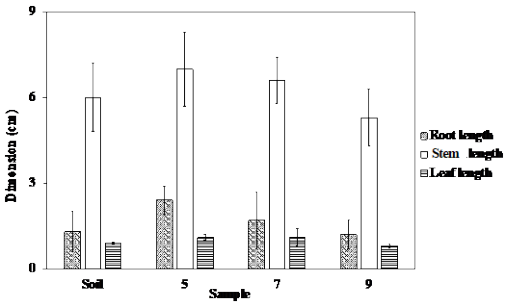


Figure 8: Effects of chitin hydrogels prepared with different LiCl contents on plant germination

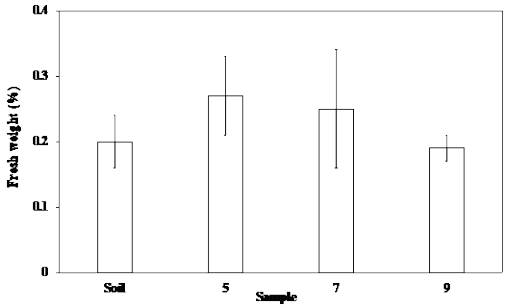


Figure 9: Fresh weight of plants germinated on chitin hydrogels prepared with different LiCl content

Biodegradation studies of chitin hydrogels

In the agricultural and horticultural industries, the timely degradation of materials is a critical consideration, as prolonged persistence of these substances in the soil can give rise to significant environmental concerns.[27] In this study, we assessed the biodegradation behaviour of the chitin hydrogel within soil environments, employing measurements of weight loss and scanning electron microscopy (SEM) analysis.

Figure 10 presents the degradation behaviour of various chitin hydrogel specimens when subjected to soil burial at room temperature for different durations. It is evident that the chitin hydrogel containing 5% LiCl/DMAc experienced degradation rates of 75.1%, 54.5%, and 19.0% after 5, 10, and 15 days, respectively.

During the biodegradation process, the crosslinking densities in the hydrogel network decreased, leading to structural alterations and eventual collapse of the hydrogel network,[28] as visually confirmed by digital photos (Fig. 11A), and both surface and cross-sectional SEM images (Fig. 11B). As can be seen in Figure 11, there was no significant difference in the structure of the chitin hydrogel from day 1 to day 5. However, when the chitin hydrogel was buried in the soil for 10 days, the surface area did not appear more porous, while its cross-sectional area actually looked denser, compared to the corresponding images to day 1 and 5. Moreover, higher magnification was required to observe the morphological changes on day 15. Here, the surface and cross-sectional morphology of the chitin hydrogel was visibly more compact because of the release of water held inside. This phenomenon can be attributed to the hydrophilic nature of the chitin hydrogel, which promotes moisture absorption, thereby facilitating microbial growth during degradation and resulting in increased hydrogel weight loss.[29,30] In contrast, the chitin hydrogel specimens containing 7% and 9% LiCl/DMAc demonstrated lower degradation rates, compared to the 5% sample, under identical conditions. This observation suggests that an increase in the LiCl/DMAc ratio leads to reduced hydrophilicity in the hydrogel, rendering it more resistant to degradation within the soil environment, compared to hydrogels with lower LiCl/DMAc ratios (5% and 7%).

Another beneficial aspect of the degradability of these hydrogels in the soil can be that, upon decomposition, the nitrogen element in the composition of chitin could also serve as a fertilizer supplement, promoting the growth of the plants in the surrounding soil environment. The decomposition of chitin and its derivatives can contribute nitrogen to the soil, acting as an organic nitrogen source that supports plant nutrition and growth. This aspect adds an additional benefit to the agricultural use of chitin-based materials.

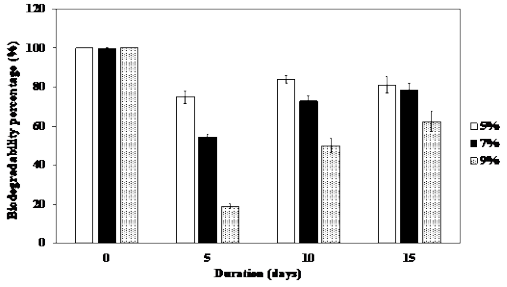
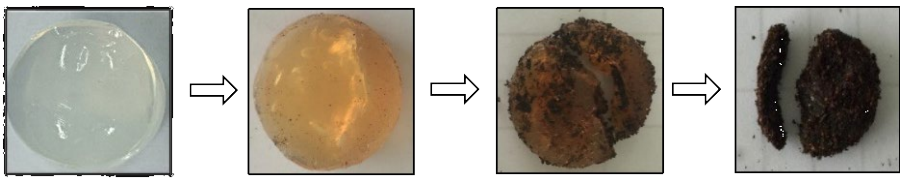


