INTERFACIAL MICROMECHANICS OF BACTERIAL CELLULOSE
BIO-COMPOSITES USING RAMAN SPECTROSCOPY

A thesis submitted to The University of Manchester for the degree of
Doctor of Philosophy
in the Faculty of Engineering and Physical Sciences.

2011

FRANCK QUÉRO

SCHOOL OF MATERIALS
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Abstract

The University of Manchester
Franck Quéro
Doctor of Philosophy
Interfacial micromechanics of bacterial cellulose bio-composites using Raman spectroscopy
December 2011

An improved method to evaluate Young’s modulus of bacterial cellulose (BC) nanofibrils is presented. This estimation takes into account polarisation configurations, nanofibril orientation and tensile deformation axis direction. A range of 79 - 88 GPa has been obtained showing their great potential to be used as reinforcement in composite materials.

BC bio-composites, constituted of a BC layer embedded in-between two matrix layers, have been prepared by compression moulding. The stress-transfer from the matrix to the reinforcement has been quantified using Raman spectroscopy. This has been carried out by following the shift of the Raman band initially located at a wavenumber position of ~1095 cm$^{-1}$. Polylactide (PLA) was chosen as matrix material due to its biodegradability and bio-sourced origin. Transparent polylactide films were obtained in specific processing conditions to suppress crystallisation. This allowed the laser to penetrate the matrix and interact with the upper layer of BC networks. Several factors that could affect the interface in these composites have been studied.

The influence of the culturing time of BC networks on the composite interfaces has been investigated. Higher Raman band shift rates with respect to strain and stress have been measured for composites manufactured using BC networks having a low culturing time. This led to enhanced coupling between PLA and the upper layer of BC networks. Scanning electron microscopy imaging of the tensile fracture surface of these composites revealed that delamination between the BC layers was occurring rather than failure at the BC/PLA interface.

Cross-linking of BC networks using glyoxal was performed to consolidate their layered structure. Raman spectroscopy was used to probe the stress-transfer of unmodified and cross-linked BC networks. These data revealed that cross-linked materials exhibit an enhanced stress-transfer both in the dry and wet states compared to unmodified BC networks.

Cross-linked BC networks were used to design composites but no significant stress-transfer improvement was observed. As a result, maleated polylactide (MAPLA) was produced and used as a matrix material in order to consolidate the interface between PLA and both the upper and lower layer of cross-linked BC networks. Composites designed using cross-linked BC networks and MAPLA showed a significant stress-transfer improvement over composites designed using unmodified BC networks and PLA. Also the determination of the bulk tensile mechanical properties of the composites revealed a significant increase of relative Young’s modulus. This increase is thought to be due to reduced molecular mobility at both the cross-linked BC/MAPLA interface and between cross-linked BC layers. This is further supported by scanning electron imaging of the tensile fracture surfaces.
Declaration

I declare that no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Franck Quéro
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Acknowledgements

I would like to express my deep and sincere gratitude to my supervisor, Prof. Stephen J. Eichhorn, for his continuous encouragement, supervision and support. This thesis would not have been possible without his help and guidance. I also would like to thank Prof. Robert J. Young and Prof. Alexander Bismarck for their occasional advice and guidance.

I extend my sincere gratitude to Prof. Hiroyuki Yano and Prof. Masaya Nogi for providing bacterial cellulose networks. Thanks are also addressed to Dr. Geert Vanden Poel, Dr. Bahij Sakakini and Dr. Koon-Yang Lee for their cooperation and assistance with some of the experimental work.

Thanks are also extended to the financial support from the Engineering and Physical Sciences Research Council (EPSRC).

I would also like to acknowledge the help and support of all staff in the Manchester Materials Science Centre and more particularly Mr. Andrej Zadoroshnyj for his technical assistance with Raman spectroscopy. I wish to thank my friendly and cheerful fellow students in the Cellulose and Natural Materials group for their support and stimulating discussions.

Je souhaite également remercier mes parents, Brigitte Tréhin et Didier Quéro, ma soeur Carole Quéro, ma nièce Lauriane Quéro, ma grand-mère disparue Jeanne Ado ainsi que mes grand-parents, Jean Quéro et Madeleine Le Dorze pour leur amour et soutien inconditionnel. Cette these leur est dédiée.

Me gustaría, también, dar las gracias a Pamela Collao Acevedo, por su apoyo y por todos los grandes momentos que pasamos juntos viajando en Europa.

I also extend my acknowledgements to all my friends but more particularly to Luis Manuel Pessahna Ribeiro, Achilleas Tsiotas and Oliver Juszczak for their friendship and support during my stay in Manchester.
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International Conferences


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- International Conference on Interfaces and Interphases in Multicomponent Materials (IIMM), September 2010, Sheffield, United Kingdom. Oral presentation: Cross-linking of Bacterial Cellulose Networks using Glyoxalisation.


CHAPTER 1
INTRODUCTION

1.1 Generalities

One can define a composite material as a mixture of solid materials, namely; metals, ceramics and polymers as illustrated in Figure 1.1. Each of these materials can be used either as matrix and/or reinforcement. The matrix is usually the dominant phase, surrounding lower amounts of reinforcement materials. The matrix is usually ductile whereas the reinforcement phase is commonly stiff, and can impart enhanced mechanical properties to the matrix material among other physical properties. For instance, glass or carbon fibres are usually used as mechanical reinforcement in more ductile polyester or epoxy polymer matrices. Another classic example of a composite material is reinforced concrete. In that particular case the matrix is concrete and the reinforcement is metal which increases the strength. Such a composite is called a ceramic-matrix composite. In the same way, when a polymer or a metal is chosen as a matrix, the materials are respectively referred as polymer-matrix and metal-matrix composites.

Figure 1.1 Classes of solid materials for the design of composite materials.
Composite materials can be divided in three main families, namely, particle-reinforced, fibre reinforced and structural composites (see Figure 1.2). The form of composite material reported in this thesis can be classified as a fibre-composite material.

![Classification of composite materials](image)

**Figure 1.2** Classification of composite materials. Reproduced from Callister (2007).

The mechanical reinforcement in composites is governed by how well the stress is transferred from the matrix to the reinforcement. This is mainly dependent on both chemical and physical interactions between the phases, where the interface plays a key role. This dissertation is focused on polymer composites where both the matrix and the reinforcement are polymers.

Polymers are natural or synthetic substances made of macromolecules, an assembly of covalently-bonded molecules (Young and Lovell 1991). Polymers have existed in a natural form since life began such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins and polysaccharides playing important roles in biological systems (Young and Lovell 1991). Other common natural polymers are silk, rubber, collagen, chitin, keratin and cellulose only to name a few (Young and Lovell 1991). They have been used since the beginning of mankind, for example for clothing, housing, tools and weapons (Young and Lovell 1991).
Cellulose is the main available source of natural polymer and can be obtained from plants, tunicates (Ranby 1952) or bacteria (Brown 1886). It is particularly useful to be used as a bio-sourced reinforcement in composite materials. They are commonly called bio-composites but also sometimes green composites if the matrix material is also bio-sourced. These composites have the advantage of being obtained from renewable resources and are potentially fully biodegradable. They have been recently reconsidered due to environmental concerns and the need to be more oil-independent.

1.2 The Research Project

The main purpose of this project was to produce low cost bacterial cellulose bio-composites and optimise their mechanical and stress-transfer performances. Another challenge was to evaluate the potential of Raman spectroscopy to probe the interface between bacterial cellulose and a synthetic biopolymer.

This project has been carried out in the framework of a joint research project funded by the Engineering and Physical Sciences Research Council (EPSRC) with both the Polymer and Composite Engineering (PaCE) Group led by Professor Alexander Bismarck and the Biological Systems Engineering Laboratory (BSEL) led by Professor Athanasios Mantalaris, both based at Imperial College in London. This research project is also the result of a collaboration with Professor Hiroyuki Yano from the Research Institute for the Sustainable Humanosphere, Kyoto University and Professor Masaya Nogi, Institute of Science and Industrial Research in Osaka University.
The contribution of each collaborator to this thesis is clearly identified in the following Chapters. All other experimental work and results presented in this dissertation were respectively performed and obtained by Franck Quéro, author of this thesis.

1.3 Problem Statements

i. How is it possible to improve the method of evaluation of Young’s modulus of bacterial cellulose fibrils using Raman spectroscopy?

ii. Is Raman spectroscopy a relevant technique to probe the interface in bacterial cellulose/polylactide composites?

iii. Does the culturing time of bacterial cellulose networks influence the interface of bacterial cellulose/polylactide composites?

iv. Does the cross-linking of bacterial cellulose networks through glyoxalisation efficiently consolidate their layered structure?

v. Do cross-linked bacterial cellulose networks enhance the stress-transfer properties of bacterial cellulose/polylactide composites?

vi. Does the modification of polylactide using maleic anhydride consolidate efficiently the bacterial cellulose/polylactide interface?

vii. What are the effects of both modification of bacterial cellulose networks and polylactide on the mechanical performances of composites?

1.4 Objectives

i. Produce bacterial cellulose/polylactide composites using a compression moulding technique.
ii. Characterise bacterial cellulose networks and bacterial cellulose/polylactide composites and in particular their mechanical and micromechanical properties.

iii. Obtain information on the micromechanics of bacterial cellulose/polylactide interfaces using Raman spectroscopy.

iv. Investigate the influence of the culturing time of bacterial cellulose networks on the interfacial micromechanics of bacterial cellulose composites.

v. Investigate the influence of glyoxalisation on the micromechanics of bacterial cellulose networks in the dry and wet states.

vi. Investigate the influence of glyoxalisation and maleation on the micromechanics of bacterial cellulose/polylactide composite interfaces using Raman spectroscopy.

1.5 Structure of Thesis

- Chapter 2 presents general information on natural cellulose, bacterial cellulose, fundamental principles of Raman spectroscopy as well as a background of the available methods to design bacterial cellulose-based composites and nanocomposites.

- Chapter 3 provides an evaluation of the influence of the exposure of bacterial cellulose networks to a near-infrared laser on the intensity and on the wavenumber position of the Raman band located at ~1095 cm$^{-1}$.

- Chapter 4 presents a method to evaluate Young’s modulus of bacterial cellulose fibrils in fibrous networks.

- Chapter 5 is an evaluation of the interface between polylactide and bacterial cellulose networks having various culturing times.
○ Chapter 6 reports the influence of glyoxalisation on the mechanical performance of bacterial cellulose networks in the dry and wet states.

○ Chapter 7 deals with the effect of glyoxalisation and maleation on the mechanical performance of bacterial cellulose/polylactide composites.

○ Chapter 8 are the conclusions drawn from this study, and also suggestions for future work.

1.6 References


CHAPTER 2
LITERATURE REVIEW

2.1 Natural Cellulose

2.1.1 Introduction

Polymers, metals and ceramics are the three classes of materials available to design composite and nanocomposite materials. Polymers are natural or synthetic substances made of macromolecules, an assembly of covalently-bonded molecules (Young and Lovell 1991). These macromolecules have existed in a natural form since life began e.g. deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins and polysaccharides playing important roles in biological systems (Young and Lovell 1991). Other common natural polymers are silk, rubber, leather, chitin, wool or cellulose to cite but a few examples. Cellulose is the most abundant polymer on earth, and is found mainly in the plant kingdom (Klemm et al., 1998). In addition to being abundant, cellulose is light and cheap, which makes it an attractive material for industrial applications. The vegetal form is the main source of cellulose, where it is typically associated with phenolic materials and carbohydrates such as lignin, pectins and hemicelluloses. It is also, to a lesser extent, associated with waxes, aromatic compounds, fats, lipids or water-soluble sugars. All together they form complex vegetal structures such as wood, natural fibres or seeds, to cite but a few examples. Cellulose is also present in sea animals, called tunicates (Ranby 1952), and can be produced by micro-organisms such as bacteria, algae and fungi.
Wood has been used and commercialised for centuries as a construction material. Cellulose fibres have also been used to design textiles and paper materials. Chemically-modified cellulose is another successful example of cellulose commercialisation. John and Isaiah Hyatt invented the first commercially successful thermoplastic polymer in 1870 based on nitrocellulose (Young and Lovell 1991).

As stated previously, cellulose is mainly provided by vegetation which forms complex hierarchical structures such as wood. All plants have common structural similarities, but specificities as well. They are typically formed from layers, each having different thickness, cellulose fibril orientations and content. This offers different ranges of textures and physical properties to each layer, which further leads to the whole plant structure having specific properties.

Figure 2.1a represents the hierarchical structure of a cotton fibre. The outer layer is the cuticle and is a waxy cellulose-free coating having a protection function. It contains pectins and proteinaceous materials. The first layer, containing cellulose, is the primary wall, and has a thickness of ~50 nm. It is made of fibrils having a diameter of ~10 nm forming a cellulose fibril network. The secondary wall is a double layer consisting of two layers; namely the S1, sometimes called the "winding layer", and the S2 layer, referred to as sometimes as the "main body". It has a thickness of ~100 nm. Its cellulose microfibrils are aligned parallel to each other, and at an angle of 40 - 70° to the fibre axis. The main body consists of concentric layers of cellulose fibrils oriented at 70 to 80° to the fibre axis. The inner layer is referred to as the lumen wall. It separates the secondary wall from the tube-like lumen.
Figure 2.1b shows the hierarchical structure of a delignified spruce wood fibre. The outer layer is called the primary wall, which is formed during the surface growth of the cell wall. The secondary wall, similar to a cotton fibre, is made of two layers, S1 and S2, each having different cellulose microfibril orientations. The S1 layer has a thickness of ~300 nm. The S2 layer is much thicker (several μm) and contains most of the cellulose mass.

**Figure 2.1** Scheme of the "morphological architecture" of (a) a cotton fibre and (b) a delignified spruce wood fibre (Krässig 1993; Klemm et al., 1998); C-cuticle (rich in pectins and waxes), L-lumen, ML-middle lamellae, P-primary wall, R-is the reversal of the fibril spiral, S1-secondary wall ("winding layer") and S2-secondary wall ("main body").

These natural composites are a great source of inspiration, and biomimetic approaches can be a way to improve the physical properties of man-made natural and synthetic composite materials.
2.1.2 Sources of Cellulose

As previously mentioned, several sources of cellulose are available; namely from plants, tunicates and micro-organisms such as bacteria, algae and fungi. Cellulose from plants is the main source of cellulose with a production of $10^{11}$-$10^{12}$ tonnes per year (Klemm et al., 1998). It can be extracted from softwoods and hardwoods, seeds or plant fibres. The main drawback of plant cellulose is that heavy chemicals and mechanical treatments are required in order to separate cellulose fibrils from other constituents of plants such as hemicelluloses and lignin.

Figure 2.2 shows the required delignification process to isolate cellulose fibrils from other constituents. Wood, containing 30 to 40 % of cellulose, is first reduced into chips. Then two chemical processes can be used; either a sulphite or a sulphate process.

![Delignification process of wood. Reproduced with modification from Klemm et al. (1998).](image-url)
The resulting product is then bleached using either chlorine or chlorine-free chemicals. A bleached dissolving pulp containing 90 to 95% of cellulose is finally obtained after being washed, dried and packed. Depending on the plant type, which have different cellulose contents as reported in Table 2.1, the separation process of cellulose from other wood constituents can be more or less harsh, but also time consuming and expensive.

**Table 2.1 Chemical composition of some typical cellulose-containing natural materials. Reproduced from Klemm et al. (2003).**

<table>
<thead>
<tr>
<th>Sources</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Lignin (%)</th>
<th>Extract (%)</th>
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<td>25-35</td>
<td>16-24</td>
<td>2-8</td>
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<td>40-44</td>
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<td>Wheat straw</td>
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</tbody>
</table>

One of the advantages of using plant cellulose is that it can be produced on an industrial scale, whereas the production of cellulose from other sources is, for now, only available on a laboratory scale. It is also, up to now, the cheapest way to mass
produce cellulose. High purity cellulose fibrils can be extracted from sea animals called tunicates (Ranby 1952). The use of tunicate cellulose for composite applications has often been reported in the literature, and is used in the form of tunicate cellulose nanowhiskers. Tunicate cellulose, like other sources of cellulose, can be transformed into nanowhiskers through an acid hydrolysis process (Favier et al., 1995). This procedure removes the amorphous regions to obtain cellulose with a higher crystallinity. The reinforcement potential of tunicate cellulose nanowhiskers has been evaluated in order to bring about the relevance of converting cellulose nanofibres into cellulose nanowhiskers. This has recently been discussed in a review, where it is mentioned that cellulose nanowhiskers are a promising material, but further development is necessary for large-scale industrial production and property consistency (Eichhorn 2011).

Cellulose can also be produced by micro-organisms such as bacteria, algae and fungi. Similarly to tunicate cellulose, bacterial cellulose has the advantage of being available in a very pure form, without the presence of lignin or hemicelluloses or other plant constituents. The number of purification treatments of bacterial cellulose is consequently reduced. Usually, only typical alkali solutions treatments using sodium hydroxide are used in order to remove impurities, such as remaining cell debris coming from the bacterial cellulose synthesis. This purification step has been found to improve some physical properties of bacterial cellulose sheets, such as thermal stability, thermo-mechanical and mechanical properties (George et al., 2005 and 2008; Gea et al., 2011). Another advantage of bacterial cellulose is that the energy involved in its production is very low, especially compared to the production of microfibrillated cellulose (MFC) which requires high energy consumption in order to convert cellulose fibres into cellulose nanofibres.
For some applications, such as wound dressings or acoustic diaphragms, BC sheets can be used without a matrix material. However for composite materials, additional processing, and sometimes chemical modification steps are required. Several preparation methods to prepare bacterial cellulose composites and nanocomposites have been proposed in the literature, and these will be discussed in Section 2.2.6.

2.1.3 Molecular Structure

Cellulose is a linear syndiotactic homopolymer made of D-anhydroglucopyranose units, linked together by $\beta$-$(1\rightarrow4)$-glycosidic bonds (Klemm et al., 1998). The chemical composition of cellulose was discovered in 1838 by Anselm Payen, a French chemist, as being $\text{C}_6\text{H}_{10}\text{O}_5$ (Payen 1838). Cellulose has a linear chain conformation and is formed by the elimination of water between two hydroxyl groups, resulting in the formation of a glycosidic bond or oxygen bridge (Klemm et al., 1998). The molecular structure of cellulose is reported in Figure 2.3.

![Molecular Structure of Cellulose](image)

**Figure 2.3 Molecular structure of cellulose. Reproduced with modifications from Klemm et al. (1998).**

As shown in Figure 2.3, cellulose has a hydroxyl-group-rich structure. Each anhydroglucose unit has a primary hydroxyl group (positioned at $C_6$) and two
secondary hydroxyl groups (positioned at C\textsubscript{2} and C\textsubscript{3}) associated with it. Owing to the presence of hydroxyl groups, cellulose can be chemically modified by various reactions e.g. esterification or etherification to name but a few examples (Klemm \textit{et al.}, 1998). The possible methods for chemical cross-linking of cellulose will be discussed in Section 2.1.6.

