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Glycerol plasticised chitosan: A study of biodegradation via carbon dioxide evolution and nuclear magnetic resonance

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ABSTRACT

The biodegradation of neat chitosan, glycerol plasticised chitosan films and their corresponding clay-based nano-biocomposites has been studied in simulated aerobic soil and composting environments using a respirometric method. The rate of biodegradation was much faster in soil and all test samples achieved close to 100% biodegradation within 70 days. During biodegradation under aerobic composting conditions the neat chitosan samples achieved approx 65% biodegradation and the plasticised chitosan samples achieved >85% biodegradation within 180 days. Additionally, nano-clay additives had no significant effect on the overall biodegradability of the chitosan-based materials during composting. High-resolution solid-state NMR studies were performed to examine the chemical structures of the plasticized chitosan and their nano-biocomposites. NMR studies indicated that the glycerol plasticizer was extracted into wet compost within first few days while acetic acid remained through strong hydrogen bonding with chitosan during the degradation process.

1. Introduction

Chitosan, a deacetylated form of chitin, is a natural polymer with repeating units of β-(1-4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). Chitosan has applications in agriculture, biomedical and drug delivery systems due to its antimicrobial [1–3], biocompatibility [4,5] and good mucoadhesive properties [4]. It also possesses huge potential as a natural, renewable and biodegradable alternative to petroleum-based plastics. Chitosan-based films have gained popularity in e.g., the food packaging industry, especially as edible films or coatings [6,7]. These films are suggested to improve food conservation and quality by forming a barrier against moisture [8], oxygen [9] and CO_2 [10]. These film properties depend on several parameters such as the chitosan molecular weight and the degree of deacetylation, the organic acid used for its solubilisation and the possible presence of plasticizer.

Generally, two methods are used to produce films based on polysaccharides; i) the solvent casting method – despite certain limitations, at the present the only process capable of producing chitosan films; and ii) the melt processing method of extrusion and kneading under thermo-mechanical treatment with plasticizers. The melt processing method is usually preferred for large-scale production of polymeric films, although its adaptation for processing polysaccharides based materials remains challenging. Chitosan, like many other polysaccharides, has very low thermal stability, degrades without melting and thus is considered infusible. We have previously developed and reported a combinatorial and innovative melt-processing route to produce plasticized chitosan sheets using glycerol [11]. The present study aims at investigating biodegradation properties of these materials under simulated aerobic soil and composting conditions.

Addition of nanoclay to natural polymers to form nano-biocomposites, is known to influence the material behaviour such as mechanical properties, water absorption, fire resistance and biodegradability [12–18]. In the present study, the effect of addition of nanoclay (unmodified and organically modified) on biodegradability of chitosan-based nano-biocomposites films was investigated under simulated aerobic composting conditions according to standard test method AS ISO 14855-1. The chemical structures of the biodegradation residues were examined by high-resolution solid-state NMR spectroscopy.

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2. Materials and methods

Two types of chitosan were used in the experimental work and their characteristics are shown in Table 1. ChitoClear® was received as a white powder (91% dry matter content) with particle diameter <1 mm (100% through mesh 18). KiOnutrime-Cs® was provided as sandy brown colour powder (92% dry matter content) and with particle diameter <1 mm. ChitoClear® was used as the matrix of chitosan-based nano-biocomposites, while KiOnutrime-Cs® was used as the organomodifier for the nanoclay. The Dellite® LVF sodium montmorillonite (MMT–Na^+) was supplied by Laviosa Chimica Mineraria S.p.A. (Italy) and has a cationic exchange capacity (CEC) of 1050 μequiv/g. Glycerol (99.5% purity, from Novance, France), acetic acid (Fluka, Sigma–Aldrich), and sodium hydroxide (Carlo Erba Réactifs – SdS, France), and sodium bromide (Sigma–Aldrich) were used as received.

2.1. Sample preparation

2.1.1. Organomodification of montmorillonite

Chitosan solution was prepared by adding 4.754 g (dry basis) of the KiOnutrime-Cs chitosan to 500 mL of 1% (v/v) acetic acid. The solution was stirred at room temperature overnight. The pH of the solution was then adjusted to 4.9 with NaOH solution. In parallel, a stock of well-dispersed clay suspension was prepared by adding 20 g of MMT–Na^+ into 500 ml of water and treating with sonication at 60 °C for 4 h. Then, the chitosan solution and the MMT–Na^+ suspension were mixed together and the mixture was stirred at 60 °C for 24 h. The mixture was centrifuged at 3000 rpm for 15 min, and then the supernatants were discarded. The precipitate was washed with distilled water and centrifuged again at the same condition, which was repeated twice to make it free from acetate. Hence, the final paste of chitosan-organomodified MMT was obtained with moisture content of 94.6%. Here, the mass ratio of chitosan and clay were thus determined to achieve a monolayer of chitosan absorbed into the nanoclay interlayer spacing through a cationic procedure with respect to the CEC of the nanoclay [19].

