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Effect of pectin and hemicellulose removal from hemp fibres on the mechanical properties of unidirectional hemp/epoxy composites

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ABSTRACT

The objective of this study was to investigate the effect of pectin and hemicellulose removal from hemp fibres on the mechanical properties of hemp fibre/epoxy composites. Pectin removal by EDTA and endo-polygalacturonase (EPG) removed epidermal and parenchyma cells from hemp fibres and improved fibre separation. Hemicellulose removal by NaOH further improved fibre surface cleanliness. Removal of epidermal and parenchyma cells combined with improved fibre separation decreased composite porosity factor. As a result, pectin removal increased composite stiffness and ultimate tensile strength (UTS). Hemicellulose removal increased composite stiffness, but decreased composite UTS due to removal of xyloglucans. In comparison of all fibre treatments, composites with 0.5% EDTA + 0.2% EPG treated fibres had the highest tensile strength of 327 MPa at fibre volume content of 50%. Composites with 0.5% EDTA + 0.2% EPG → 10% NaOH treated fibres had the highest stiffness of 43 GPa and the lowest porosity factor of 0.04.

1. Introduction

Cellulosic fibres are natural resources with important properties such as low density, high specific tensile strength and stiffness, and a high aspect ratio (average length over diameter of the fibres) [1]. Due to these properties and concerns about the environment, the application of natural cellulosic fibre reinforced polymer composites has received considerable attention in recent years [2,3].

Hemp (*Cannabis sativa*) is a fast growing crop, which produces strong fibres that primarily lie beneath the epidermis in the cortex and form a ring in the phloem parenchyma [4]. Like wood fibres, hemp fibre cell walls are natural composites composed mainly of three classes of polysaccharides: cellulose, hemicellulose and pectins. Cellulose consists of β-1,4-linked glucan chains and is organized into microfibrils interlocked by xyloglucan (XG) [5]. The cellulose microfibrils and the cross-linked XG chains are generally considered as the two main components which provide cell wall strength. Pectins fill the spaces between cellulose and XG [5]. The pectins function as glue packing the microfibrils into final fibres that are approx. 20 mm in length and 10–40 μm in diameter [6]. The fibres make up the fibre bundles with varied sizes which are in turn organized into a fibre layer inside the cortex. Pectins and lignin in the middle lamellae (ML) join the fibres together [4,7].

The most abundant pectic polysaccharide in plant cell walls is homogalacturonan (HG), which is a linear homopolymer of α-1,4-linked galacturonic acid that comprises approx. 70% of pectin. Besides HG, the pectic polysaccharides are mainly comprised of rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II). RG I represents 20–35% of pectic substrates and consists of a backbone of alternating α-1,4-D-galacturonic acid and α-1,2-L-rhamnose units; the latter are usually decorated with homopolymeric side chains of β-D-galactose and α-L-arabinose [8]. RG II makes up 5–10% of the pectin, and consists of a HG backbone of 1,4-linked α-D-galacturonic acid residues decorated with different types of side branches [9].

The physicochemical properties of pectin are largely dependent on the degree of methyl and acetyl esterification. Low-methoxyl (LM) pectin (i.e. HG) has sufficient carboxyl groups for the formation of calcium-mediated interactions between two neighbouring pectin chains, as described by the "egg-box" model [10]. However, high-methoxyl (HM) pectin does not contain sufficient polygalacturonic acid residues un-methylated at the C-6 position to form a stable structure through calcium-mediated interactions. Instead, hydrogen bonding and hydrophobic interactions have been

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suggested important forces in maintaining a stable structure for HM-pectin [11,12].

The principle behind fibre processing for the application of natural fibres in composites is to remove the non-cellulosic components (e.g. pectin, hemicellulose and lignin) to obtain well separated and cellulose rich fibres before use as reinforcement in composites. Traditional fibre processing methods such as field retting and water retting, however, are largely dependent on weather conditions (especially rainfall and temperature) and may damage the fibres if fibres are over retted [4,13]. Enzymatic treatment, involving mainly pectinolytic enzymes, may offer an alternative method to degrade pectin from hemp fibre strips and provide a solution to the limitations of traditional fibre retting methods.

In treatment of fibre with enzymes, pectic polymers are released from ML and fibre cell walls by using pectinases (e.g. endo-polygalacturonase) that randomly hydrolyze the glycosidic bonds of the HG backbone to liberate monomeric, dimeric or oligomeric fragments [14]. Addition of chemical chelators (e.g. ethylenediaminetetraacetic acid (EDTA)) has been shown to promote enzyme catalyzed degradation of HG from cellulosic fibres during enzymatic treatments [15,16]. The enhanced enzymatic degradation of HG results from the capacity of chemical chelators to form complexes particularly with calcium in pectin [17]. Furthermore, alkaline extraction with 10% NaOH is widely used for the isolation of hemicellulose from lignocellulosic biomass to obtain cellulose of high purity [18].

The objective of this study was to investigate the effect of sequential removal of pectin (e.g. homogalacturonan) and hemicellulose (e.g. xyloglucan) on the mechanical properties of fibres and their subsequent use in unidirectional hemp fibre/epoxy composites. Pectin removal from hemp fibres was carried out using EDTA alone and in combination with monoactive pectinase enzyme. In some experiments hemicellulose was also sequentially removed using 10% NaOH.