The position of the intramolecular bonds between O(3)–H and O(5’) and between O(2’)–H and O(6’) and the intermolecular bond between O(3) and O(6’)–H are reported in Figure 2.4.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{cellulose_hydrogen_bonds.png}
\caption{The intra and intermolecular hydrogen bonding occurring in natural cellulose. Reproduced with modifications from Klemm \textit{et al.} (1998). Black lines represent the covalent backbone structure of cellulose. Small black cercles represent oxygen atoms. Blue and red dotted lines represent respectively the inter and intramolecular hydrogen bonding.}
\end{figure}

The presence of hydroxyl groups also favours hydrogen bonding between cellulose polymer chains (intermolecular hydrogen bonding) and also within individual
polymer chains (intramolecular hydrogen bonding). Both intra and intermolecular hydrogen bonding impart stiffness to the cellulose macromolecule. Intramolecular hydrogen bonding contributes more to the axial stiffness of individual cellulose polymer chains while intermolecular hydrogen bonding imparts mechanical cohesion between cellulose polymer chains. Intermolecular hydrogen bonding may also occur between cellulose fibrils, which could explain the good mechanical properties of some cellulose films formed from these materials. This means that hydrogen bonding is involved at multiple scales. It is also the reason why cellulose cannot be dissolved but why it also swells in most common solvents.

2.1.4 Crystal Structure

Natural cellulose is a semi-crystalline biopolymer meaning it is composed of both crystalline and amorphous regions. The crystalline fraction is the predominant phase in natural cellulose and also contributes, in addition to hydrogen bonding, to its high stiffness. The crystalline elastic modulus of cellulose I has been estimated to be ~138 GPa (Sakurada et al., 1962; Nishino et al., 1995). A quantification of the crystalline fraction or ratio in a natural cellulose sample is commonly called the crystallinity. A common approach to determine this is to use powder X-ray diffraction (Segal et al., 1959). Other techniques such as CP/MAS C$^{13}$ solid-state NMR (Atalla and Vanderhart 1984) and Fourier-Transform IR spectroscopy (Nelson and O'Connor 1964) are also relevant tools.

The crystalline structure of natural cellulose, also called cellulose I, has been one of the most studied subjects in polymer science. Whatever its source, cellulose has the same chemical composition, but can differ in terms of crystal structure and crystallinity. The crystal structure of cellulose I was first reported by Nishikawa
and Ono (1913). The first model for the unit cell of cellulose I was proposed by Meyer and Mark (1929) (see Figure 2.5).

![Unit cell of cellulose I according to the Meyer-Misch model (Klemm et al., 1998).](image)

**Figure 2.5** Unit cell of cellulose I according to the Meyer-Misch model (Klemm et al., 1998).

This model assumed a monoclinic unit cell with two antiparallel cellobiose chain segments along the fibre axis. This has been later confirmed by Meyer and Misch (1937). One practical use of this model is the calculation of the dimensions of the cellulose unit cell as reported in Table 2.

**Table 2.2** Unit cell dimensions of various cellulose allomorphs. Reproduced from Krässig (1993) and Klemm et al. (1998).

<table>
<thead>
<tr>
<th>a-axis (Å)</th>
<th>b-axis (Å)</th>
<th>c-axis (Å)</th>
<th>Lattice angle γ (°)</th>
<th>Cellulose polymorph</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.85</td>
<td>8.17</td>
<td>10.34</td>
<td>96.4</td>
<td>cellulose I</td>
</tr>
<tr>
<td>9.08</td>
<td>7.92</td>
<td>10.34</td>
<td>117.3</td>
<td>cellulose II</td>
</tr>
<tr>
<td>9.9</td>
<td>7.74</td>
<td>10.3</td>
<td>122</td>
<td>cellulose III</td>
</tr>
<tr>
<td>7.9</td>
<td>8.11</td>
<td>10.3</td>
<td>90</td>
<td>cellulose IV</td>
</tr>
</tbody>
</table>
The Meyer-Misch model is, however, obsolete now since it has been found later that cellulose I has a two chain parallel structure.

Gardner and Blackwell (1974) proposed that cellulose I has a monoclinic lattice with two parallel chains. Later in 1984, the crystal structure of natural cellulose was investigated using solid-state carbon-13 nuclear magnetic resonance. The investigators found that cellulose I is a composite of two distinct crystal forms; namely cellulose I$_a$ (triclinic) and cellulose I$_β$ (monoclinic). They mentioned that the presence of two distinct forms of cellulose I was independent of the cellulose source (Atalla and Vanderhart 1984). They also noticed that the proportion of I$_a$ and I$_β$ allomorphs may vary depending on the cellulose source (Atalla and Vanderhart 1984). Cellulose I$_a$ is more dominant in algae and bacteria, whilst cellulose I$_β$ is more dominant in plants like ramie (Atalla and Vanderhart 1984). The existence of two natural cellulose crystalline forms was confirmed later using TEM in combination with electron diffraction (Sugiyama et al., 1991). They reported a triclinic unit-cell having a single cellulose chain with a P$_1$ space group with a = 6.74 Å, b = 5.93 Å, c = 10.36 Å, α = 117º, β = 113º and γ = 81º for cellulose I$_a$ and a monoclinic unit-cell two-chain monoclinic P2$_1$ unit cell with a = 8.01 Å, b = 8.17 Å, c = 10.36 Å, α = 90º, β = 90º and γ = 97º for cellulose I$_β$.

Other crystalline forms of cellulose exist, called regenerated celluloses, such as celluloses II, III, IV (Marchessault and Sarko 1967). Some of these forms of cellulose can be occasionally found in nature. Cellulose II can for instance be produced by some bacteria (Bureau and Brown 1987). Regenerated cellulose fibres, such as rayon, are obtained by dissolving cellulose in an appropriate solvent and then by regeneration through wet spinning.
As reported in Table 2.2, the unit cell dimensions of other cellulose crystalline forms differ from cellulose I, which can influence the bulk properties of cellulose. In this section, only the natural form of cellulose is discussed because bacterial cellulose is the form used in this study.

### 2.1.5 Mechanical Properties

One of the physical properties that makes natural cellulose an attractive material to be used as reinforcement for composites is its good mechanical properties. Young’s modulus of cellulose nanofibrils from various sources has been estimated on the nano and micro scales using Raman spectroscopy, X-ray diffraction and a cantilever AFM method. For instance Young’s modulus of bacterial cellulose nanofibrils, microcrystalline cellulose, tunicate and cotton nanowhiskers has been estimated using Raman spectroscopy and found to be respectively 114, 25, 143 and 57-105 GPa (Eichhorn and Young 2001; Šturcová et al., 2005; Hsieh et al., 2008; Rusli and Eichhorn 2008). Using a cantilever AFM technique, Young’s modulus of bacterial cellulose nanofibrils has been estimated to be 78 ± 15 GPa (Guhados et al., 2005). The elastic modulus of crystalline regions of cellulose I was determined using X-ray diffraction (Sakurada et al., 1962). A value of 137 GPa was found for bleached ramie (Sakurada et al., 1962). This value was also confirmed later using purified ramie (Nishino et al., 1995). The elastic modulus of crystalline regions of cellulose I was estimated by subjecting a ramie fibre under tensile deformation. At each tensile deformation increment, an X-ray pattern was recorded. Typical shifts towards a lower diffraction angle for several reflections e.g. (002) and (004) were observed. Then the lattice spacing at each tensile deformation increment was calculated using the Bragg’s law (1913) which is defined as
with \( n \) is a positive integer and corresponds to the number of wavelengths, \( \lambda \) is the wavelength of the X-ray source, \( c \) is the lattice spacing of the material’s crystal structure and \( \theta \) is the angle between the initial X-ray wave and the scattering planes.

The crystal deformation of cellulose I can be calculated using the formula

\[
\varepsilon = \frac{\Delta c}{c_0} \times 100
\]  

(2.2)

with \( \varepsilon \) the crystal deformation and \( c_0 \) and \( \Delta c \) are respectively the initial and the change in lattice spacing. The elastic modulus was then calculated using the formula

\[
E = \frac{\sigma}{\varepsilon}
\]

(2.3)

where \( E \) and \( \sigma \) are respectively the elastic modulus of crystalline regions and the stress in the crystalline regions. The stress in the crystalline regions is supposed to be equal to the stress applied to the sample.

The value obtained from X-ray diffraction is higher than the value obtained from Raman spectroscopy and AFM. X-ray diffraction takes into account only crystalline regions whereas Raman spectroscopy and AFM techniques take into account both amorphous and crystalline regions. So the values obtained using
Raman spectroscopy and AFM possibly reflect an average of the contribution of both crystalline and amorphous regions.

For various reasons, Young’s modulus values obtained at a macroscale are largely inferior compared to values obtained at a nano or microscale, but still compare well to most polymers. Typical values for Young’s modulus of bacterial cellulose sheets are in the range of 10 up to 30 GPa (Yamanaka et al., 1989; Nishi et al., 1990; Hsieh et al., 2008).

2.1.6 Chemical Cross-Linking

As mentioned in Section 2.1.3, cellulose has a hydroxyl group-rich structure which allows it to be chemically modified. Acetylation is one of the most utilised ways to modify cellulose for the mass production of cellulose acetate. Cross-linking is used in the textile industry to chemically modify cellulose (Klemm et al., 1998). Chemical cross-linking of cellulose is particularly useful to impart good mechanical properties in the wet state. When cellulose is exposed to water, its intermolecular and possibly its intramolecular hydrogen bonding is disturbed due to competitive hydrogen bonding formation between hydroxyl groups of the polymer and hydrogen bonding formation between hydroxyl groups of cellulose chains and water molecules (Klemm et al., 1998). Consequently the mechanical properties of cellulose decrease. But if cellulose polymer chains have been previously cross-linked, then covalent bonds, which are not affected by the presence of water, substitute hydrogen bonding and so the mechanical properties can be preserved.

The physical properties of chemically cross-linked cellulosic materials depend on the cross-link density, constitution and length distribution of cross-links in the cellulose structure. This depends on the nature of the cross-linker utilised, and the
chemical reaction parameters. It is also important to note that when heterogeneous cross-linking modification is performed, the number of accessible hydroxyl groups is limited due to the high crystallinity of cellulose (Krässig 1993); thus limiting the cross-linking density for instance.

Cellulose can be inter and intramolecularly cross-linked by covalent and/or ionic reactions. The main routes for these cross-linking reactions to occur have been cited by Klemm et al., (1998) and are:

- Recombination of cellulose macro-radicals formed chemically or by irradiation.
- Reaction of anionic cellulose derivatives by metal cations.
- Oxidative cross-linking by formation of disulphide bridges.
- Formation via urethane bridges by reaction of hydroxyl groups with isocyanates.
- Crosslinking via ester groups formed by reaction with polycarboxylic acids
- Formation of ether bonds with a multifunctional etherification agent.

The latter cited route is the most utilised scientifically and industrially and has been used in this study through the chemical cross-linking of bacterial cellulose networks with glyoxal. These molecules can form either or both acetal and hemiacetal bonds via reaction of dialdehydes with hydroxyl groups belonging to cellulose chains. Acetal bonds are a particular type of the ether bond family. The selected cross-linking agents have the advantage of being produced either from fossil or from renewable resources. More details on the cross-linking agent used and on the cross-linking chemical reaction will be given in Chapter 6.
2.2 Natural Cellulose Produced by Bacteria

2.2.1 Introduction

Natural cellulose produced by bacteria is referred to as bacterial cellulose (BC). It is an extra-cellular product which was discovered by Brown (1886 and 1886). He described BC sheets as being "very tough, especially if an attempt is made to tear it across its plane of growth". Brown renamed what he firstly called the "vinegar plant" as "bacterium xylinum". These bacteria are able to produce, under specific conditions (Schramm and Hestrin 1954), an almost pure form of cellulose, chemically identical to the cellulose produced by plants and tunicates. Several genera from different family of bacteria namely Acetobacter, Enterobacter, Rhizobium, Agrobacterium and Sarcina, to cite but a few examples, are all cellulose producers (Brown 1989; Jonas and Farah 1998). Acetobacter xylinum, now reclassified as Gluconacetobacter xylinum, are by far the best known species. Bacterial cellulose is also famous for being extensively used as a raw material for nata-de-coco, an indigenous dessert food of the Philippines (Lapuz et al., 1967; Iguchi et al., 2000).

2.2.2 Biosynthesis

Bacterial cellulose is produced by specific genera of bacteria in specific conditions through a biological process called biosynthesis, where carbon compounds such as glucose, hexoses or glycerol are converted into cellulose. Acetobacter xylinum has been used as a model bacterium in order to understand how bacterial cellulose is synthesised. It is a multi-step process (at least four steps) where enzymes, catalysts and precursors play a very important role. A simplified version of this bioprocess is reported in Figure 2.6.
The carbon compound, which can be glucose for example, is firstly converted into glucose-6-phosphate by the enzyme gluconase. This is followed by the isomerisation of glucose-6-phosphate into glucose-1-phosphate by the enzyme phosphoglucomutase. Then glucose-1-phosphate is converted into uridine diphosphate-glucose-1-phosphate (UDPG) by the enzyme pyrophosphorylase. In *Acetobacter xylinum*, this enzyme is activated by cyclic nucleotide (c-di-GMP). The cellulose synthase activator c-di-Guanosine monophosphate (c-di-GMP) is synthesised in *Acetobacter xylinum* by the enzyme diguanylate cyclise. Its concentration is regulated by the action of phosphodiesterases. Finally, UDPG is polymerised into cellulose by an enzyme called cellulose synthase.

The cellulose synthase enzyme, a nucleotide, plays a key role in the synthesis of cellulose and its production is regulated at the genetic level. Analysis of the nucleotide sequence has indicated that the bacterial cellulose synthesis (*bcs*) operon (functioning unit of genomic DNA containing a cluster of genes) is 9217 base pairs long and is composed of four genes, *bcsA*, *bcsB*, *bcsC* and *bcsD* (Ross *et al.*, 1991).
The bcsA gene is thought to initiate the polymerisation of UDPG into cellulose (Ross et al., 1991). The bcsB gene is thought to be an activator-binding subunit (Ross et al., 1991). The bcsC and bcsD genes are positioned outside of the surface of the membrane (Ross et al., 1991). They are thought to play a decisive role in both the crystallisation and extrusion of the cellulose. Results from genetic tests such as gene disruption analyses established that bcsA, bcsB, bcsC and bcsD genes are necessary to maximise bacterial cellulose production in Acetobacter xylinum (Ross et al., 1991).

BC synthesis is a rather quick process which enables bacteria to produce cellulose in a relatively short time. It has been reported that a single bacterium cell can polymerise up to 200,000 glucose molecules per second (Schramm and Hestrin 1954; Ross et al., 1991). Schramm and Hestrin (1954) were the first to introduce the use of a culture medium to control and optimise BC synthesis. They used a typical aqueous culture medium composed in (%, w/v) of glucose 2.0, bactopeptone (Difco) 0.5, yeast extract (Difco) 0.5 at an initial pH of 6.0 (Schramm and Hestrin 1954). These aerobic bacteria are able to convert glucose, fructose and other carbon compounds into cellulose through a complex biological process that we have previously described in this Section (Ross et al., 1991). The synthesis of cellulose in Acetobacter xylinum occurs between the outer membrane and the cytoplasmic membrane by a cellulose-synthesising complex which is in association with pores at the surface of the bacterium (Jonas and Farah 1998). Actually one can consider these bacteria as "biological extruders" (Jonas and Farah 1998). Figure 2.7 illustrates how cellulose molecules, synthesised inside the bacterium, are spun out through some sort of "biological dyes", also called cellulose export components (Iguchi et al., 2000). The protofibrils have diameters of ~1.5 nm (Jonas and Farah
1998), which then form ribbon-shaped-like cellulose fibrils having dimensions of ~80×4 nm (Brown 1989).

**Figure 2.7** Schematic illustration of BC biogenesis and fibril formation. Reproduced with modifications from (Brown 1989). Not to scale.

BC can either be produced under static or agitated conditions, as illustrated in Figure 2.8. In static culture a pellicle of BC is formed at the air/liquid interface and gets thicker day after day, creating a more or less layered structure. These layers have been described as mats of overlapping and intertwisted cellulose nanofibres (Jonas and Farah 1998). Only the bacteria, in contact with oxygen, produce cellulose, while others are in a sort of "sleeping state" (Iguchi et al., 2000). This means that if all the bacteria could be in contact with oxygen, the yield of BC production could be much higher. This is the reason why agitated cultures were later introduced.
BC cultured in agitated conditions is a more attractive process from an industrial point of view. The oxygen is directly supplied into the culture medium so bacteria do not need to reach the surface to find oxygen. This means that all the bacteria present in the culture medium can be better supplied with oxygen, and therefore produce higher yields of BC (Vandamme et al., 1998). The macroscale morphology of BC cultured in agitated conditions is different from that cultured in static conditions. It has been described as irregular granules or pellets, homogeneously dispersed in the culture medium (Bielecki et al., 2005). This form of BC also shows differences in its nano/microscale morphology. For instance BC nanofibres cultured in static cultures have a diameter of ~100 nm, whereas agitated cultures allow the production of BC nanofibres with diameter of ~200 nm. They also exhibit differences in terms of crystallinity, crystallite size and cellulose I\textsubscript{α} content leading to different physical properties (Watanabe et al., 1998). Both static and agitated conditions, for now, do not provide sufficient production yields of BC, but further improvements are still possible.
In this study only BC networks cultured in static conditions were used, so the following sections will only deal with this form of BC.

### 2.2.3 Structure and Morphology

The chemical composition of cellulose is similar whatever its source. BC has similar chemical composition when compared to plant or tunicate cellulose. It has, however, different macromolecular structure, physical properties and morphology e.g. microfibril dimensions (see Table 2.3).

**Table 2.3 Range of microfibril diameters of various cellulose samples. Reproduced from Fink et al. (1990) and Klemm et al. (1998).**

<table>
<thead>
<tr>
<th>Material</th>
<th>Microfibril diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial cellulose</td>
<td>4-7</td>
</tr>
<tr>
<td>Cotton linters</td>
<td>7-9</td>
</tr>
<tr>
<td>Ramie</td>
<td>10-15</td>
</tr>
<tr>
<td>Dissolving pulp</td>
<td>10-30</td>
</tr>
<tr>
<td>Valonia cellulose</td>
<td>10-35</td>
</tr>
</tbody>
</table>

The proportion of cellulose I\textsubscript{a} and I\textsubscript{b} also differs between cellulose sources (Atalla and Vanderhart 1984). As described in Figure 2.7, BC nanofibrils, also called ribbons, are an assembly of protofibrils having a width of ~1.5 nm (Jonas and Farah 1998). Their length has been reported to be ~10 µm (Yamanaka et al., 1989); it is however rather difficult to measure the length of these fibrils since they form a network and the beginning and the end of a single nanofibril is not obvious to determine. The widths and thicknesses of BC nanofibrils have also been reported to be in the range 70 - 133 nm and 3 - 4 nm respectively (Bielecki et al., 2005).
BC nanofibrils do not form a linear structure but present some sort of "three-way branching points" along their length forming a network (Yamanaka et al., 1989). This type of structure, offers specific physical properties to BC networks, and is unique and different to plant and tunicate cellulose fibrils.