Figure 10: Biodegradability percentage of chitin hydrogels prepared with different LiCl content



A)

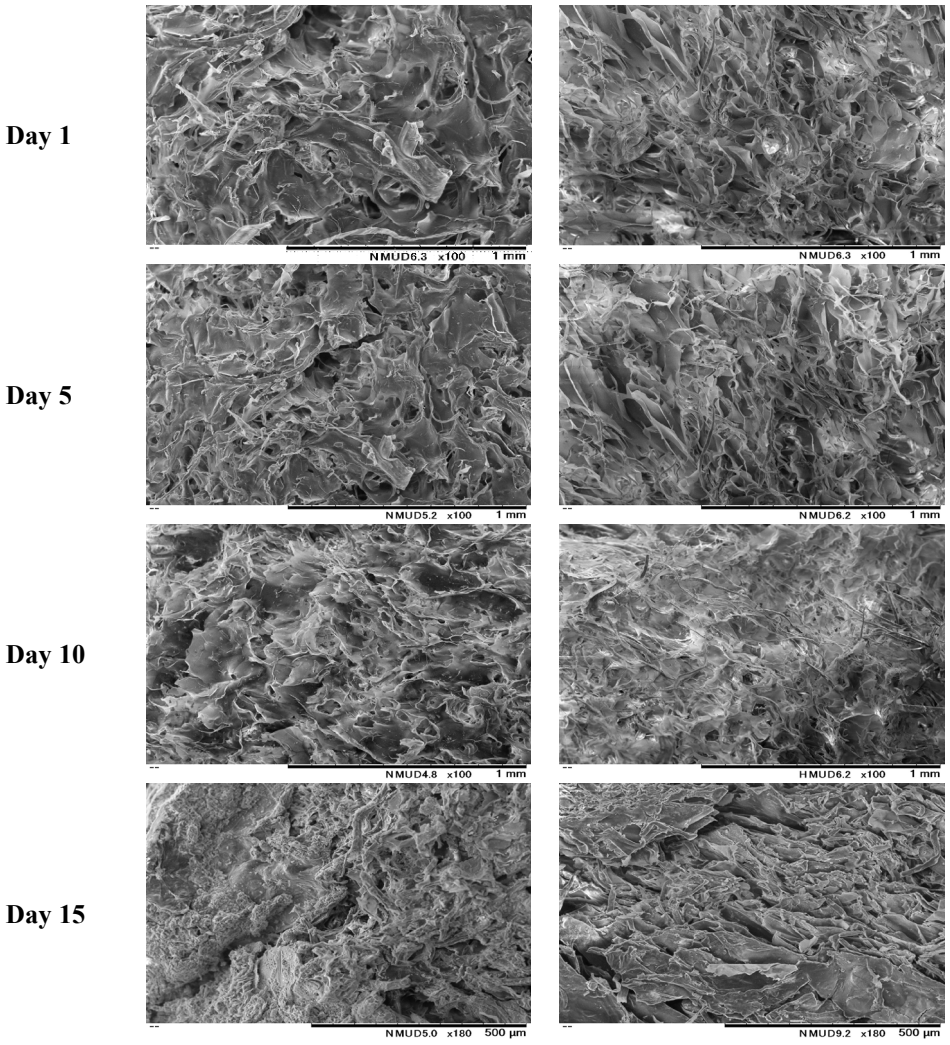


Figure 11: (A) Biodegradation process and (B) SEM surface (left) and cross-sectional (right) images of chitin hydrogels prepared with 5% LiCl after 5, 10 and 15 days of soil burial

CONCLUSION

Chitin extraction from shrimp shells was carried out successfully, yielding a process efficiency of 19.22%. This study also determined the optimal solvent for dissolving chitin, revealing that a suitable content of 5% LiCl proved to be the most effective. The variation in LiCl concentration had a discernible impact on both the properties of the chitin hydrogel material and its influence on plant growth. The growth exhibited by root, stem, and leaf length, as well as the fresh plant weight, demonstrated significant disparities among seed development in the presence of the 5% LiCl/DMAc chitin hydrogel, and in the other hydrogels or in the soil. Moreover, the biodegradability behaviour of the chitin hydrogel in soil environments was examined, with a focus on the impact of the LiCl/DMAc ratio on the degradation rate of the hydrogel, indicating substantial weight loss and structural changes during degradation. These findings underscore the potential utility of the prepared chitin hydrogel, which is a safe and biodegradable material, as a viable alternative to traditional soil for nurturing seed germination and plant growth. This innovation holds promise for various applications within the realm of plant cultivation and agriculture.

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