2.1.2. Preparation of chitosan-based nano-biocomposites

The preparation procedure of chitosan-based nano-biocomposites used here was similar to that in a previous work, including modifications relating to the addition of nanoclay [11]. Seven samples with different formulations and/or preparation methods were prepared, with the details listed in Table 2. In summary, glycerol was manually mixed with the chitosan powder and then acetic acid aqueous solution (3%, v/v) was added to the glycerol/chitosan mixture. For unplasticised formulation, acetic acid was directly added to the chitosan powder and mixed. For nano-biocomposites, nanoclay (in the form of either paste or dried powder) was added to glycerol/chitosan mixture first, manually mixed and then acetic acid aqueous solution (3%, v/v) was added to the chitosan–glycerol–nanoclay mixture with continuous mixing to obtain a paste with final chitosan concentration of 25 wt%.

The mixtures with different formulations were then thermo-mechanically kneaded in a Haake Rheocord 9000 internal batch mixer with twin roller rotors at 80 °C for 15 min, with a rotor speed of 100 rpm. The resulting materials were compression moulded at 110 °C under a pressure of 160 bar for 15 min (with a venting process after 8 min), then immediately cooled at room temperature for 5 min. After compression moulding, chitosan sheets of approximately 2 mm thickness were obtained. The sheets were then conditioned in desiccators at 57% relative humidity (achieved with saturated NaBr solution) and ambient temperature for one month to achieve the equilibration of the materials with constant moisture contents. The films were successfully made for all formulations (with or without glycerol) however the unplasticised chitosan films shrank a lot and became curled and rigid during ageing. Detailed properties of all these films will be published in a separate paper (draft under preparation).

Table 1
Chitosan source and properties (as provided by the supplier).

Commercial name	KiOnutrime-Cs®	ChitoClear™
Supplier	KitoZyme	Primex
Source	Aspergillus niger (mushroom)	Pandalus borealis (shrimp)
Molecular Mass	15,000 Da	250,000–300,000 Da
Degree of deacetylation	78–80%	96%

2.2. Source of inoculum

The soil sample was collected from a grass field covered with grass, mainly *Pennisetum clandestinum* Kikuyu with some local weeds (CSIRO Highett campus, Victoria, Australia). No herbicide or pesticide has been reportedly used and the site has not been under use or any construction over the past years. Samples were collected and sieved through 8 mm sieve, and a subsample was sent to ALS laboratories (Victoria, Australia) for analysis. The soil characteristics were pH 5.6, dry weight 82%, volatile solids 6.7% and C/N ratio of 13 on an oven-dry basis.

Approximately 2–3 months old mature compost samples were collected from a commercial composting facility (Natural Recovery Systems, Victoria, Australia). It is an in-vessel composting facility which has been converting kerbside garden organics and a range of commercial and industrial food wastes into high quality compost for over a decade. The compost sourced was sieved using a screen size of 8 mm to obtain a homogeneous mix, free from large inert objects such as glass, stones or pieces of metals. A subsample was sent to ALS laboratories (Victoria, Australia) for analysis. The compost characteristics were pH 7.5, dry weight 52%, volatile solids 44% of dry weight, and C/N ratio of 10 on an oven-dry basis.

2.3. Aerobic biodegradation

The test films were analysed for total dry solids, volatile solids and total organic carbon content and values were used (1) to calculate quantity of material to be used in the test so as to yield suitable amount of carbon dioxide for the determination, and (2) to calculate theoretical amount of carbon dioxide (Equation (1)), which is used to determine biodegradation percentage (Equation (2)).

Prior to the testing, the film samples were reduced in size to achieve approximately 2 cm × 2 cm maximum surface area of each individual piece of the test material. The test was conducted in triplicate including the blank (soil/compost only), test material (inoculum with test material) and reference material (inoculum with cellulose). Biodegradation experiments under simulated soil environment consisted of 'blank' bioreactor (3 L glass vessel) containing approximately 800 g soil on dry weight basis. The 'test' bioreactors contained 800 g of soil and 10 g of test material, both on dry weight basis, and test material was replaced with cellulose in the case of the 'reference' bioreactors. The contents of all bioreactors were well mixed and placed inside an in-house built respirometer unit [20]. The temperature was maintained at 30 ± 2 °C throughout the test.

Biodegradation under aerobic composting environment consisted of 'blank' bioreactors each contained 600 g of total dry solids of compost inoculum. The bioreactors filled with 'test material' each contained 600 g of total dry solids of compost inoculum and 100 g of dry solids of test material and the 'reference' bioreactors were filled with 100 g of cellulose powder and 600 g total dry solids of compost inoculum. These contents were mixed thoroughly