Figure 2.9 presents a comparison between the structure of BC microfibrils and the "fringed micelle", which was proposed for the structure of plant cellulosates.

![Image](image.png)

**Figure 2.9** Schematic model of BC microfibrils on the right drawn in comparison with the "fringed micelles" of plant cellulose on the left (Iguchi et al., 2000).

One can see that the structure of BC microfibrils is even more highly oriented than for plant cellulose. This might explain why BC and plant cellulose have different mechanical properties. This is an example of highly aligned microfibrils from nature which is what is sometimes aimed at, for instance during fibre spinning where high fibre alignment is targeted.
2.2.4 Mechanical Properties

BC produced in static conditions has a layered sheet-like structure. In the literature, the mechanical properties of BC are almost exclusively reported for sheets. One of the first values obtained for Young’s modulus of BC sheets was 15 GPa, which is really high for 2 dimensional (2D) organic materials (Yamanaka et al., 1989). A maximum value of 30 GPa was obtained for treated BC sheets (with 5 % NaOH and NaClO) (Nishi et al., 1990). However a rather unexpected value as low as 130 MPa has also been reported (Astley et al., 2003). More recently Young’s modulus of BC sheets cultured for 3 days was reported and found to be \( \sim 10 \) GPa (Hsieh et al., 2008). With such good mechanical properties, the mechanical reinforcement of polymeric resin materials is potentially good, opening the possibility to use BC sheets as reinforcement for composite applications. A summary of work already done will be discussed in Section 2.2.6.

A very recent work has shown that a two-step purification process improved significantly the structural, mechanical, thermal and morphological behaviour of BC sheet (Gea et al., 2011). An alkali treatment, using 2.5 weight % (wt.%) of NaOH, was found to improve significantly the mechanical properties of BC sheets; an increase of Young’s modulus, tensile strength and strain at failure was observed (Gea et al., 2011). A second treatment using 2.5 wt.% of NaOCl led to further mechanical properties improvements. Finally the authors obtained a value of \( 18.8 \pm 2.0 \) GPa for the Young’s modulus for two-step treated BC sheets. To conclude, values of \( 7.6 \pm 1.2 \) GPa, \( 14.1 \pm 1.6 \) GPa and \( 18.8 \pm 2.0 \) GPa were measured for respectively untreated BC, single-step and double-step treated BC sheets.

More delicate direct measurements of Young’s modulus of BC filaments have been reported. A value of \( 78 \pm 17 \) GPa has been measured using an AFM cantilever
method for a range of BC nanofibres diameters (Guha et al., 2005). More recently, Raman spectroscopy has been used to estimate the Young’s modulus of BC filaments. A value of 114 GPa has been reported (Hsieh et al., 2008).

2.2.5 Applications of Bacterial Cellulose

BC finds applications in various domains (Klemm et al., 2006). It is used in healthcare and veterinary medicine owing to its biocompatibility. Commercial products have been developed for wound dressing, temporary skin (Fontana et al., 1990) and connective tissue replacement (Curti et al., 1989; Klemm et al., 2001; Bodin et al., 2007; Bodin et al., 2007). Some current research has focused on the healing effect of BC for human and animal medicine (Cockbill and Turner 1995; Goodrich et al., 2000; Czaja et al., 2006). BC has also been used as a scaffold material for cell cultivation in tissue engineering (Svensson et al., 2005). BC also finds applications in the food industry. As said previously, BC is found in nata-de-coco, an indigenous food from the Philippines. It is also used as an emulsifying agent in meatballs, referred to as kung-wan (Lin and Lin 2004) and as a clarification agent (filter) for fruit juices (Krystynowicz et al., 2000). BC can also be added to ice-cream, where it plays the role of stabiliser to resist meltdown and heating during storage (Okiyama et al., 1993). BC finds also applications in the electronics sector as biosensors, optical devices for screens and displays, as shown in Figure 2.10 (Shah and Malcolm Brown 2005; Yano et al., 2005) and as an acoustic diaphragm for loudspeakers and headphones (Nishi et al., 1990). The paper industry has also been interested in BC for producing high strength paper (Hioki et al., 1995; Basta and El-Saied 2009; Cheng et al., 2011). Two Japanese
companies (Ajinomoto Co. and Mitsubishi Paper Mills) are currently working on the development of microbial cellulose for paper products (patent JP 63295793).

**Figure 2.10** Luminescence of an organic light-emitting diode deposited onto a transparent BC nanocomposite (Nogi and Yano 2008).

It is important to add that since BC finds so many applications, one needs to increase the yield of production through genetic selection of bacteria, and to also develop more efficient processes to culture BC on an industrial scale. This has already been discussed for BC cultured in agitated culture (Watanabe *et al.*, 1998). Up to now, the industrial production of BC still requires development and this will require close collaboration between biologists and materials scientists.

In this study, BC was used as reinforcement to produce bio-composites, thanks to its bio-origin and excellent mechanical properties.

### 2.2.6 Bacterial Cellulose for Composite Application

Some of the physical properties of biopolymers *e.g.* thermoplastics including polylactide (PLA) or polyhydroxyalcanoate (PHA), are not yet sufficient to match the properties of polymers made from oil resources. One possibility is to enhance
the properties of biopolymers by associating them with a reinforcement phase. The final product is then called a composite or a nanocomposite material. Cellulose-containing natural materials (natural fibres) or cellulose in its almost pure form (nanocellulose), is a material of choice to be used as a reinforcement in a composite material especially when environmentally friendly aspects are targeted, coupled with high performance with low weight penalties. Cellulose has good mechanical properties, as discussed in Section 2.2.4, and is therefore an excellent candidate to reinforce either petroleum-based or bio-based thermoplastics or thermosets polymers. Even more important is the excellent specific mechanical properties of cellulose e.g. Young’s modulus and tensile strength divided by the density of cellulose (Eichhorn et al., 2010). This is particularly relevant to design light weight composite materials. The density of crystalline cellulose is \( \sim 1.5 \text{ g.cm}^{-3} \) while other materials such as steel and glass having respectively a density of \( \sim 7.8 \text{ g.cm}^{-3} \) and \( \sim 2.5 \text{ g.cm}^{-3} \) (Ashby and Jones 1989). Steel and glass cannot compete with the specific mechanical properties of cellulose. Furthermore the use of cellulose is even more relevant for the design of bio-composites, where both reinforcement and matrix have to be entirely biodegradable and made from renewable resources. As already mentioned in Section 2.1.2, they are several reasons for choosing BC as a reinforcement over other sources, with the main reasons being their high purity, good mechanical properties as well as energetic and environmental reasons. The following paragraph describes several methods that have been proposed to design BC composites and nanocomposites.

Transparent BC nanocomposites have been designed by vacuum impregnation of solvent-treated BC networks with acrylic resin or epoxy resin (Yano et al., 2005; Nogi and Yano 2008). The authors did not give much detail about the impregnation
procedure, given that its application was commercially sensitive. Further property improvement was obtained by the same group, who chemically modified BC nanofibres through acetylation (Ifuku et al., 2007). They reported, for a specific acetylation degree, a higher transparency and reduced hygroscopicity by one third, over untreated composites. The physical properties of cellulose-containing composites and nanocomposites are known to be sensitive to moisture (Berglund and Peijs 2010) and new methods are always welcome to overcome this problem.

BC can also be used as a natural fibre interfacial agent for composite materials. This has been investigated by depositing nanosized BC on the surface of natural fibres such as sisal fibres for interface improvement with green resins (Pommet et al., 2008). The authors reported an improvement of the tensile strength when natural fibres were coated with BC and, especially when polylactide (PLA) is used as a matrix material (Juntaro et al., 2007). An increase of interfacial sheer stress between BC-coated sisal fibres and PLA was also reported (Juntaro et al., 2008).

Another example of the use of BC by the same research group is the design of porous PLA nanocomposites, forming three-dimensional (3D) scaffolds for tissue engineering application (Blaker et al., 2010). The authors reported that they can produce these materials by combining an ice-microsphere templating technique with a thermally induced phase separation (TIPS) technique in the presence of BC nanowhiskers. They mention that they can control the pore structure, interconnects and surface area in these materials, which could lead to better expansion of cell cultures in this form of 3D-environment-scaffold material compared to scaffolds designed using only a TIPS process.

All-cellulose nanocomposites have been proposed as a way to avoid the sorting of material types during recycling. All-BC nanocomposites have been prepared by
surface selective dissolution of BC pellicles (Soykeabkaew et al., 2009). After immersion of BC pellicles in lithium chloride/N,N-dimethylacetamine (Li/DMAc) for different times, all-BC nanocomposites with different ranges of optical and mechanical properties were obtained. An immersion time of 60 min allowed an impressive increase of both the work of fracture and the transparency. A similar approach has been used to produce all-aramid composites by partial dissolution of aramid fibres (Zhang et al., 2010). The main drawback of this method is the use of expensive and non-environmentally friendly solvents. Since the matrix and the reinforcement phase are the same, one does not need to separate materials for different waste streams, so recycling and disposal is facilitated. A potential drawback is that both the matrix and the reinforcement are made from BC, which is for now is considered to be an expensive material. This is because of the low yield of the bacterial process. The price of dry-based BC has been reported to be ~30 US dollars/kg (Iguchi et al., 2000). The German company FZMB GmbH prices wet-based BC (94 wt.% of water) as ~5 euros/kg.

Another method that has been proposed to prepare BC/PLA nanocomposites, uses a method referred to as thermally induced phase separation (TIPS), followed by a conventional extrusion process (Lee et al., 2009). This group reported an increased in the mechanical properties over neat PLA, and non-modified BC/PLA nanocomposites, when BC is modified with lauric acid. It is important to add that the modification of BC as well as the nanocomposite preparation was very time consuming and also involved the use of costly and non-environmentally chemicals. A positive point is the use of an extrusion process, which is interesting in terms of industrially scaled and conventional composite production. BC/poly(ε-caprolactone) nanocomposites have also been prepared by mixing particulate and
fibrous freeze-dried bacterial cellulose (PBC and FBC respectively) with PCL using a *melt-compound process* (Gea *et al*., 2010). Both PBC and FBC showed a significant improvement of the mechanical properties over PCL. Nanocomposites prepared from FBC nanofibres showed higher tensile strength and strain at break than the ones prepared with PBC. This result was attributed to the higher aspect ratio of FBC nanofibres (Gea *et al*., 2010).

*Solvent casting methods* are another way to produce BC nanocomposites. BC sheets are immersed in a polymer solution for several hours and then dried to evaporate the solvent. BC/cellulose acetate butyrate (CAB) composites have been produced by solvent evaporation casting (Gindl and Keckes 2004). The authors performed cyclic tensile loading–unloading experiments at incremental strain levels. They observed an increase in the elastic modulus, when increasing the number of tensile loading–unloading cycles. They attributed this to the reorientation of initially randomly oriented BC nanofibres. BC/PLA nanocomposites were also produced using a solvent evaporation casting method (Kim *et al*., 2009). After evaporation of the chloroform, the authors obtained transparent BC/PLA nanocomposites. The authors reported an increase in the mechanical properties of PLA after incorporation of BC nanofibres. Young’s modulus and the tensile strength were respectively increased by 146 % and 203 % respectively. A similar approach was used to produce maleated BC/PLA composites (Li *et al*., 2010). Non-modified BC and BC modified with maleic anhydride sheets were immersed for 24 hours in a solution containing 10 wt.% of PLA in chloroform. After solvent evaporation, some of the samples were further hot-pressed in a compression moulder. This further processing step was found to
increase the mechanical properties of the materials. Also maleation of BC resulted in a significant enhancement of the mechanical properties.

Another recent work involving a solvent casting method and the design of composites for artificial blood vessel applications has been recently reported (Brown et al., 2011). BC pellicles were immersed in a bovine fibrin/ionic liquid/dimethyl sulfoxide solution. The authors performed mechanical tests and reported a significant change in ultimate tensile stress and cyclic creep strain level when varying the fibrin/BC weight percentage ratio.

In-situ methods have been recently proposed for the preparation of BC-containing nanocomposites or composites. This method can be used when the polymer matrix is soluble in the culture medium of Acetobacter xylinum, which is basically water. BC composites have been prepared by adding xyloglucan or pectin in the culture medium of Acetobacter xylinum. BC composites and BC alone were deformed using a tensile tester and analysed using small-angle X-ray scattering (Astley et al., 2003). This allowed the reorientation of cellulose microfibrils to be followed during tensile deformation. The presence of xyloglucan or pectin was found to not affect the reorientation process (Astley et al., 2003). Astley et al. (2003) also proposed a theoretical model in order to predict reorientation behaviour of BC nanofibrils in these composites. An in-situ method has been also used to produce BC/polyvinyl alcohol nanocomposites (Gea et al., 2010). Polyvinyl alcohol (PVA) was introduced directly in the culture medium of Acetobacter xylinum. These so-called "in-situ" nanocomposites were characterised and compared to nanocomposites made by immersion of BC sheets in an aqueous PVA solution. Mechanical testing revealed an expected decrease of Young’s modulus of BC when PVA is added. Higher strain at failure and work of fracture were also
obtained. The *in-situ process* allowed the preservation of the high tensile strength of BC, while nanocomposites made from an impregnation process (solvent casting) showed a significant decrease of the stress at failure. Another interesting effect was the transparency of the nanocomposites, even with a small amount of PVA added; respectively 3.7 and 1.4 % for "impregnated" and "in-situ" nanocomposites. Also one of the advantages of "in-situ" preparation over the "impregnation" method is that it reduces the preparation time of the nanocomposites, and consequently the cost, since only one preparation step is required. The authors also suggested that the presence of PVA around BC nanofibres disrupts the hydrogen bonding between BC nanofibres. The presence of fibre/fibre hydrogen bonding can be beneficial for mechanical properties of the nanocomposites, but at such high weight fractions of BC (96.3 and 98.6 %) the percolation ratio is likely to be reached, so BC fibre/fibre interactions may still occur. This would need to be demonstrated experimentally. A further possibility to reduce the cost of this material would be to optimise the weight fraction of BC, if controllable, and determine the percolation ratio.

BC/poly(ethylene oxide) (PEO) composites have also been designed using this "in-situ" method. The polymer was added, in different quantities, to the growth medium of *Acetobacter xylinum* to vary the composition of the composites. The authors observed a good dispersion of BC nanofibres in the polymer matrix which hindered, along with the presence of debris cells, its crystallisation. The melting point and crystallinity of the matrix was found to be lower when compared to the neat resin. An increase of the degradation temperature peak and the tensile storage modulus of poly(ethylene oxide) was also observed, especially above 50 °C.

The main methods to prepare BC composites and nanocomposites have been presented. They can also be used when other source of cellulose fibres or
nanofibres are considered. The design of model BC laminated composites using compression moulding has never yet been reported. This could present some advantages over previously cited methods e.g. quick design, BC weight fraction control through the control of the layer thicknesses, composite process industrially scalable using a lamination process and full advantage of cellulose fibre/fibre hydrogen bonding interaction without the necessity to reach a percolation ratio. Opaque or transparent composites could be designed by varying the culturing time/thickness of BC networks. A further advantage of designing laminated composites is the possibility to easily detect the Raman signals belonging to cellulose, which are useful for later molecular deformation studies. This is achievable in a composite material containing ~ >15 wt.% of cellulose, but signals can be detected even at low concentrations, such as 3-4 wt.%. For cellulose nanocomposites where BC nanofibres are dispersed in the matrix, a cellulose content of at least 15 wt.% is necessary. This will be shown in Chapter 4.

2.3 Raman Spectroscopy

2.3.1 Introduction

Raman spectroscopy, like infra-red spectroscopy, belongs to the family of vibrational spectroscopic techniques. This non-destructive technique is widely used for the chemical and physical analysis of materials. Chemical bonds and functional groups are usually either or both Raman and infra-red active, depending on the symmetry or asymmetry of molecules or moieties. For example a molecule of water will show a strong infra-red signal, but a low signal will be measured using Raman spectroscopy; meaning that water is considered an infra-red active molecule. Consequently Raman spectroscopy allows the study water-containing
samples, without interference with other bands in the spectrum. Raman spectroscopy is able to detect moieties that are not detectable using infra-red spectroscopy. In other words, Raman and infra-red spectroscopic techniques give complementary information about the chemical structure of a substance. Raman spectroscopy is based on a change of polarisability due to bond vibrations whereas infra-red spectroscopy is based on a change of dipole moment.

The Raman spectroscopic technique is mainly used to both identify and quantify bonds and functional groups in a substance. Other applications are the examination of the morphology of materials, and recently this technique has been successfully applied to the micromechanical deformation of polymer fibres and composites (Young and Eichhorn 2007). The following sections contain information taken primarily from Ferraro, Nakamoto and Brown (2003) and also from Smith and Dent (2005).

### 2.3.2 The Raman Scattering Effect

The existence of inelastic scattering of photons was first postulated in 1923 by the Austrian physicist Adolf Smekal. He proposed that photons could be inelastically scattered by vibrational transitions of molecules (Smekal 1923). This phenomenon has been then experimentally observed for the first time in 1928 by two Indian scientists, Sir C.V. Raman and K.S. Krishnan (Raman and Kirshnan 1928; Raman and Kirshnan 1928). In their experiments they focused sunlight on either a purified liquid or a dust-free vapour sample using a telescope and a second lens. In order to detect the modified scattered radiation, they used the method of complementary light-filters. They observed, in addition to the elastic component of the radiation (Rayleigh scattering), a modified or inelastically scattered radiation, with an altered
frequency. They further discovered that this effect was more or less strong depending on the sample tested (some substances contain more or less Raman active moieties). In other words, when a solid, a liquid or a gas, is exposed to a monochromatic source of light, a fraction of the scattered radiation has a modified frequency. This effect is now called the \textit{Raman Effect}.

Some of the first substances to be studied were benzene, toluene and carbon tetrachloride (Raman and Krishnan 1929). The first Raman spectra reported for polymers were obtained by Signer and Weiler (1932) and then by Gehman and Osterhof (1936). They respectively obtained spectra for polystyrene and rubber. The use of Raman spectroscopy at that time was quite difficult. Many years later, owing to technological advances of excitation sources, optics and detection systems, Raman spectroscopic techniques have become more widely used, allowing additional in-depth structural information about polymer materials to be discovered.

A diagram of the Rayleigh and Raman scattering processes is reported in Figure 2.11. When an incident laser source, composed of photons (elementary light particles) interacts with a substance, the photons can either be absorbed, scattered or may not interact (they transmit through the substance). Incident photons have an initial energy and frequency, and if their electric field interacts with the outer electrons of the substance’s molecules, the photons energy is transferred to the target molecules according to the equation:

\[ \Delta E = h\nu_0 = h\frac{c}{\lambda_0} \]  

(2.4)
where $\Delta E$ is the energy difference between the ground state and the excited states, $h$ is Planck’s constant ($6.62 \times 10^{-34}$ J s) and $\nu_0$ is the frequency of the incident photons, $c$ is the speed of light ($\sim 3 \times 10^8$ m s$^{-1}$) and $\lambda_0$ is the wavelength of the incident light source.

\[ E = h \nu_0 \]

**Figure 2.11** Diagram of the Rayleigh and Raman scattering processes with $m$ and $n$ representing the vibrational or ground states of the molecule. Reproduced with modifications from Smith and Dent (2005).