2. Materials and methods

2.1. Raw material and processing

2.1.1. Plant material

Hemp (*Cannabis sativa* L.), variety USO-31, was grown in France (N48.8526°, E3.0190°(WGS84)) as described in detail by Liu et al. [4]. Hemp stem pieces with a length of 150 ± 10 mm were randomly collected from the stems. Before treatment, hemp bast fibre strips were manually peeled from the stem pieces, gently rinsed with warm water (40 °C) to remove dirt and then dried at 50 °C for 12 h. Field retting was carried out on whole stems for 20 days after harvest [4] as a comparison to the treatments investigated in this study.

2.1.2. EDTA treatment

EDTA treatment was carried out on bast fibre strips using different concentrations of EDTA ($C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$) (0.5, 0.75, 1, 2, and 3%, w/v) for 4 h in a water bath at 40 °C and agitation of 100 rpm. The liquid (cm^3) to fibre (g) ratio was 40:1. Before treatment, the pH of the EDTA solution was adjusted to 6.0 with 5 mol dm^{-3} NaOH. Each treatment was done in triplicate (4 g bast fibre strips/replicate). At the end of each treatment, the wet bast fibre strips were rinsed with MilliQ water and then dried at 50 °C for 12 h.

2.1.3. Enzymatic treatment

Enzymatic treatments using monoactive endo-polygalacturonase (EPG) with and without EDTA were performed on hemp bast fibre strips. The enzymatic treatments were carried out for 4 h at pH 6.0 in 25 mmol dm^{-3} citrate buffer in a water bath at 40 °C and agitation of 100 rpm. The liquid (cm^3) to fibre (g) ratio was maintained at 40:1. For enzymatic treatment in the presence of EDTA, 0.5% EDTA was added to enzyme solutions, and pH of the mixture was adjusted to 6.0 with 5 mol dm^{-3} NaOH.

The recombinant EPG was applied at a dosage of 0.2% (g protein/g dry matter). The EPG, derived originally from *Emericella nidulans*, was produced by fermentation of a *Pichia pastoris* clone (principally as described previously [19]) transformed with the gene AN4372.2 obtained from the Fungal Genetic Stock Center, Kansas State University, USA [20]. After the fermentation, the supernatant was recovered by centrifugation, and then subjected to a 0.4-μm sterile filtration, and finally the concentrated enzymes (or crude enzyme) after ultrafiltration with a 10 kDa cutoff membrane (Millipore, Sartorius) were used. At the end of enzyme treatment, the wet bast fibre strips were rinsed with MilliQ water and then dried at 50 °C for 12 h. Control treatments (i.e. without enzymes) were performed under the same conditions to determine the influence of non-enzymatic processing.

Using the above conditions, the activity of EPG was 945 U cm^{-3} (17 U mg^{-1} protein). The EPG activity was determined by measuring formation of reducing ends using 2 g dm^{-3} polygalacturonic acid as substrate [21] with enzyme to substrate ratio of 5:1 (v/v). The amount of reducing sugars that were liberated was measured by using 4-hydroxybenzoic acid hydrazide (PAHBAH) as colorimetric agent and glucose as standard [22]. One unit (1 U) of EPG activity is defined as the volume of the crude enzyme solution (cm^3) required to liberate 1 μmole reducing ends (glucose equivalents) per minute under the conditions applied in enzyme treatment. Protein content of the crude enzyme solutions was determined according to Bradford [23] using Bovine serum albumin as standard.

2.1.4. Sodium hydroxide treatment

After treatment with 0.5% EDTA and 0.2% EPG, the bast fibres were treated with 10% NaOH in tight plastic bags and held for 4 h in a water bath at 60 °C and agitated of 100 rpm. The liquid (cm^3) to fibre (g) ratio was 40:1. At the end of each treatment, the wet bast fibre strips were rinsed with MilliQ water and dried at 50 °C for 12 h.

2.1.5. Manufacturing of composites

The treated hemp bast fibre strips were manually aligned to allow the fibres to be processed into unidirectional composites. Bundles of fibre strips were firstly cut to a length of 140 mm, and the fibre strips were then justified to a bunch of fibre strips with masses in the range 0.6–2.3 g. Bunches of fibre strips were then put in each mould chamber. Afterwards, a press beam was placed on the top of the fibre strips in each chamber, and two insert beams were used to fix the height of the mould chambers to 2 mm. Epoxy resin (Araldite® LY 1568) and its amine hardener (Aradur® 3489) were mixed at a 100/28 mass ratio and degassed in a vacuum oven. The setup for the vacuum infusion and moulding processing has been described by Liu et al. [24]. After demoulding, composite samples with dimensions 140 mm × 10 mm × 2 mm were obtained and then glass fibre/epoxy tabs with lengths of 50 mm were mounted on composite specimens using epoxy glue (DP 460).

2.2. Characterization and testing of fibres and composites

2.2.1. Chemical composition analysis

The dried bast fibres were ground with a microfine grinder (IKA, MF 10.1; IKA®-Werke GmbH) to a particle size of 1 mm. Ground samples were extracted in a Soxhlet apparatus as described by Liu et al. [4], and then the extractive-free fibres were hydrolysed