If scattering occurs, incident photons are predominantly elastically scattered. This phenomenon is called the Rayleigh scattering, where incident and scattered photons have similar vibrational frequencies. The target molecule returns to its initial energy state. At the same time, a fraction of the incident photons are inelastically scattered. Most of the inelastically scattered photons have a lower frequency than the incident photons, and this is referred to as Stoke’s scattering. At the end of the interaction, the target molecule is promoted to a higher energy state. Sometimes the molecules are already in an excited state, and as soon as the photons are scattered, the molecule returns to the ground state. In that case the photons are scattered at a higher frequency than the incident photons. This is called anti-Stoke’s
scattering. Both Stoke’s and anti-Stoke’s scattering phenomena are more generally referred to as Raman scattering.

Two theories can be used to describe the Raman scattering; namely the "classical theory" and the "quantum theory". These theories are described in Sections 2.3.3 and 2.3.4.

2.3.3 The Classical Theory of Raman Scattering

The classical theory of Raman scattering is based on the wave theory of light. The electric field strength \( E \) of the electromagnetic waves of the incident laser source propagates according to the equation

\[
E = E_0 \cos(2\pi v_0 t) \tag{2.5}
\]

where \( E_0 \) is the vibrational amplitude, \( v_0 \) is the frequency of the incident light and \( t \) is time. When the electric field of the incident light source interacts with a di-atomic molecule, a dipole moment \( P \) is generated due to its polarisability, and is expressed by the equation

\[
P = \alpha(q)E \tag{2.6}
\]

where \( \alpha(q) \) is a proportionality constant called the polarisability, and is a function of the nuclear displacement \( q \). If the initial vibration of the di-atomic molecule is referred to as \( v_m \), \( q \) is expressed by the equation

\[
q = q_0 \cos(2\pi v_m t) \tag{2.7}
\]
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where \( q_0 \) is the vibrational amplitude. For small amplitudes of vibration, \( \alpha \) is a linear function of \( q \), and can be re-expressed by the equation

\[
\alpha(q) = \alpha_0 + \left( \frac{\partial \alpha}{\partial q} \right)_0 q_0 + ...
\]  \hspace{1cm} (2.8)

where \( \alpha_0 \) is the polarisability at the equilibrium position and \( (\partial \alpha / \partial q) \) is the rate of change of \( \alpha \) with respect to the change in \( q \), evaluated at the equilibrium position.

By combining Equations 2.6, 2.7 and 2.8 one can obtain the equation

\[
P = \alpha_0 E_0 \cos(2\pi v_0 t) + \left( \frac{\partial \alpha}{\partial q} \right)_0 q_0 E_0 \cos(2\pi v_0 t) \cos(2\pi v_m t) = \alpha_0 E_0 \cos(2\pi v_0 t)
\]

\[
+ \frac{1}{2} \left( \frac{\partial \alpha}{\partial q} \right)_0 q_0 E_0 [\cos(2\pi(v_0 + v_m) t) + \cos(2\pi(v_0 - v_m) t)]
\]  \hspace{1cm} (2.9).

According to the "classical theory", the first term of Equation 2.9 represents an oscillating dipole that radiates light of frequency \( v_0 \) (Rayleigh scattering). The second term corresponds to the Raman scattering of frequency \( v_0 + v_m \) (anti-Stokes) and \( v_0 - v_m \) (Stokes). If \( (\partial \alpha / \partial q)_0 \) is zero, the vibration is not Raman-active. In other words, Raman scattering will occur if the rate of change of polarisability \( \alpha \) of the vibration is not zero.

The "classical theory" of Raman scattering is deficient in that it does not take into account the quantised nature of vibrations. In addition it is not able to explain as much about the relationship between molecular properties and Raman scattering as the quantum theory does.
2.3.4 Quantum Mechanical Approach of Raman Scattering

This section contains information from a review published by Tanaka and Young (2006). The quantum mechanical theory of Raman scattering has been introduced because the "classical theory" could not explain some phenomena. For instance, the intensity of Stokes Raman scattering has been discovered to be usually higher compared to the intensity of anti-Stokes Raman scattering. This observation disproves the rule of classical electromagnetic theory which states that the intensity of the light is proportional to the fourth power of its wavenumber (Bowley et al., 1989). Only the quantum mechanical approach can explain this behaviour.

The vibrational energy level \( \varepsilon_v \) for a harmonic oscillation can be expressed using the equation

\[
\varepsilon_v = (v + \frac{1}{2})\nu_m
\]  

(2.10)

which is a solution of the Schrödinger wave equation, where \( v \) is a vibrational quantum number. Energy transitions of \( v = 1, 2 \ldots \) are allowed during Raman scattering. The probability of the transition is higher for the \( v = 1 \) than other transitions as the \( v = 1 \) transition results in a small energy change. Normally, the initial state of the molecules is at the vibrational energy of the ground state \( (v = 0) \) except when the temperature is very high. The population of molecules with a vibrational energy \( \varepsilon_v \) can be elucidated using the Maxwell-Boltzmann distribution law. The equation
further describes the correlation of the Stokes and anti-Stokes scattering intensities with \( I \) the intensity, \( N \) the number of molecules with a vibrational energy, where \( \varepsilon_v \) and \( \varepsilon_{v'} \) are the energy levels of the molecule before and after transition respectively. Additionally, \( \nu_o \) is the frequency of the incident light, \( \nu_m \) is the frequency of the vibration and \( k \) and \( T \) are Boltzmann’s constant and temperature, respectively. At ambient temperatures, it is expected that the probability of the vibrational energy transition from \( \nu = 0 \) to \( \nu = 1 \) is high during the Raman scattering.

To summarise, the quantum theory tells us that photons are more likely to be scattered following the description of the Stoke Raman scattering, and that is why its intensity is higher compared to the intensity of anti-Stokes Raman scattering.

### 2.3.5 Polarised Raman Spectroscopy

This section contains information from journal articles published by Bower (1972 and 1975). Polarised Raman spectroscopy, as shown in Figure 2.12, is obtained by using a polarised laser source and by placing an analyser before the Raman scattering is detected.

As stated previously, Raman scattering can occur when a source of light interacts with a sample. The photons contributing to both the source of light and the Raman scattering are oscillating in all directions. Polarised Raman spectroscopy means that only the photons propagating in a particular direction are selected. In other words, when a monochromatic source of light interacts with a sample, a dipole moment \( P \) is generated. This dipole moment is dependent on the angle
between the electric vector of the light source and the magnitude of the polarisability. If both incident and scattered photons are selected according to the same direction then the molecular orientation of polymers can be studied.

\[ I \propto \cos^2 \theta \]  

(2.12).

Polarised Raman scattering can be obtained by using a polarised laser excitation and a polarisation analyser so as to filter the back-scattered light. The intensity is then expressed as

\[ I \propto \cos^2 \theta \]  

(2.12).

**Figure 2.12** Experimental arrangement used for polarised Raman spectroscopy measurements. Reproduced from Smith and Dent (2005).
\[ I \cos^2 \theta \cos^2 \theta' \]  

where \( \theta' \) is the angle between the scattered light and the analyser polarisation directions (Bower 1972 and 1975). When the incident and scattered lights are both polarised in the sample axis direction, \( \theta' = \theta \) and the intensity can be expressed as

\[ I \cos^4 \theta \]  

These equations will be defined in more detail in Chapter 4, describing particular examples of polarisation configurations; commonly called "VV" and "VH" configurations. Polarised Raman spectroscopy has been used in Chapter 4.

### 2.3.6 Polarisation Configurations

It is possible to use various polarisation configurations when using Raman spectroscopy. For this purpose, a half-wave retardation plate and a polariser analyser were inserted in the path of the laser within the Raman spectrometer before the back-scattered photons were detected. This had the effect of rotating the back-scattered light polarisation in a specific direction. When the polarised photons from the laser enter in interaction with a material, its electric field interacts with the electronic cloud associated with its molecules. A dipole moment, as described in Section 2.3.3, is induced. The photons constituting the Raman component of the scattered radiation, after interaction of the laser with the sample, are radiated in all directions, with no specific polarisation. This means that if no polariser analyser is inserted in the Raman spectrometer, the spectrum that one obtains is representative
of photons vibrating with no specific polarisation direction. This configuration is
often denoted as "VN" and was used in Chapters 3, 5, 6 and 7.

If a polariser analyser is inserted before the detector, then only the Raman
scattered photons aligned parallel to the electric vector of the incident light will be
detected (see y-axis in Figure 2.13). In other words, it means that both the laser and
the back-scattered light are oriented in the y-axis direction. This polarisation
configuration is denoted as "VV", where the Raman spectra obtained will contain
information from cellulose chains, within fibrils, aligned in the polarisation
direction. This polarisation configuration was used in Chapter 4 to study the
orientation of cellulose nanofibrils in flax fibres and BC networks.

A second half-wave plate, in addition of the polariser analyser, can be used to
rotate the vibration direction of the photons by 90° (see x-axis Figure 4.13). This
gives information on the cellulose fibrils oriented perpendicularly to the laser
polarisation. This configuration is often denoted as "VH". The different filter
arrangements or combinations corresponding to different polarisation
configurations are reported in Table 2.4 and in Figure 2.13.

Table 2.4 Different polarisation configurations in the Raman spectroscopic system.
Reproduced with modification from Kao (2008).

<table>
<thead>
<tr>
<th>Configuration</th>
<th>VN</th>
<th>VV</th>
<th>VH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident laser polarisation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>Out</td>
<td>Out</td>
<td>Out</td>
</tr>
<tr>
<td>Second</td>
<td>Out</td>
<td>Out</td>
<td>In</td>
</tr>
<tr>
<td>Polarisier analyser</td>
<td>Out</td>
<td>In</td>
<td>In</td>
</tr>
</tbody>
</table>
2.3.7 Cellulose Molecular Identification

Cellulose has been characterised for the first time using Raman spectroscopy in 1970. Material from the cell walls of the algae *Valonia ventricosa* were used (Blackwell *et al.*, 1970). A typical Raman spectrum is reported in Figure 2.14.

**Figure 2.14** Typical Raman spectrum obtained from unoriented *Valonia ventricosa* (Blackwell *et al.*, 1970).
This form of cellulose is highly crystalline, which allowed the authors to obtain well resolved spectra. They proposed the first vibrational assignment of Raman bands for cellulose (see Table 2.5).

**Table 2.5** Raman band assignments for Valonia ventricosa. Reproduced from Blackwell et al. (1970).

<table>
<thead>
<tr>
<th>Raman band (cm⁻¹)</th>
<th>Assignment</th>
<th>Raman band (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3398</td>
<td>O–H stretching</td>
<td>1432</td>
<td>CH₂ bending</td>
</tr>
<tr>
<td>3374</td>
<td>&quot;</td>
<td>1407</td>
<td>O–H deformation</td>
</tr>
<tr>
<td>3369</td>
<td>&quot;</td>
<td>1377</td>
<td>C–H deformation</td>
</tr>
<tr>
<td>3354</td>
<td>&quot;</td>
<td>1359</td>
<td>&quot;</td>
</tr>
<tr>
<td>3339</td>
<td>&quot;</td>
<td>1337</td>
<td>O–H deformation</td>
</tr>
<tr>
<td>3307</td>
<td>&quot;</td>
<td>1319</td>
<td>CH₂ wagging</td>
</tr>
<tr>
<td>3295</td>
<td>&quot;</td>
<td>1293</td>
<td>CH₂ twisting</td>
</tr>
<tr>
<td>3277</td>
<td>&quot;</td>
<td>1234</td>
<td>(Anti–symmetric ring mode)</td>
</tr>
<tr>
<td>3235</td>
<td>&quot;</td>
<td>1204</td>
<td>&quot;</td>
</tr>
<tr>
<td>2972</td>
<td>C–H stretching</td>
<td>1174</td>
<td>&quot;</td>
</tr>
<tr>
<td>2932</td>
<td>CH₂ anti–symmetric stretching</td>
<td>1152</td>
<td>C–O stretching, ring</td>
</tr>
<tr>
<td>2920</td>
<td>C–H stretching</td>
<td>1122</td>
<td>&quot;</td>
</tr>
<tr>
<td>2907</td>
<td>&quot;</td>
<td>1090</td>
<td>&quot;</td>
</tr>
<tr>
<td>2889</td>
<td>&quot;</td>
<td>1071</td>
<td>&quot;</td>
</tr>
<tr>
<td>2867</td>
<td>&quot;</td>
<td>1057</td>
<td>&quot;</td>
</tr>
<tr>
<td>2850</td>
<td>CH₂ symmetric stretching</td>
<td>1035</td>
<td>&quot;</td>
</tr>
<tr>
<td>1479</td>
<td>O–H deformation</td>
<td>997</td>
<td>&quot;</td>
</tr>
<tr>
<td>1454</td>
<td>&quot;</td>
<td>910</td>
<td>C(1)–H(β) deformation</td>
</tr>
</tbody>
</table>
Specifically they first reported a Raman band located at ~1090 cm$^{-1}$, and assigned it to C-O stretching ring modes. Another important advancement in the assignment of cellulose Raman bands is the work by Wiley and Atalla (1987) using a microprobe, which allowed new information to be discovered. In this study, they used fibres extracted from cell walls of *Valonia macrophysa* and ramie fibres. They reported that the Raman bands located at ~997, 1034, 1057, 1095, and 1123 cm$^{-1}$ must correspond to C-C and C-O stretching motions because these bands were the most intense when the electric vector of the incident light was parallel to the fibre axis (Wiley and Atalla 1987). This work confirmed that vibrational motions of C-O moieties in the backbone of cellulose contribute to the presence of the Raman band located at ~1095 cm$^{-1}$. Later on, another piece of work by Edwards *et al.* (1997) used several types of cellulose natural fibres (flax, jute, cotton, kapok, ramie...), revealing that a Raman band, this time reported to be located at ~1096 cm$^{-1}$, also corresponded to asymmetric vibrational motions of the C-O-C glycosidic bond (Edwards *et al.*, 1997).

A more recent work by Gierlinger *et al.* (2006) on single wood fibres has shown that the same Raman band, this time reported to be located at ~1097 cm$^{-1}$, is dominated by the stretching motions of C-O bonds within the backbone of the cellulose macromolecules. Gierlinger *et al.* (2006) also refer another work by Agarwal (1999) on softwood-cellulose using FT-Raman spectroscopy, where the C-C and C-O stretching within the backbone of cellulose is mentioned (Agarwal 1999). To summarise, C-C, C-O and C-O-C moieties are all likely to contribute to the presence of the Raman band located at ~1095 cm$^{-1}$. 
2.3.8 Cellulose Molecular Deformation

In addition to being a tool to identify the molecular material structure, Raman spectroscopy can also be used to study the molecular deformation or micromechanics of polymeric films and fibres. The first molecular deformation study using Raman spectroscopy was performed on monocrystalline fibres of polydiacetylene (Mitra et al., 1977). This study revealed that two Raman bands initially located at ~1498 and 2104 cm\(^{-1}\), respectively corresponding to the vibrational motions of C=C and C≡O moieties, shifted towards a lower wavenumber under the application of external tensile deformation. They concluded, after theoretical calculations in agreement with experimental data, that these shifts towards a lower wavenumber are due to bond anharmonicity (Mitra et al., 1977).

A review by Young (1995) reports a detailed explanation about the stress-induced Raman band shifts. The stress-induced change in the vibrational frequencies of Raman bands can be described in terms of the so-called Morse function (see Figure 2.15) using the equation

\[ U(\Delta r) = D[1 - \exp(-\eta\Delta r)] \]  
\[ (2.15) \]

where \( U \) is the potential energy, \( \Delta r \) is the displacement of the interatomic distance, \( D \) is the dissociation energy and \( \eta \) is a constant for a particular molecule.

The force constant of vibration \( F \) is given by the second derivation of equation 2.15 with respect to \( \Delta r \) and is expressed using the equation

\[ F = \frac{d^2U}{d^2\Delta r} = 2D\eta^2[2\exp(-2\eta\Delta r) - \exp(-\eta\Delta r)] \]  
\[ (2.16) \]
The force constant is a function of $\Delta r$, and is reduced when the interatomic distance is increased. This induces stress-induced Raman band shifts toward a lower wavenumber position when samples are submitted to external tensile deformation. This shift is proportional to the square root of the force constant.

![Figure 2.15](image_url)

**Figure 2.15 The Morse potential function for a C-O diatomic molecule.**

A new field of investigation was consequently opened and other polymeric fibres and materials were subsequently studied such as poly($p$-phenylene benzobisoxazole) fibres (Young et al., 1990), aramid fibres and composites (Andrews and Young 1993) and high modulus polymer fibres such as Kevlar®, Twaron® and Technora® (Yeh and Young 1999), to cite but a few examples.

The first investigation involving the use of Raman spectroscopy to investigate the molecular deformation of cellulose fibres was done by Hamad and Eichhorn (1997). This work was done on regenerated cellulose fibres (cellulose II) - then marketed as Tencel® fibres. They showed that Raman bands initially located at $\sim$895 and 1095 cm$^{-1}$ shifted towards a lower wavenumber upon the application of a
macroscopic external tensile strain or stress. This shift was attributed to the molecular deformation of cellulose chains. It is important to note that the high intensity Raman band initially located at ~895 cm$^{-1}$ is specific to cellulose II fibres. A similar approach was used to study the molecular deformation of hemp and flax fibres (Eichhorn et al., 2000; Eichhorn et al., 2001) and then on regenerated cellulose fibres (Cordenka®, Enka® and Lyocell®) (Eichhorn et al., 2001). This allowed a close comparison of the deformation mechanisms of cellulose I and II fibres (Eichhorn et al., 2001). These studies also revealed that the Raman band initially located at ~1095 cm$^{-1}$ shifts towards a lower wavenumber under the application of external tensile strain (Eichhorn et al., 2000). Further investigation showed that the Raman band shift rate of the Raman band initially located at ~1095 cm$^{-1}$ of cellulose I and II fibres is proportional to the modulus of the fibre (Eichhorn et al., 2001); this makes it a stress dependent effect. Invariant shift rates with stress were also noted (Eichhorn et al., 2001). Consequently, the structure of natural cellulose fibres was modelled using a modified series aggregate model (uniform stress), as shown in Figure 2.16. This model has been developed to explain the elastic response of highly oriented fibres (Northolt and Hout 1985).

This study by Eichhorn et al. (2001) also reported studies of the deformation mechanisms of cotton paper and pine wood using Raman spectroscopy. The study of pine wood showed that it behaves like a composite material, with cellulose fibrils acting as reinforcement (load-bearing component); since the Raman band initially located at ~1095 cm$^{-1}$ shifted towards a lower wavenumber. The Raman band initially located at ~1600 cm$^{-1}$ belonging to lignin was found not to shift, meaning that lignin acts like a matrix and has no load-bearing role in wood. Investigation of cotton paper revealed that the deformation mechanism is separated
into three phases: elastic deformation (linear shift of the Raman band initially located at $\sim 1095 \text{ cm}^{-1}$), fibre debonding with full non-linear relaxation of the stress and then complete fracture of the sample (Eichhorn et al., 2001).

Figure 2.16 Schematic diagram of (a) modified series aggregate model and (b) possible physical structure of a semicrystalline cellulose fibre (Eichhorn et al., 2001).

Raman spectroscopy has been further used to investigate the micromechanics of wood materials in the form of spruce latewood fibres (Gierlinger et al., 2006). In this work, they studied in-situ molecular deformation and orientation under tensile deformation. They also showed that it is possible to limit the effect of relaxation phenomena for the purposes of micromechanical studies. As already reported by Eichhorn et al. (2001), the Raman band initially located at $\sim 1095 \text{ cm}^{-1}$ was found to shift towards a lower wavenumber, whereas the Raman band located at $\sim 1600 \text{ cm}^{-1}$ did not shift significantly towards a lower wavenumber, again highlighting the respective role of cellulose and lignin in woody materials. A shift rate of $-6.1 \text{ cm}^{-1}$
GPa$^{-1}$ was found for the Raman band initially located at 1095 cm$^{-1}$ which was a bit higher than the values of -4.5 cm$^{-1}$ GPa$^{-1}$ reported by Eichhorn et al. (2001). Another interesting observation was the return to the original Raman band position after fibre failure, which is an indication of the elastic nature of the deformation mechanism. Additionally, a reduction of the intensity ratio of the Raman bands located at ~1127 and ~1097 cm$^{-1}$ was observed under increasing tensile strain. This has been related to a widening of the torsion angle of the glycosidic C-O-C bonding.

In addition to cotton paper, the micromechanics of another type of cellulose network, bacterial cellulose (BC), has been recently investigated using Raman spectroscopy. These networks, made of nanosized fibres, were deformed using a customised tensile rig, and the Raman band initially located at ~1095 cm$^{-1}$ found again found to shift towards a lower wavenumber. A Raman band shift of -1.8 cm$^{-1}$ %$^{-1}$ was reported for these materials, which is a bit higher than the value of 1.5 ± 0.2 cm$^{-1}$ %$^{-1}$ measured for cotton paper (a similar network of cellulose fibres) (Eichhorn et al., 2001). From this work, Hsieh et al. (2008) also estimated the Young’s modulus of single BC filaments, based on the Raman band shift rate of BC networks, and a universal calibration obtained for aligned cellulose I fibrils (Eichhorn et al., 2001). They also used a relationship derived by Krenchel (1964) for the stress-transfer efficiency of a network of fibres. However, experimental effects such as the culturing time of BC, the laser polarisation direction, tensile deformation angle and back-scattered polarisation configurations (polarised Raman spectroscopy) were not taken into account. In Chapter 4, work will be presented that takes into account these parameters.
Other natural fibres have more recently been investigated, such as coir and celery (Bakri and Eichhorn 2010). These fibres exhibit high strain to failure, compared to say flax or hemp fibres. For this reason, coir and celery fibres could be used for composites with higher toughness (Bakri and Eichhorn 2010).

This section has reported that the micromechanics of natural and regenerated cellulose fibres, wood, cotton paper, BC networks, to cite but a few examples, can be investigated using Raman spectroscopy. The influence of the culturing time and chemical cross-linking modification on the micromechanics of BC networks has never been reported before, and this is what will be reported in Chapters 5 and 6.

2.3.9 Cellulose Molecular Deformation in Composites

The first use of Raman spectroscopy to probe the interface between a polymer fibre and a polymer matrix was achieved on a polydiacetylene fibre/epoxy resin composite (Galiotis et al., 1983). This pioneering study eventually led to the investigation of microcrystalline cellulose, natural fibres, cellulose nanowhiskers or cellulose networks embedded in polymer matrices (epoxy resin, polyester, polyvinyl acetate, polylactide or even cellulose). Eichhorn and Young (2001) have reported a study of the micromechanical properties of microcrystalline cellulose/epoxy resin composites using a 4-point bending deformation rig. They managed to estimate Young’s modulus of microcrystalline cellulose by following the shift towards a lower wavenumber of the Raman band initially located at ~1095 cm\(^{-1}\). They obtained a Raman band shift rate of \(-0.8 \pm 0.1\) and \(-0.9 \pm 0.3\) cm\(^{-1}\) \%\(^{-1}\) for respectively smeared and thin film composites. This led to a value of 25 ± 4 GPa for Young’s modulus of microcrystalline cellulose. They also concluded that microcrystalline cellulose is not such a good reinforcing agent compared to other
sources of cellulose such as flax and hemp fibres. One reason for this was the relatively low degree of crystallinity of microcrystalline cellulose. Another reason may be the low aspect ratio of many of the particulate forms of microcrystalline cellulose.

The same authors reported the micromechanical properties of hemp/epoxy resin composites (Eichhorn and Young 2004). Microdroplets of epoxy resin were formed around hemp fibres in order to study the stress-transfer from the epoxy resin to the hemp fibres. At first, they studied the deformation of single hemp fibres in order to obtain calibration data, obtaining the relationship between Raman band shift and both strain and stress. Then different levels of tensile deformation were applied to the hemp fibres surrounded by droplets of epoxy resin. Raman spectra were then recorded along this model composite, and the Raman band positions of the band initially located at ~1095 cm$^{-1}$ were determined and then converted into strain and stress levels using the calibration obtained from single hemp fibres. Finally the authors obtained both strain and stress mapping in the hemp/epoxy resin composite for several levels of tensile deformation. They also determined the interfacial shear stress along the composite using a simple force balance approach (Eichhorn and Young 2004). A few years later, the stress-transfer between regenerated cellulose fibres and epoxy and regenerated cellulose fibres and polyester was evaluated, following the same Raman spectrometric method (Mottershead and Eichhorn 2007). The authors proposed two types of model composites; namely droplet-fibre and thin flat film-fibre geometries. The latter allowed the authors to get rid of optical distortion coming from the curvature of the droplet fibre geometry. Film-fibre geometries seem, however, to give rise to residual stress effects which may disrupt the interface during composite preparation. Additionally, it was not possible
to conclude whether the regenerated cellulose fibre/epoxy interface is significantly stronger or weaker than the regenerated cellulose fibres/polyester interface.

Following these works on the micromechanics of microcrystalline cellulose and natural cellulose fibres composites, the reinforcement efficiency of cellulose nanowhiskers has also been evaluated using Raman spectroscopy. Tunicate cellulose nanowhiskers having high aspect ratio, surface area and crystallinity, have been firstly investigated after embedment in an epoxy resin (Šturcová et al., 2005). The Raman band, from tunicate nanowhiskers, initially located at ~1095 cm\(^{-1}\) could be detected through the transparent epoxy resin which allowed the authors to follow the shift of this Raman band during deformation of the sample using a 4-point bending rig. A Raman band shift rate of -2.4 cm\(^{-1}\) %\(^{-1}\) was obtained which led to an estimation of 143 GPa for the Young’s modulus of single tunicate cellulose whiskers, by first considering the nanowhiskers to have a two-dimensional (2D) random orientation. This was verified by measuring the intensity of the Raman band located at ~1095 cm\(^{-1}\) as a function of the sample rotation angle, which was found not to vary significantly. Cotton cellulose whiskers/epoxy resin nanocomposites were then investigated, and a Raman band shift rate of -0.9 cm\(^{-1}\) %\(^{-1}\) was found (Rusli and Eichhorn 2008). This value is much lower than the one obtained from tunicate nanowhiskers, possibly because cotton nanowhiskers have a lower aspect ratio and also a lower crystallinity compared to tunicate nanowhiskers and not because they actually have a lower modulus. An estimate of the Young’s modulus of cotton whiskers was found to be in the range 57 - 105 GPa, considering the nanowhiskers to have respectively a 2D and 3D orientation distribution.

Another study by Rusli et al. (2010) revealed information about the stress-transfer process between tunicate nanowhiskers and polyvinyl acetate. The authors
found that the Raman band shift rate was dependant of the orientation of the cellulose nanowhiskers, which were found to be sometimes highly aligned and sometimes randomly oriented depending on the area chosen on the sample. They also found that environmental conditions, such as high temperature (above the glass transition temperature of the matrix), and extreme humidity could suppress the stress-transfer process from the matrix to the cellulose nanowhiskers. Another recent work by Rusli et al. (2011) has again shown the relevance of Raman spectroscopy to probe interfacial properties of whisker cellulose nanocomposites. They have shown that tunicate whiskers having higher aspect ratio induce higher stress-transfer efficiency compared to lower aspect ratio cotton nanowhiskers. They have also reported that it is possible to suppress the stress-transfer process by controlling the surface charge of cellulose nanowhiskers using different acid hydrolysis chemicals.

Another recent study by Pullawan et al. (2010) reports the use of Raman spectroscopy applied to the micromechanical investigation of all-cellulose nanocomposites. Microcrystalline cellulose was dissolved and used as the matrix material, while cotton cellulose nanowhiskers were used as reinforcement. This study revealed that in addition to following the stress-transfer from the matrix to the reinforcement by following the shift of the Raman band initially located at \(~1095\,\text{cm}^{-1}\), it is also possible to follow the matrix molecular deformation by following the shift towards a lower wavenumber of the Raman band initially located at \(~895\,\text{cm}^{-1}\).

After careful consideration of the available literature about the study of the micromechanical properties of cellulose-based composites and nanocomposites using Raman spectroscopy, it is concluded that investigations have mostly been
performed on either natural fibre composites or on cellulose nanowhiskers embedded in polymer matrices. The micromechanical properties of model laminated cellulose bio-composites, particularly from bacterial sources have never been investigated using Raman spectroscopy and this is what is reported in Chapters 5 and 7.

2.4 References


3.1 Objectives

It is necessary to verify whether the intensity and the wavenumber position of the Raman band located at \( \sim 1095 \text{ cm}^{-1} \) belonging to cellulose are influenced by the extended exposure of bacterial cellulose networks (BC) and composites to the laser. This will give a clear indication if the reported shifts in this position, and the change in intensity with rotation, are functions of molecular deformation and orientation respectively. The recording of both intensity and laser position have been taken at fixed and random laser spot positions on the samples. BC networks were then deformed in tension to verify if the shift towards a lower wavenumber of the Raman band initially located at \( \sim 1095 \text{ cm}^{-1} \) is due to data scattering or due to molecular deformation. This has also been performed at fixed and random laser spot positions on the samples.

3.2 Materials and Methods

3.2.1 Materials

Prof. Hiroyuki Yano’s group from the Research Institute for the Sustainable Humanosphere at Kyoto University in Japan and Prof. Masaya Nogi (now at the Institute of Science and Industrial Research at Osaka University, Japan) produced and provided the BC networks. They used the following method to produce these networks.
CHAPTER 3

The biosynthesis of BC networks was carried out at a temperature of 27 °C in static conditions using *Gluconacetobacter xylinum* (no. 13693; National Institute of Technology and Evaluation, Tokyo, Japan) and Hestrin-Schramm medium (Hestrin and Schramm 1954; Schramm and Hestrin 1954). A chemical treatment was performed by boiling BC networks in a 2 % sodium hydroxide solution for 2 h. The BC pellicles, having diameters of ~35 mm, were subsequently washed with de-ionised water. The bulk water was then removed from the BC pellicles by compression at 2 MPa and 120 °C for 4 min. BC pellicles cultured for 6 and 18 days are respectively referred to as BC6 and BC18 in this Chapter.

PLA pellets (PLA L9000; density 1.25 g cm\(^{-3}\)) were supplied by Biomer® (Krailing Germany).

3.2.2 Experimental Methods

3.2.2.1 Sample Preparation

BC6 networks were cut into strips of ~1 mm in width using a razor blade. BC6 strips were then mounted onto 20 mm gauge length paper testing cards as shown in Figure 3.1.

*Figure 3.1 Schematic of a BC strip mounted on a paper testing card.*
At first, the samples were fixed on the testing cards using two small pieces of adhesive tape and then by using a slow curing two-part cold-curing epoxy resin (Araldite®, Huntsman, UK) to prevent slippage of BC strips during tensile deformation tests.

The epoxy resin was prepared by mixing, for 1 minute, an equal amount of resin and hardener, as recommended by the manufacturer’s datasheet (Huntsman, UK). Tensile deformation tests were performed at least 48 hours after the sample preparation. This sample preparation procedure was used for all materials undergoing tensile deformation testing.

3.2.2.2 Composite Preparation

A razor blade was used to cut BC18 networks into strips of ~1 mm width. PLA pellets were dried overnight at 40 °C and transparent films, approximately 160 µm in thickness, were prepared by compression moulding at a temperature of 180 °C and 12 MPa pressure. The samples were cooled down at cooling rate of ~55 °C min⁻¹. BC18 networks have been embedded in PLA also by compression moulding. These samples were prepared by taking BC18 networks strips which were then moulded between two PLA films again at a temperature of 180 °C and 12 MPa pressure. The samples were then mounted on paper testing cards as described in the previous Section.

3.2.2.3 Instrumentation

As already mentioned in Section 2.3.2, when the laser interacts with a sample, photons interact with its molecules. Several interactions can occur: absorption, transmission, emission and scattering (Rayleigh and Raman). The Raman scattered
light (photons) is then detected by the CCD (charge-coupled device) camera having passed through the optical microscope, several filters and mirrors. Since the CCD camera is typically connected to an acquisition system, and a computer equipped with processing software, Raman spectra can be obtained. Figure 3.2 reports a detailed illustration of the Renishaw® Raman spectrometer system 1000 used in this Chapter, and in Chapters 4, 5, 6 and 7.

Figure 3.2 Schematic of the Renishaw® Raman spectrometer optical system 1000 coupled to an optical microscope. Renishaw® Service Manual (1997). The dotted line indicates the laser pathway.

The Renishaw® Raman spectrometer system 1000 comprises the following components:

A: laser attenuation filter wheel (NDF wheel)

B: laser alignment mirror (first internal beam steering mirror)
Data reported in this thesis were obtained using a Raman system 1000 spectrometer, coupled with a 785 nm near-infrared laser if not mentioned. An Olympus BH-1 microscope with a ×50 long working distance objective lens was used to focus the laser beam on the surface of the samples to a spot size of ~1-2 µm. 100 % of the laser power was used which corresponds to ~26 mW at the sample surface. Raman spectra were recorded in the range of 1050 to 1150 cm$^{-1}$ using a high gain, an exposure time of 30 seconds and 4 accumulations. The intensities and the wavenumber positions of the Raman band initially located at
~1095 cm\(^{-1}\) were all determined using a mixed Gaussian/Lorentzian function and an algorithm based on the work of Marquardt (1963). An example of the fitting process is reported in Figure 3.3 which also defines the intensity and the wavenumber position of the Raman band located at ~1095 cm\(^{-1}\).

![Figure 3.3](image)

**Figure 3.3** A typical form of the Raman band located at ~1095 cm\(^{-1}\) obtained from a BC18 network, fitted using a mixed Gaussian-Lorentzian function and a linear baseline function.

### 3.2.2.4 Calibration

The calibration procedure has to be carried out prior any spectrum is recorded using the Raman spectrometer. It is performed to verify the accuracy and consistency of the recorded data. Silicon is generally used as a calibration substance and generates a sharp, well-defined Raman band located at 520 ± 1 cm\(^{-1}\) (see Figure 3.4).
Figure 3.4 A typical Raman spectrum showing the band located at ~520 cm\(^{-1}\) for a silicon standard, used for the calibration of the Raman spectrometer.

The calibration procedure was achieved using 100 % laser power (~26 mW at the sample surface), a high gain and an exposure time of 10 s with 1 accumulation. In order to determine accurately the peak position of the Raman band located at ~520 cm\(^{-1}\), a mixed Gaussian/Lorentzian function and an algorithm based on the work of Marquardt (1963) were used. The necessary calibration offset value was put into the software, if the peak position was higher or lower than 520 ± 1 cm\(^{-1}\). The procedure was then repeated in order to check if the peak position was in the 520 ± 1 cm\(^{-1}\) wavenumber range.

3.2.2.5 Effect of the Extended Laser Exposure and Spot Position on the Intensity

The effect of extended exposure to the laser on the intensity of the Raman band located at ~1095 cm\(^{-1}\) has been investigated with the Raman spectrometer configured in a "VN" configuration. Details on polarisation configurations are
given in Chapter 2, Section 2.3.6. It was important to know if the extended laser exposure could contribute or not to a change of Raman band intensity. The laser was simply focused on a BC18 network and Raman spectra were recorded at several time intervals corresponding to the acquisition time of a single Raman spectrum, an exposure time of 30 s with 4 accumulations (2 min). In total 36 spectra were recorded which corresponds to the number of spectra necessary to rotate a sample from 0 to 360° with steps of 5°. The effect of the laser spot position on the intensity of the Raman band initially located at ~1095 cm\(^{-1}\) was also studied. Raman spectra were recorded by moving the laser spot position randomly on the sample surface. The deformation axis of the sample was positioned parallel to the laser polarisation direction (y-axis direction as shown in Figure 3.5).

![Figure 3.5 A schematic of different polarisation configuration effects on the polarisation direction. Reproduced with modifications from Kao (2008).](image)

3.2.2.6 Effect of the Extended Laser Exposure and the Laser Spot position on the Raman Band Position

The effect of extended exposure to the laser on the position of the Raman band located at ~1095 cm\(^{-1}\) has been investigated using the Raman spectrometer configured in a "VN" configuration. This was important to know if the laser power,
and subsequent heating effects, can contribute or not to a change of the Raman band position. For that purpose, the laser was focused on a BC18 network and at the interface of a BC18/PLA composite. The Raman spectra were recorded at several time intervals corresponding to the acquisition time of a single Raman spectrum. The deformation axis of the sample was positioned parallel to the laser polarisation direction (y-axis direction as shown in Figure 3.5).

3.2.2.7 Raman Band Shift Statistical Measurements

Statistical measurements were performed to verify whether the shift towards a lower wavenumber of the Raman band initially located at \( \sim 1095 \text{ cm}^{-1} \) is due to tensile deformation or caused by scatter. Strips of BC6 networks were deformed with strain increments of 0.5 % using a customised deformation rig (Deben Microtest, Deben, Bury St Edmonds, UK) equipped with a 2 kN load cell. At each increment, 30 Raman spectra were recorded, firstly with the laser positioned at a fixed point, and then randomly along the sample. The Raman spectrometer was set in "VN" configuration with the deformation axis of the sample parallel to the laser polarisation direction (y-axis direction as shown in Figure 3.5).

3.3 Results and Discussion

3.3.1 Effect of the Extended Laser Exposure and the Laser Spot Position on the Intensity

Figure 3.6 reports a typical Raman spectrum for BC18 networks where the Raman band located at \( \sim 1095 \text{ cm}^{-1} \) is highlighted. This Raman band corresponds to C-O and C-O-C moieties in the cellulose backbone structure (Wiley and Atalla 1987; Edwards et al., 1997; Gierlinger et al., 2006). Figure 3.7 reports the effect of the extended exposure of the sample to the laser on the intensity of the Raman band
located at \( \sim 1095 \text{ cm}^{-1} \). This has been done by focusing the laser spot on a fixed position.

**Figure 3.6** Typical Raman spectra obtained from 300 to 1600 cm\(^{-1}\) for BC18 networks with the Raman band initially located at \( \sim 1095 \text{ cm}^{-1} \) highlighted.

**Figure 3.7** Effect of extended exposure of a sample to the laser and its position on the intensity of the Raman band located at \( \sim 1095 \text{ cm}^{-1} \) for a normalised intensity scale from 0.75 to 1.05 for BC18 networks.
One can see that extended exposure of the laser to a BC sample has a significant effect on the intensity of the Raman band located at ~1095 cm$^{-1}$, after 36 spectra have been acquired. This number of spectra corresponds to same number acquired when recording Raman spectra from 0 to 360° every 5° steps, and is at least 1h and 12 min of laser exposure time. This graph also shows that changing the laser spot position induces data scattering. For this reason, when the molecular orientation was investigated, a fixed laser position was chosen.

Figure 3.7 further shows the importance of attempting to always select the same sample area, because at random laser positions it would be impossible to observe a decrease of the normalised intensity as a function of the number of recorded spectra.

Figure 3.8 reports similar data but at a scale typically used when intensity changes are observed corresponding to oriented cellulose fibrils in a natural fibres such as flax. These data are reported in Section 4.3.2, and Figure 4.9. As a consequence the effect of the laser exposure on the intensity of the Raman band located at ~1095 cm$^{-1}$ can be neglected compared to the intensity change due to cellulose fibril orientation after 36 spectra have been acquired.
Figure 3.8 Effect of extended exposure of a sample to the laser and its position on the intensity of the Raman band located at ~1095 cm\(^{-1}\) for a normalised intensity scale from 0 to 1.1 for BC18 networks.

3.3.2 Effect of the Extended Laser Exposure and the Laser Spot Position on the Raman Band Position

Figures 3.9 and 3.10 report respectively the influence of the effect of the exposure of a BC18 networks and a BC18/PLA composite to the laser on the position of the Raman band initially located at ~1095 cm\(^{-1}\). It is clear that there is a significant effect after 36 spectra have been recorded with the Raman band being shifted towards a lower wavenumber. Furthermore, it is noted that changing the laser spot position induces some scattering. For this reason, a fixed laser position was used when investigating the molecular deformation of these materials, although it is always difficult to maintain the same position on the sample. No significant effect is, however, observed in the composite material since the data are scattering.
Figure 3.9 Influence of the extended exposure of the sample to the laser and its position on the wavenumber position of the Raman band initially located at ~1095 cm$^{-1}$ for a Raman band shift scale from -0.225 to 0.175 for BC18 networks.

Figure 3.10 Influence of the extended exposure of the sample to the laser and its position on the wavenumber position of the Raman band initially located at ~1095 cm$^{-1}$ for a Raman band shift scale from -0.225 to 0.175 for BC18/PLA composites.
Figure 3.11 shows the same data but at a scale typically used when Raman band shifts are observed for samples under tensile deformation, as it will be shown later in Section 4.3.3, and Figure 4.14.

![Graph showing Raman band shift](image)

**Figure 3.11** Influence of the extended exposure of the sample to the laser and its position on the wavenumber position of the Raman band initially located at ~1095 cm\(^{-1}\) for a Raman band shift scale from -2.0 to 0.2 for BC18 networks.

This shows that the Raman band shift due to the laser exposure is much smaller than Raman band shifts due to tensile deformation. Consequently the effect of the laser exposure on the Raman band shift of the Raman band located at ~1095 cm\(^{-1}\) can be neglected compared to the Raman band shift due to tensile deformation after 36 spectra have been acquired. Figure 3.12 reports similar data but for a BC18/PLA composite. As observed for BC18 networks, the effect of the laser exposure to the BC/PLA interface on the Raman band shift of the Raman band located at ~1095 cm\(^{-1}\) can be also neglected.
Figure 3.12 Influence of the extended exposure of the sample to the laser and its position on the wavenumber position of the Raman band initially located at \( \sim 1095 \text{ cm}^{-1} \) for a Raman band shift scale from -2.0 to 0.2 for BC18/PLA composites.

3.3.3 Raman band shift statistical measurements

Statistical measurements were performed on BC6 networks to verify whether the shift towards a lower wavenumber of the Raman band initially located at \( \sim 1095 \text{ cm}^{-1} \) is due to deformation, or whether it could be caused by scattering in the data. Figure 3.13 reports a typical Raman spectrum for BC6 networks where the Raman band located at \( \sim 1095 \text{ cm}^{-1} \) is highlighted. This Raman band has been found to shift towards a lower wavenumber under tensile deformation and is consequently particularly useful to study the micromechanical properties of cellulose containing materials (Eichhorn et al., 2010). A typical shift towards a lower wavenumber position for BC6 networks is reported in Figure 3.14. The origin of this shift is reported in details in Section 2.3.8.
Figure 3.13 A typical Raman spectrum in the range of 300 to 1600 cm$^{-1}$ for BC6 networks; the Raman initially located at ~1095 cm$^{-1}$ is highlighted.

Figure 3.14 A typical shift towards a lower wavenumber of the Raman band initially located at ~1095 cm$^{-1}$ upon tensile deformation for a BC6 network.

Figures 3.15 and 3.16 report a statistically significant shift towards a lower wavenumber of the Raman band located at ~1095 cm$^{-1}$. At a fixed laser spot
position (Figure 3.15) the shift is even clearer compared to when the laser spot is positioned at random positions (Figure 3.16). When the Raman spectra are acquired at random laser spot positions, a slight overlapping of the distributions of Raman band positions is observed. This result is not surprising since variability of the position of this Raman band is expected, given local strain variations. This slight overlapping might also be due to a stress relaxation effect occurring during experiments, affecting in particular the positions of bands acquired at the end of each collection period at each deformation point. The variability of the Raman band position, depending on the laser spot position, demonstrates the importance of always choosing the same spot position during a molecular deformation test.

![Graph showing distributions of Raman band position](image)

**Figure 3.15** Distributions of the position of the Raman band initially located at ~1095 cm⁻¹ peak, for readings taken at a fixed spot position at different levels of tensile deformation for BC6 networks.
Figure 3.16 Distributions of the position of the Raman band initially located at ~1095 cm\(^{-1}\) peak, for readings taken at random spot positions at different levels of tensile deformation for BC6 networks.

### 3.4 Conclusions

To conclude, exposure to the laser has been found to change the intensity and the position of the Raman band located at ~1095 cm\(^{-1}\). These changes have however been found to be much smaller than intensity and Raman band position changes due to cellulose fibril orientation and tensile deformation respectively. This was found to be true after at least 36 spectra were recorded; the same number of consecutive spectra taken during a rotation experiment. This means that significant changes in intensity and Raman band position are observed due to molecular orientation and deformation, and not due to data scattering during the exposure of BC networks to the laser.

Shifts in the position of the Raman band initially located at ~1095 cm\(^{-1}\), have been found to arise from tensile deformation, and not due to data scatter. Exposure to the laser was found to not significantly affect the position of the Raman band.
Initially located at ~1095 cm\(^{-1}\) in BC18 networks and BC18/PLA composites. The shift towards a lower wavenumber position of the Raman band initially located at ~1095 cm\(^{-1}\) when samples are deformed cannot be due simply to scatter in the data arising from other influences. Keeping the laser spot at a fixed position has shown that scattering of the data can be reduced.

### 3.5 References


CHAPTER 4

EFFECTIVE YOUNG’S MODULUS OF BACTERIAL CELLULOSE NANOFIBRILS USING RAMAN SPECTROSCOPY

4.1 Introduction

Fibres provided by nature such as flax, hemp, jute or ramie, are available to produce cheaper and lighter composites with higher specific mechanical properties compared to some oil and mineral-based composites (Eichhorn et al., 2010). Classic examples of traditional composites are glass fibre/polyester or glass fibre/epoxy composites. The high energy required to produce glass fibre is costly compared to the production of plant fibres. The use of natural fibres, however, has some limitations, such as dimensional and property inconsistencies, relatively low tensile strength and limited thermal stability of the fibres (Cheng et al., 2007; Tingaut et al., 2009; Eichhorn et al., 2010).

Micro or nano-sized cellulose fibrils or fibres are an alternative reinforcement for polymer resins, where variability is thought to be reduced by breaking down the larger scale structures of the plant. These cellulose fibrils can be combined with resins on their own, or with micron sized natural fibres to form hierarchical composites (Blaker et al., 2011). Terms and sizes of cellulose fibres according to terminology and morphology are reported in Table 4.1. They play an important structural role by giving necessary stiffness to plants. In addition to being extracted from the vegetal kingdom, cellulose fibrils can also be extracted from sea animals called tunicates (Ranby 1952) and are produced by some bacteria (Brown 1886). Before using these micro or nano-sized cellulose fibrils for man-made polymer composite applications, one needs to evaluate their physical properties to know
which form of cellulose fibrils offer the best reinforcement potential. New or improved characterisation methods are necessary in order to measure absolute property values such as Young’s modulus.

Table 4.1 Components of microfibrillated cellulose. Reproduced from Chinga-Carrasco (2011).

<table>
<thead>
<tr>
<th>Biological structure</th>
<th>Technological terms</th>
<th>Diameter (µm)</th>
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<td>Tracheid</td>
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</tbody>
</table>

A direct method to evaluate Young’s modulus of cellulose nanofibrils is to use an AFM cantilever method (Guhados et al., 2005). Another is to use Raman spectroscopy. Recently, Young’s modulus of single bacterial cellulose (BC) nanofibrils has been estimated using the Raman spectroscopic technique; a value of 114 GPa was obtained (Hsieh et al., 2008). Similarly the stiffness of cotton and tunicate cellulose nanowhiskers has been evaluated (Šturcová et al., 2005; Rusli and Eichhorn 2008). Tunicate cellulose nanowhiskers were found to be stiffer than cellulose nanowhiskers prepared from cotton. Raman spectroscopy has been more recently used to evaluate the effective Young’s modulus of carbon nanotubes (CNT) embedded in a polyvinyl alcohol (PVA) matrix (Deng et al., 2011). In this work, the authors demonstrated the relevance for taking into account polarisation configurations, carbon nanotube orientation and tensile deformation axis direction. These experimental parameters were not taken into account in the first evaluation.
of Young’s modulus of BC nanofibrils using Raman spectroscopy (Hsieh et al., 2008).

In this Chapter, the study of the influence of polarisation configurations, nanofibril orientation and specimen rotation angle on the stress-transfer properties of BC networks is reported. An improved method for the estimation of Young’s modulus of a single BC nanofibril is proposed. In addition, a method for evaluating Poisson’s ratio of BC networks is addressed by fitting experimental data with a theoretical model.

4.2 Materials and Methods

4.2.1 Materials

BC networks were prepared as described in Chapter 3, Section 3.2.1 with a culturing time of 18 days.

Flax fibres were provided by FH Reutlingen, Denmark. The steam exploded fibres were taken out from bundles and hydrogen peroxide treated for 48 h. This procedure is useful to lessen fluorescence from the laser.

4.2.2 Experimental Methods

4.2.2.1 Density Measurements

The density of BC networks was evaluated by measuring their thickness using a micrometer. Their diameter was determined using a calliper. The surface area of BC sheets was calculated by considering BC pellicles as perfect disks using the equation

\[ S = \pi R^2 \]  

(4.1)
with $S$ and $R$ being respectively the surface and the radius of BC pellicles. Each dimension measurement was performed 20 times on each sample, and the averages were used to calculate the volumes of the networks by multiplying the thickness of the networks by their surface area. A precision balance was used to weigh the BC pellicles. The density of BC networks was calculated using the equation

$$\rho = \frac{m}{V}$$

where $\rho$ is the density, $m$ is the weight and $V$ is the volume. Average density values and standard deviations were determined from 3 samples.

### 4.2.2.2 Field Emission Scanning Electron Microscopy (FEG-SEM)

BC networks were gold-coated at 40 mA for 4 min using a sputter coating process. Their surface morphology was subsequently imaged at a 4 kV acceleration voltage using a scanning electron microscope (Philips XL-30 FEG-SEM). BC nanofibrils' average diameters were determined from the measurements of 20 nanofibrils. Minimum and maximum values were used to obtain a range of fibril diameters.

### 4.2.2.3 Raman Spectroscopy

#### 4.2.2.3.1 Molecular Orientation of BC Networks and Flax Fibres

The molecular orientation in flax fibres and BC networks was investigated using the Raman spectrometer set in a "VV" configuration. Prior to rotating the samples, the central position of the sample was determined, and at each angle increment care was taken to check that the laser was always close to the same sampling area.
position. A rotation stage was used to rotate the samples from 0 to 90°. The experimental set-up used for this investigation is illustrated in Figure 4.1.

Figure 4.1 Experimental set-up used in this study to investigate the molecular orientation of flax fibres and BC networks.

Every 5° step, a Raman spectrum of the studied material was acquired. Normalisation of the intensities was performed by dividing the data by the intensity at 0° for BC networks. For flax fibres, normalisation of the intensities was carried out by dividing the data by the maximum intensity value. This maximum is thought to correlate closely with the microfibril angle of this fibre type and was found to be positioned at ~5°. Experiments were carried out three times for each set of samples and means are presented. The maximum standard deviation value associated with average normalised intensity data was found to be 0.3 and 0.2 respectively for flax fibres and BC networks. The orientation of BC networks has been also investigated
at 0 and 1.5 % tensile strain increments. This has been performed in the same previously cited conditions.

4.2.2.3.2 Molecular Deformation of BC Networks

The molecular deformation of BC networks was determined using a Raman system 1000 spectrometer, coupled with a 785 nm near-infrared laser. 100 % of the laser power was used, which corresponds to ~26 mW at the sample surface. The influence of polarisation configurations ("VV" and "VH"), the orientation of cellulose nanofibrils and the tensile deformation axis angle on the stress-transfer was investigated. A customised deformation rig (Deben Microtest, Deben, Bury St Edmonds, UK) equipped with a 2 kN load cell (see Figures 4.2 and 4.3) was used to deform BC networks. Strain was calculated from the displacement of the mobile clamp.

Figure 4.2 Experimental set-up used to investigate the molecular deformation of BC networks.
The samples were deformed from 0 up to 1% and rotated from 0 to 90° using 10° steps at each 0.1% tensile strain increment. Four accumulations and an exposure time of 30 s were utilised to record Raman spectra. Experiments were carried out three times for each set of samples and means and standard deviations are presented.

![Figure 4.3 A schematic representation of the Deben® rig.](image)

**4.3 Results and Discussion**

**4.3.1 Surface Morphology and Physical Properties of BC Networks**

Figures 4.4a and 4.4b report respectively FEG-SEM images of the surface of BC networks at magnifications of ×5000 and ×20 000. One can see that BC networks are made of cellulose nanofibrils also called nanoribbons as previously described in the literature (Yamanaka et al., 1989; Lee et al., 2009). Figure 4.5b indicates the laser spot diameter compared to the size of BC nanofibrils. The reason for this will be justified in Section 4.3.2. Table 4.2 reports some physical characteristics of BC networks.
Figure 4.4 Scanning electron microscope micrographs for BC networks (a) at a magnification of ×5000 and (b) at a magnification of ×20 000. The black circles indicate the laser spot diameter (~1-2 µm).

Table 4.2 Some physical properties of BC networks.

<table>
<thead>
<tr>
<th>Thickness (mm)</th>
<th>Density (g cm(^{-3}))</th>
<th>Diameter range (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.083 ± 0.005</td>
<td>1.1 ± 0.04</td>
<td>50-140</td>
</tr>
</tbody>
</table>

4.3.2 Molecular Orientation of BC Networks and Flax Fibres

Figure 4.5 reports typical Raman spectra for a flax fibre and a BC network. These spectra are characterised by the presence of a Raman band located at ~1095 cm\(^{-1}\) corresponding to the vibrational motions of C-O bonds and possibly C-O-C bonds in the backbone structure of cellulose (Wiley and Atalla 1987; Edwards et al., 1997; Gierlinger et al., 2006). This Raman band is sensitive to the local orientation of cellulose macromolecules constituting BC nanofibrils (Wiley and Atalla 1987; Rusli et al., 2010). The orientation of BC fibrils can be studied by rotating the specimen using a rotation stage and by recording a Raman spectrum in the region of 1050 to 1150 cm\(^{-1}\) at each angle increment using polarised Raman spectroscopy ("VV" configuration). This method has been used to study the orientation of
cellulose nanowhiskers in polyvinyl acetate-based composite materials (Rusli et al., 2010). Another use of the Raman band located at \( \sim 1095 \text{ cm}^{-1} \) is the study of the stress-transfer or micromechanics of cellulose-containing materials, but this will be discussed in Section 4.3.3.

Figure 4.5 Typical Raman spectra obtained in "VV" configuration in the range of 300 to 1600 cm\(^{-1}\) for flax fibres and BC networks. The Raman band located at \( \sim 1095 \text{ cm}^{-1} \) is highlighted. The spectra have been obtained for \( \varphi = 0^\circ \) to the laser polarisation axis.

Figure 4.6 reports typical Raman spectra for flax fibres at different specimen rotation angles, 0, 30, 60 and 90°. One can clearly see a significant change in the intensity of the Raman band located at \( \sim 1095 \text{ cm}^{-1} \). The study of the orientation of cellulose fibril orientation is based on the fact that the intensity of particular Raman band corresponding to specific moieties (C-O and C-O-C) in the cellulose structure depends on the orientation of cellulose chains within nanofibrils. Assuming that the cellulose chains are highly aligned in the nanofibrils and that they are aligned in the
polarisation direction, the intensity of the studied Raman band is maximised. When the cellulose chains are perpendicular to the polarisation direction, the intensity will be minimised. This arises due to the directions and the magnitudes of the Raman tensor components of different molecular vibrations coincident with the laser and back-scattered light polarisation (Tanaka and Young 2006). Such a change in the intensity of the Raman band located at ~1095 cm\(^{-1}\) is typical of high cellulose fibril alignment in a natural fibre (Bakri and Eichhorn 2010) or in a cellulose-containing composite materials (Rusli et al., 2010). As a consequence one can conclude that flax fibres contain highly aligned cellulose fibrils. This has been investigated so as to know the relationship between the intensity of the Raman band located at ~1095 cm\(^{-1}\) and the rotation angle of a fibre, containing cellulose I (analogous to a BC fibril).

![Figure 4.6 Typical Raman spectra obtained from flax fibres in the region of the Raman band located at ~1095 cm\(^{-1}\) using a "VV" laser polarisation configuration. Only angles of $\phi = 0, 30, 60$ and $90^\circ$ to the laser axis are presented here.](image-url)
Figure 4.7 reports similar curves to those reported in Figure 4.6, but now for BC networks. In this case, the intensity of the Raman band located at ~1095 cm\(^{-1}\) does not change significantly with the specimen rotation angle. This is because, even if the specimen rotation angle is changed, there are always BC nanofibrils oriented in the polarisation direction, and therefore cellulose chains within these fibrils. It is also important to note that this is also because the diameter of the laser spot (~1-2 μm) is much larger than the diameter of an individual BC fibril. If the laser spot size was of an equivalent size to a BC nanofibril, orientation effects would probably be observed. The laser spot diameter is compared to the BC fibril diameter in Figure 4.4b. It can be concluded that BC networks comprise randomly oriented nanofibrils, but this will be confirmed by plotting the detailed intensity obtained at each 5° angle increment.

**Figure 4.7** Typical Raman spectra obtained from BC networks in the region of the Raman band located at ~1095 cm\(^{-1}\) using "VV" laser polarisation configuration. Only angles of φ = 0, 30, 60 and 90° to the laser axis are presented here.
Each intensity value at a 5° rotation angle increment has been plotted as a function of the specimen rotation angle, as shown in Figure 4.8. One can clearly see the high alignment of cellulose chains along flax fibres, with the intensity at an angle offset of ~6° to the 0° axis. So the maximum intensity is not obtained when the flax fibre is strictly aligned in the polarisation direction. This means that the cellulose fibrils are not perfectly aligned with the main flax fibre axis. Similar results have also been obtained by an independent study on flax fibres (Bakri and Eichhorn 2010).

**Figure 4.8** The normalised intensity of the Raman band located at ~1095 cm$^{-1}$ as a function of the angle $\phi$ between the polarisation direction of the laser and the tensile deformation direction. The maximum standard deviation values associated with average normalised intensity data were found to be 0.3 and 0.2 respectively for flax fibres and BC networks.

This offset is thought to arise from the cellulose chains, or fibrils within the cell wall of the flax fibre, being at an angle to the axis of the fibre; the so-called microfibril angle. The angle between the main fibre axis and the cellulose fibril...
orientation direction was determined by fitting the experimental data using the equation

\[ I = a + b \cos^4(\varphi + \theta) \]  \hspace{1cm} (4.3)

where \( I \) is the intensity of the Raman band located at \( \sim 1095 \text{ cm}^{-1} \), \( a \) and \( b \) are constants corresponding to the derivatives of the polarisability tensors as a function of normal coordinates (Wiley and Atalla 1987). \( \theta \) is the microfibril angle and \( \varphi \) is the specimen rotation angle. The fitting of the experimental data was optimised for \( a = 0.08 \), \( b = 0.88 \) and \( \theta = 6.4^\circ \). This value of \( \theta \) is close to a value reported for a flax fibre of \( 9.6 \pm 2.5^\circ \) (Charlet et al., 2009).

It is clear from Figure 4.8 that nanofibrils within BC networks do not have any preferential alignment, confirming their random orientation. This result is in accordance with the SEM images reported in Section 3.3.1. Similar results concerning the random orientation of tunicate cellulose nanowhiskers embedded in a PVAc matrix have been obtained (Rusli and Eichhorn 2008; Rusli et al., 2010).

4.3.3 Molecular Deformation of BC Networks

As mentioned in Section 4.3.2, the Raman band initially located at \( \sim 1095 \text{ cm}^{-1} \) can be used to investigate the stress-transfer properties of cellulose-containing materials. A typical Raman spectrum for BC networks is reported in Figure 4.9. The shift of this particular Raman band can be monitored as a function of tensile deformation. A typical shift towards a lower wavenumber of the Raman band initially located at \( \sim 1095 \text{ cm}^{-1} \) is reported in Figure 4.10 for a BC network in "VV"
configuration. Similar shift has been also observed when using a "VH" configuration.

This shift towards a lower wavenumber position is typical for cellulosic material deformed in tension, and has already been reported previously in the literature. The shift in this peak position is related to the molecular deformation of the cellulose backbone (Eichhorn et al., 2001; Bakri and Eichhorn 2010; Rusli et al., 2010). These shifts arise due to the fact that when nanofibrils are deformed in tension, the interatomic distance is altered, and so the polarisability of C-O and C-O-C moieties present in the backbone structure of cellulose I is also altered. Detailed shifts for BC networks obtained in "VV" and "VH" configurations are reported in Figure 4.11 as a function of tensile strain.

Figure 4.9 Typical Raman spectra obtained from BC networks in the region of the Raman band located at ~1095 \( \text{cm}^{-1} \), using "VV" and "VH" polarisation configurations at \( \phi = 0^\circ \) to the laser polarisation axis.
Figure 4.10 A typical shift in the wavenumber position of the Raman band initially located at ~1095 cm\(^{-1}\) for a BC network subjected to tensile deformation at \(\phi = 0^\circ\) to the laser polarisation axis using a "VV" configuration.

Figure 4.11 Detailed shifts in the wavenumber position of the Raman band initially located at ~1095 cm\(^{-1}\) as a function of tensile deformation for BC networks at \(\phi = 0^\circ\) to the laser polarisation axis ("VV" and "VH" configurations).
Raman band shift rates with respect to strain of $S(0)_{vv} = -1.1 \pm 0.1 \text{ cm}^{-1} \text{ %}^{-1}$ and $S(0)_{vH} = -0.8 \pm 0.1 \text{ cm}^{-1} \text{ %}^{-1}$ have been obtained when the tensile deformation axis is oriented at $\phi = 0^\circ$ to the polarisation direction of the laser. As previously mentioned, it is possible to use several polarisation configurations when using Raman spectroscopy to investigate the molecular deformation of materials. The laser can be polarised as well as the back-scattered light. These different configurations can be very useful, for instance, for studying the orientation of cellulose fibrils in composites and networks. Polarisation configurations can sometimes help to enhance the detection of the Raman band initially located at $\sim 1095 \text{ cm}^{-1}$ in composite materials having low cellulose content.

Study of the stress-transfer mechanisms that may occur in cellulose-containing materials does not only depend on the polarisation configurations selected. In addition there are other factors that need to be taken into account, such as fibril and tensile deformation axis directions. The relationship between these factors is described in Figure 4.12 and their influence on the stress-transfer has been studied experimentally. The experimental data are then explained using a theoretical model previously used to study the influence of these experimental parameters on the stress-transfer occurring in carbon nanotube/PVA nanocomposites (Deng et al., 2011).
Figure 4.12 Schematic of a single fibril at angles between the fibril direction and strain axis ($\theta$), the tensile deformation direction and the laser polarisation axis ($\phi$), and the fibril direction and the laser axis ($\alpha$) in a 2D plane of a cellulose network.

Figure 4.13 reports the influence of the tensile deformation direction ($\phi$) on the Raman band shift rate for BC networks at $\phi = 0, 30, 60$ and 90° in "VV" configuration. When the specimen is rotated at these angles the Raman band shift rate significantly changes. For an angle of 90°, the Raman band shift rate is low because only the transverse stresses are detected. The Raman band shift rate depends on many experimental parameters so consistency is extremely important when comparing the stress-transfer of several materials. As one can see in Figure 4.14, the Raman band shift rates measured in "VH" configurations are much less influenced by the tensile deformation direction ($\phi$) and are almost constant. So in "VV" configuration the Raman band shift rate is dependent on the specimen rotation angle ($\phi$) whereas in "VH" configuration it seems to be independent. More detailed data will be shown later in this Section to confirm this statement.
Figure 4.13 Variation of the wavenumber position of the Raman band initially located at ~1095 cm$^{-1}$ as a function of tensile deformation for BC networks rotated every 10° increments with "VV" configuration. Only angles of $\phi = 0$, 30, 60 and 90° to the laser axis are presented here.

Figure 4.14 Variation of the wavenumber position of the Raman band initially located at ~1095 cm$^{-1}$ as a function of tensile deformation for BC networks rotated every 10° increments with "VH" configuration. Only angles of $\phi = 0$, 30, 60 and 90° to the laser axis are presented here.
A theoretical model has been previously proposed in order to model the influence of polarisation configurations, fibre orientation and tensile deformation axis direction on the Raman band shift rate (Deng et al., 2011). This has been done on carbon nanotubes/PVA nanocomposites (Deng et al., 2011). This model is now described.

The relationship between the Raman band shift rate \( S(\theta) \) and the fibre orientation \( \theta \), in model single short-fibre composites, is given by the equation (Andrews et al., 1992; Cooper et al., 2001; Wood et al., 2001; Deng et al., 2011)

\[
S(\theta) = S_0 (\cos^2 \theta - \nu \sin^2 \theta)
\]  

(4.4)

where \( S_0 \) is the band shift rate for a single fibre aligned parallel to the tensile deformation axis direction and \( \nu \) is Poisson’s ratio of the material. For uniformly dispersed and 2D in-plane materials, the Raman band shift rate can be considered as an intensity weighted average of the contribution of single BC nanofibrils oriented at different directions in the structure of the network (Wood et al., 2001). The average Raman intensities at an angle \( \phi \), for "VV" and "VH" polarisation configurations, can be calculated using the equations (Cooper et al., 2001; Deng et al., 2011)

\[
I_{VV} = \frac{1}{\pi} I_0 \int_0^\pi \cos^4 (\theta + \phi) d\theta = \frac{3}{8} I_0
\]

(4.5)

\[
I_{VH} = \frac{1}{\pi} I_0 \int_0^\pi \cos^2 (\theta + \phi) \sin^2 (\theta + \phi) d\theta = \frac{I_0}{8}
\]

(4.6)
where \( I_0 \) and \( I'_0 \) are, respectively, the maximum intensities for "VV" and "VH" polarisation configurations. As a consequence, the Raman shift band rates for "VV" and "VH" configurations are given by the following equations (Deng et al., 2011)

\[
S_{VV}(\varphi) = \frac{1}{\pi} \int_{\theta} I_0 \cos^2(\theta + \varphi)S_0(\cos^2(\theta) - \nu \sin^2(\theta))d\theta
\]

\[
= \frac{S_0}{2} (1 - \nu) + \frac{S_0}{3} (1 + \nu) \cos(2\varphi) \tag{4.7}
\]

\[
S_{VH}(\varphi) = \frac{1}{\pi} \int_{\theta} I_0 \sin^2(\theta + \varphi)S'_0(\cos^2(\theta) - \nu \sin^2(\theta))d\theta
\]

\[
= \frac{S_0}{2} (1 - \nu) \tag{4.8}
\]

The solutions of equations 4.5, 4.6, 4.7 and 4.8 were determined numerically using the Wolfram Mathematica® software version 8. One can see that these solutions are dependant of Poisson’s ratio of the studied material.

Poisson’s ratio for cellulose is dependent on the source; e.g. values of \( \nu = 0.38 \) and 0.46 have been respectively reported for single ramie and flax fibres (Peura et al., 2008; Nishiyama 2009). For network comprised of cellulose microfibrils, Poisson’s ratio has been reported to be between -0.26 and -1.17 using a X-ray microdiffraction technique (Peura et al., 2008). The in-plane Poisson’s ratio has never been reported and was, therefore, considered as unknown. It has been, however, obtained from the theoretical fitting of experimental data. Consequently, this method can be used to determine Poisson’s ratio of these materials. From the theoretical fitting to the experimental data reported in Figures 4.17 and 4.18, BC
networks were found to have a Poisson’s ratio of -0.1. These data are reported in Table 4.3. Negative values have already been reported for networks of fibres, but never for Poisson’s ratio of BC networks. This indicates that BC networks "broaden" in the transverse direction during stretching. These materials are referred to as "auxetic" materials (Evans 1991). The origin of this "auxetic" behaviour is not yet clear, but it could arise from re-entrant mechanisms. Further experimental investigation is, consequently, necessary to obtain a better understanding of these mechanisms.

Figure 4.15 reports the detailed Raman band shift rates against tensile deformation direction ($\phi$) of BC networks in "VV" and "VH" configurations.

![Figure 4.15](image_url)

**Figure 4.15** The shift rates with respect to strain of the Raman band initially located at ~1095 cm$^{-1}$ observed in "VV" and "VH" configurations for BC networks as a function of the angle $\phi$. Black lines represent a model fit to the data based on the Equations 4.7 and 4.8.
The specimen rotation angle significantly influences the Raman band shift rate. Similar experimental data are reported for BC networks but in a "VH" configuration. One can see that the experimental Raman band shift rate is also dependent of the specimen rotation angle. This was really not expected since carbon nanotube/polyvinyl alcohol nanocomposite films containing randomly oriented carbon nanotubes showed invariant behaviour (Deng et al., 2011). Also the theoretical model showed that the relationship between the band shift and the deformation axis angle is constant. This model does not, however, take into account possible changes in nanofibril orientation that might occur during tensile deformation of the networks.

A higher level of BC nanofibril orientation in the tensile deformation direction has been observed for BC networks and is reported in Figure 4.16.

**Figure 4.16** Normalised intensity of the Raman band located at $\sim 1095 \text{ cm}^{-1}$ as a function of the angle $\varphi$ between the polarisation direction of the laser and the tensile deformation direction. Percentages of 0 and 1.5 % indicate the level of tensile deformation of BC networks. Black arrows indicate the tensile deformation direction.
This may explain the non-constant behaviour for the Raman band shift rates with respect to the angle of the tensile deformation axis angle $\phi$ (see Figure 4.16). Carbon nanotube/polyvinyl alcohol nanocomposite fibres containing highly aligned carbon nanotubes showed a non-constant behaviour for the Raman band shift rates with respect to the angle of the tensile deformation axis angle $\phi$ both in "VV" and "VH" configurations in the situation where $\phi = \theta$ (Deng et al., 2011).

An estimation of the effective Young’s modulus of BC nanofibrils is possible using the equation

$$E_{\text{network}} \times \frac{d(\Delta \nu)}{d\sigma} = S_0$$

(4.11)

where $S_0$ is the band shift rate for BC nanofibrils parallel to the tensile deformation axis direction.

Young’s moduli of cellulosic materials have been previously estimated using the value of $-4.3 \text{ cm}^{-1} \text{ GPa}^{-1}$ for $d(\Delta \nu)/d\sigma$ (Eichhorn et al., 2001; Eichhorn and Young 2001; Sturcova et al., 2005; Hsieh et al., 2008; Rusli and Eichhorn 2008). This value has been obtained by following the molecular deformation of natural cellulose and regenerated cellulose fibres using Raman spectroscopy (Eichhorn et al., 2001). In this Chapter, it is assumed that this calibration value can be also used for BC nanofibrils.

According to Equations 4.12 and 4.13, with a strain axis parallel to the laser polarisation direction ($\phi = 0^\circ$), $S_0$ is expressed respectively for "VV" and "VH" configurations as
The effective Young’s modulus of a single BC nanofibril has been calculated using Krenchel’s relationship (1964)

\[ E_{\text{network}} = \eta_0 E_{\text{fibril}} \]  

Equations 4.11-4.15 allow estimating the effective Young’s modulus for a single BC nanofibril. A range of values of 79 - 88 GPa have been obtained from these equations and is reported in Table 4.3 and are lower than estimations of the crystal modulus of cellulose I, 138 GPa (Sakurada et al., 1962; Nishino et al., 1995).
Raman spectroscopy allows obtaining information about molecular deformation of both crystalline and amorphous regions. Consequently, a decreased value of Young’s modulus is obtained maybe due to averaging of the stiffness. The value of $78 \pm 17$ GPa (Guhados et al., 2005) is close to the values of $79 \pm 3$ GPa and $88 \pm 10$ GPa acquired, respectively, in "VV" and "VH" polarisation configurations.

**Table 4.3** Estimated Poisson’s ratio, measured average Raman band shift rates and calculated effective single BC fibrils Young’s modulus obtained from BC networks.

<table>
<thead>
<tr>
<th>Polarisation configuration</th>
<th>Poisson’s ratio, $\nu$</th>
<th>Average Raman band shift rate, $S_0$, (cm$^{-1}$%$^{-1}$)</th>
<th>Calculated Young’s modulus $E_{fibril}$ (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;VV&quot;</td>
<td>-0.1</td>
<td>$-1.3 \pm 0.1$</td>
<td>$79 \pm 3$</td>
</tr>
<tr>
<td>&quot;VH&quot;</td>
<td>-0.1</td>
<td>$-1.4 \pm 0.2$</td>
<td>$88 \pm 10$</td>
</tr>
</tbody>
</table>

**4.4 Conclusions**

Raman spectroscopy has been shown to be a powerful tool to study the orientation of cellulose fibrils in flax and BC networks. No significant changes in intensity were observed meaning that they comprise randomly oriented BC nanofibrils.

The micromechanical deformation of BC networks has also been investigated in two polarisation configurations ("VV" and "VH"). From this study, the effective Young’s modulus of single filaments of BC was estimated and the influence of the laser and back-scattered light polarisation configuration and both the nanofibril and deformation axis angles has been taken into account. The effective modulus of single fibrils of BC was found to be in the range of 79 - 88 GPa. The negative Poisson's ratio of BC networks may be explained by re-entrant structure mechanisms.
4.5 References


CHAPTER 5

INFLUENCE OF THE CULTURING TIME OF BACTERIAL CELLULOSE NETWORKS ON THE MICROMECHANICS OF BIO-COMPOSITE INTERFACES

5.1 Introduction

There is a growing demand to investigate environmentally friendly routes to produce composite materials for industrial applications. The aim is to replace oil-sourced composites by bio-sourced composites. This is because oil-sourced composite wastes have a negative impact on the environment. That is why bio-sourced composites are an interesting alternative since their wastes would have a limited impact on the environment through composting. Bio-sourced and potentially fully biodegradable composite materials are more commonly called bio-composites. A way to create bio-composites is by blending natural fibres, mainly constituted of cellulose with a bio-sourced polymer. Other sources of cellulose can also be used; e.g. higher plants, algae, bacteria, and sea animals called tunicates (Ranby 1952; Favier et al., 1995; Šturcová et al., 2005). Bacterial cellulose (BC), discovered by Adrian J. Brown (1886), is a possible alternative to other cellulose sources. It has been demonstrated in Chapter 4 that BC nanofibrils have a Young’s modulus of 79 – 88 GPa. Consequently, they have the potential to reinforce polymer materials and this has been reported in the literature (Astley et al., 2003; Gindl and Keckes 2004; Yano et al., 2005; Kim et al., 2009; Lee et al., 2009; Martins et al., 2009).

The stress-transfer mechanisms occurring between BC nanofibrils and polymer matrices is not completely understood and is still an important subject of research.
BC, when cultured in static conditions, forms weakly linked layered structures named BC networks (Nogi and Yano 2008). The interaction between BC networks and polymeric matrices has also not been fully investigated. Theoretical nanofibrous networks have been recently shown to have small mean pore sizes (Eichhorn and Sampson 2005). Consequently, one is not sure if polymer matrices can impregnate the structure of BC networks. Complete melt-impregnation of BC networks for efficient stress-transfer may be difficult using high molecular weight e.g. PS or PLA whose melts have high viscosity (Koyama et al., 2009).

Polylactide (PLA) is a man-made biopolymer. The physical properties of bio-composites designed with PLA have been widely investigated (Pan et al., 2007; Kim et al., 2009; Lee et al., 2009; Tingaut et al., 2009). There are, however, very few example of research of composite designed using both PLA and BC. A recent example is the production of transparent composite films obtained by combining these materials (Yano et al., 2005; Kim et al., 2009). Another recent example reports the use of BC to coat sisal fibres which resulted in an improvement of interfacial adhesion with PLA (Juntaro et al., 2007; Juntaro et al., 2008; Pommet et al., 2008). BC-containing composites could be potentially used to produce packaging materials, display devices, coatings and lenses (Yano et al., 2005; Nogi and Yano 2008; Kim et al., 2009). The biomedical sector could also benefit from these materials because both BC and PLA are biocompatibility (Klemm et al., 2001; Svensson et al., 2005; Bäckdahl et al., 2006; Czaja et al., 2006).

In this Chapter, BC networks having various culturing times and PLA have been combined to create bio-composites. The stress-transfer from the matrix material (PLA) to the nanocellulose BC networks has been quantified using Raman spectroscopy.
5.2 Materials and Methods

5.2.1 Materials

BC3 and BC6 networks, having respective culturing times of 3 and 6 days, were prepared as described in Section 3.2.1.

PLA pellets (PLA L9000; density 1.25 g cm\(^{-3}\)) were supplied by Biomer\(^{\circledR}\) (Krailing Germany).

5.2.2 Sample Preparation and Experimental Parameters

5.2.2.1 Bacterial Cellulose Networks

The samples used to study the tensile mechanical properties and the molecular deformation of BC3 and BC6 networks have been prepared as described in Section 3.2.2.1.

5.2.2.1.1 Density Measurements

The densities of BC3 and BC6 networks have been evaluated as described in Section 4.2.2.1. In a similar way, the density of BC networks having a culturing time of 2 days (BC2) has been determined.

5.2.2.1.2 Nitrogen Adsorption

Nitrogen adsorption experiments have been kindly conducted by Dr. Bahij H. Sakakini at the Centre for Nanoporous Materials (School of Chemistry, The University of Manchester) with the kind permission of Dr. Stuart M. Holmes (Senior Lecturer in Chemical Engineering and Associate Director of the Centre for Nanoporous Materials).

The total surface area of BC3 and BC6 networks was determined using the B.E.T. theory (Brunauer et al., 1938) which is an extension of the Langmuir theory.
(Langmuir 1916). An ASAP 2010 porosimeter (Software version 5.02, Micrometrics) in static mode was utilised to carry out nitrogen adsorption measurements.

### 5.2.2.1.3 Powder X-ray Diffraction

XRD patterns of BC3 and BC6 networks having respective thickness of ~8 and 35 µm were obtained using a Philips™ X’Pert powder diffractometer with a 1.79 Å Cobalt X-ray source. θ was varied from 10 to 40° in increments of 0.04°. In order to determine the crystallinity index of the BC networks Segal’s method (Segal et al., 1959) was used. This method uses the intensity of the 002 reflection compared to the intensity of the amorphous background.

The equation

\[
CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100
\]  

was used to calculate the crystallinity index (CrI) of BC3 and BC6 networks. \(I_{002}\) and \(I_{am}\) as shown in Figure 5.1, are the intensities of the crystalline peak and amorphous background peaks. \(I_{am}\) is taken at \(2\theta = 21^\circ\) if the X-ray source is cobalt (with copper, \(I_{am}\) is taken at \(2\theta = 18^\circ\)).
Figure 5.1 A typical X-ray diffraction pattern for BC6 networks with the 101, 10\(\bar{1}\) and 002 diffraction planes highlighted.

Scherrer’s equation (Scherrer 1918; Patterson 1939),

\[
L_{(002)} = \frac{K\lambda}{\beta \cdot \cos \theta} \tag{5.2}
\]

was used to determine the lateral crystallite size \(L_{(002)}\). \(\beta\) is the full width at half maximum of the 002 reflection (see Figure 5.2), \(\theta\) is the Bragg’s angle in degrees, \(K = 0.91\), \(\lambda\) is the wavelength of the X-ray source (\(\lambda = 1.79\) Å for Cobalt).

The lattice spacing \(c\) was calculated using Bragg’s law (Bragg 1913) which is defined as

\[
n\lambda = 2c \sin(\theta) \tag{5.3}
\]
where \( n \) is a positive integer and represents the order of the reflection (\( n=2 \) for the 002 reflection).

Five samples for BC3 and BC6 networks have been tested to obtain averages and standard deviations for the crystallinity index, the lateral crystal size and the lattice spacing.

### 5.2.2.1.4 Tensile Tests

The tensile mechanical properties of BC3 and BC6 networks were determined using an Instron® tensile tester (2511-111) coupled with a 50 N load cell. The compliance of the machine was measured by deforming a piece of steel in the tensile tester. A value of \( 4.4 \times 10^{-3} \) mm N\(^{-1}\) was obtained. An example of a compliance correction applied to a typical stress-strain curve for BC6 is shown in Figure 5.2. Young’s modulus and strain at failure are respectively increased and reduced by \( \sim 16 \) and \( 1.6\% \) after correction from the machine compliance. The stress at failure is however unchanged.

During testing, environmental conditions were maintained at a temperature of \( 23 \pm 1 \) °C and a humidity of \( 50 \pm 0.5\% \). The samples were conditioned during 24 hours prior to being deformed until failure using a 0.5 mm min\(^{-1}\) crosshead speed. At least 6 samples were tested for each material.
Figure 5.2 A typical stress-strain curve for BC6 networks with and without tensile test machine compliance correction.

It is important to mention that experimental data such as Young’s modulus, stress and strain at failure obtained from the stress-strain curve were obtained by considering cross-sections of the samples as being uniform along the sample strip. Poisson’s ratio of the materials was also not taken into consideration for the calculations. The sample extension was assumed to be the same as the crosshead displacement of the tensile tester. The strain was calculated by dividing the crosshead displacement by the initial sample length. This may, however, not be true locally in the sample. Consequently, engineering stress and strain have been used in this thesis. The following equations

\[
\sigma = \frac{F}{A} \tag{5.4}
\]
\[ \varepsilon = \frac{l - l_0}{l_0} \]  

(5.5)

\[ E = \frac{d\sigma}{d\varepsilon} \]  

(5.6)

have been used to calculate the engineering stress \( \sigma \) and strain \( \varepsilon \). \( F \) is the applied force, \( A \) is the sample cross-section area and \( l_0 \) and \( l \) are respectively the initial and deformed sample length. \( E \) is Young’s modulus.

### 5.2.2.1.5 Scanning Electron Microscopy

A Zeiss EVO® 60 scanning electron microscope has been utilised to image the fracture surfaces of BC3 and BC6 networks. Samples were gold coated before being imaged using an acceleration voltage of 5 kV. This has been done with the kind assistance of Mr. Michael Faulkner (senior experimental officer in the School of Materials at the University of Manchester).

### 5.2.2.1.6 Raman Spectroscopy

The molecular deformation of BC3 and BC6 networks has been quantified using a Renishaw system-1000 Raman spectrometer set in "VN" configuration (see Section 3.2.2.2). The spectrometer was coupled to a 785 nm NIR laser having a spot diameter of ~1-2 \( \mu \)m. An optical microscope equipped with a \( \times50 \) long working distance lens was used to focus the laser on the surface of the materials. The laser power at the sample surface was 26 mW. A customised deformation rig (Deben® MICROTEST™) equipped with a 2 kN load cell was used to deform the samples. An elongation rate of 0.033 mm min\(^{-1}\) has been used to deform the sample with
strain steps of 0.1%. A Raman spectrum was acquired at each strain step using an exposure time of 30 s and 4 accumulations.

5.2.2.2 Bacterial Cellulose Networks/Polylactide Composites

5.2.2.2.1 Composite Preparation

BC3/PLA and BC6/PLA composite materials were prepared as described in Chapter 3, Section 3.2.2.2. For the preparation of composites using BC3, a pressure of 1.2 MPa was used so as not to damage these thinner and lighter networks. It is important to note that PLA samples were submitted to the same thermal and mechanical cycles used for composite preparation.

An image of the samples is presented in Figure 5.3. In this Chapter, composites prepared using BC networks cultured for 3 and 6 days are respectively referred to as BC3/PLA and BC6/PLA.

Figure 5.3 An image of (a) a BC3 network, (b) a BC3/PLA composite, (c) a BC6 network, (d) a BC6/PLA composite and (e) a PLA film.
The samples used to study the tensile mechanical properties and the molecular deformation of BC3/PLA and BC6/PLA composites have been prepared as described in Section 3.2.2.1. The cellulose volume fractions for BC3/PLA and BC6/PLA composites were respectively ~4.3 and 18.1 volume % (vol.%).

5.2.2.2.2 Differential Scanning Calorimetry

A TA Q100 heat-flux differential scanning calorimeter has been used to determine the thermal properties of processed PLA films and BC3/PLA and BC6/PLA composites. Hermetic aluminium pans containing samples of ~8 mg were heated up from 25 to 200 °C and subsequently cooled and then heated up again. The samples were cooled and heated up and a rate of 10 °C min⁻¹ and under a 50 ml min⁻¹ nitrogen purge gas flow. Experiments were repeated twice and average and standard deviation values are presented. Empty pan measurements were achieved, prior to experiments, to verify the cleanliness of the furnace.

5.2.2.2.3 Tensile Tests

BC3/PLA and BC6/PLA composites and PLA have been tested as described in Section 5.2.2.1.4. Theoretical values for Young’s modulus of the composites were calculated using the equation

\[ E_c = E_n V_n + E_m (1 - V_n) \]  \hspace{1cm} (5.7)

which is commonly called "the rule of mixtures". \( E_c \) is Young’s modulus of the composite, \( E_m \) is the Young’s modulus of the matrix, \( E_n \) is the Young’s modulus of BC networks and \( V_n \) is their volume fraction.
5.2.2.2.4 Scanning Electron Microscopy
Tensile fracture surfaces of BC3/PLA and BC6/PLA composites have been imaged as described in Section 5.2.2.1.5.

5.2.2.2.5 Raman Spectroscopy
The molecular deformation of BC3/PLA and BC6/PLA composites has been investigated as described in Section 5.2.2.1.6. The laser was focused using the optical microscope at the BC3/PLA and BC6/PLA interfaces through the transparent PLA matrix as shown in Figure 5.4.

![Figure 5.4 Schematic showing how the laser was focused at the interface of BC3/PLA and BC6/PLA composite materials.](image)

5.3 Results and Discussion
5.3.1 Bacterial Cellulose Networks
5.3.1.1 Density
The densities of BC2, BC3, BC6 and BC18 networks are reported in Figure 5.5. One can see that the density of BC networks increases very quickly for low
culturing times (2, 3 and 6 days) and then plateaus for high culturing times (18 days).

![Graph showing density of BC networks as a function of culturing time](image)

**Figure 5.5** Density of BC networks as a function of their respective culturing times.

The reason for that may be because low culturing time BC networks have a more open structure and more air (density of $1.2 \times 10^{-3} \text{ g cm}^{-3}$ at 25 °C) is present in the BC networks structure. Then for networks produced using longer culturing times, less air is present in the networks due to further BC production from bacteria. At a certain culturing time, the density of BC networks appears to saturate. The use of low culturing time BC networks is potentially interesting in terms of specific mechanical properties. This will be shown later in Section 5.3.1.4.

Data reported in Figure 5.5 were fitted using the equation

$$\rho = a + b \exp(at)$$  \hspace{1cm} (5.8)
where \( \rho \) is the density of BC networks in g cm\(^{-3}\), \( t \) is the time in days and \( a \) and \( b \) are constants. An optimised fitting of the experimental data was found for \( a = 1.16 \), \( b = -1.22 \) and \( \alpha = -0.26 \). From this model the density of BC networks for a culturing time comprised between 0 and 18 days was predicted. For instance the density of BC networks cultured for 14 days would be \(~1.1\) g cm\(^{-3}\). This value will be used later in Chapter 7.

5.3.1.2 Total Surface Area

The total surface area for BC3 and BC6 networks has been estimated using nitrogen adsorption. Values of \( 95 \pm 15 \) and \( 7 \pm 2 \) m\(^2\) g\(^{-1}\) have been respectively obtained. This shows that BC3 networks have a much higher total surface area compared to BC6 networks. Consequently, it is possible that BC3 will show better impregnation and mechanical anchorage of resin compared to BC6 networks. This might lead to higher stress-transfer and mechanical properties for composites designed using BC3 networks over composites designed using BC6 networks. This has been investigated and is reported Sections 5.3.2.3 and 5.3.2.5.

5.3.1.3 Crystallinity and Crystal Morphology

Figure 5.6 reports typical powder XRD patterns for BC3 and BC6 networks. Three peaks can be observed for both BC3 and BC6 networks in the range of 10 to 40° and correspond to the 101, 10 \( \bar{1} \) and 002 diffraction planes. These diffraction planes have already been reported in the literature for BC (Cheng et al., 2009; Li et al., 2009; Lee et al., 2011). The intensity of these three peaks for BC3 networks is lower than for BC6 networks. This is because BC3 and BC6 networks have
respective thickness of ~0.007 and 0.035 mm and the intensity of these peaks is proportional to the concentration and quantity of cellulose.

![X-ray diffraction pattern](image)

**Figure 5.6** Typical X-ray diffraction patterns for BC3 and BC6 networks with the 101, 101 and 002 diffraction planes.

By determining the intensity, the peak position and the full width at half maximum of the 002 reflection and using Equations 5.3, 5.4 and 5.5, the crystallinity index (CrI), the lateral crystallite size \( L_{(002)} \) and the lattice spacing \( c \) of BC3 and BC6 crystal structure have been calculated. Close values for these crystalline characteristics have been reported by Lee et al., (2011). The values for BC3 and BC6 networks are reported in Table 5.1. Significant differences in terms of crystallinity index and lateral crystallite size between BC3 and BC6 can be seen. However one can see no difference concerning their lattice spacing.
**Table 5.1** Summary of the powder X-ray diffraction measurements for BC3 and BC6 networks obtained from the 002 reflection.

<table>
<thead>
<tr>
<th>Material</th>
<th>Crl (%)</th>
<th>FWHM (°)</th>
<th>2θ (°)</th>
<th>L_{002} (nm)</th>
<th>c_{002} (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC3</td>
<td>77.7 ± 0.9</td>
<td>1.4 ± 0.0</td>
<td>26.5 ± 0.1</td>
<td>6.6 ± 0.1</td>
<td>0.8 ± 0.0</td>
</tr>
<tr>
<td>BC6</td>
<td>81.1 ± 0.4</td>
<td>1.8 ± 0.0</td>
<td>26.5 ± 0.1</td>
<td>5.3 ± 0.1</td>
<td>0.8 ± 0.0</td>
</tr>
</tbody>
</table>

### 5.3.1.4 Tensile Mechanical Properties

Figure 5.7 shows typical stress-strain curves for BC3 and BC6 networks. One can clearly see that BC6 networks have higher Young’s modulus, stress and strain at failure and work of fracture. The detailed tensile mechanical properties of BC3 and BC6 networks are reported in Table 5.2.

**Figure 5.7** Typical stress-strain curves for BC3 and BC6 networks. Arrows indicate the failure of the samples.
Table 5.2 Summary of the tensile mechanical properties for BC3 and BC6 networks.

<table>
<thead>
<tr>
<th>Material</th>
<th>Young’s modulus (GPa)</th>
<th>Stress at failure (MPa)</th>
<th>Strain at failure (%)</th>
<th>Work of fracture (MJ m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC3</td>
<td>9.4 ± 1.0</td>
<td>109.0 ± 41.8</td>
<td>1.6 ± 0.9</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>BC6</td>
<td>13.0 ± 1.8</td>
<td>218.3 ± 39.5</td>
<td>2.4 ± 0.3</td>
<td>2.8 ± 0.8</td>
</tr>
</tbody>
</table>

From Figure 5.8, one can clearly see that BC3 networks have similar specific Young’s modulus thanks to its density of 0.7 ± 0.1 g cm⁻³ whereas BC6 networks have a density of 1.0 ± 0.0 g cm⁻³. This property is very important if one thinks about producing lighter composites with similar mechanical performance. The detailed values for the specific tensile mechanical properties of BC3 and BC6 networks are reported in Table 5.3.

![Figure 5.8](image-url) Figure 5.8 Typical specific stress-strain curves for BC3 and BC6 networks.
Table 5.3 Summary of the specific tensile mechanical properties for BC3 and BC6 networks.

<table>
<thead>
<tr>
<th>Material</th>
<th>Specific Young’s modulus (GPa cm$^3$ g$^{-1}$)</th>
<th>Specific stress at failure (MPa cm$^3$ g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC3</td>
<td>13.4 ± 2.4</td>
<td>155.7 ± 63.7</td>
</tr>
<tr>
<td>BC6</td>
<td>13.0 ± 0.0</td>
<td>218.3 ± 39.7</td>
</tr>
</tbody>
</table>

5.3.1.5 Tensile Fracture Surface

Figure 5.9 reports SEM images of the tensile fracture surface for BC3 and BC6 networks at the same scale. Both networks are made of weakly linked layers (Nogi and Yano 2008) which can delaminate when networks are submitted to external tensile deformation. Furthermore, it seems that this delamination process is even more pronounced for BC6 networks probably due to their thicker and initially more laminated structure.

Figure 5.9 Scanning electron microscope images of fracture surfaces of (a) BC3 networks and (b) BC6 networks.

This observation might explain why BC6 networks have higher stress and strain at failure and work of fracture (2.8 ± 0.8 MJ.m$^{-3}$) compared to BC3 networks (0.8 ±...
0.6 MJ.m\(^{-3}\)) as described in Section 5.3.1.4. Energy is likely to be dissipated during delamination of BC layers when BC networks are subjected to tensile deformation. Consequently it is possible that the use of BC6 networks might lead to a composite with a higher work of fracture.

### 5.3.1.6 Molecular Deformation

Figure 5.10 reports typical Raman spectra for BC3 and BC6 networks. The presence of a high intensity Raman band located at ~1095 cm\(^{-1}\) is noted. This Raman band corresponds to the vibrational motions of C-O bonds and possibly C-O-C bonds in the backbone structure of cellulose (Wiley and Atalla 1987; Edwards et al., 1997; Gierlinger et al., 2006).

![Typical Raman spectra](image)

*Figure 5.10 Typical Raman spectra in the range of 300 to 1600 cm\(^{-1}\) for BC3 and BC6 networks with the Raman initially located at ~1095 cm\(^{-1}\) highlighted.*

The intensity of the Raman band located at ~1095 cm\(^{-1}\) however, appears lower for BC3 networks compared to BC6 networks. This is because BC3 networks are much
thinner than BC6 networks. The intensity of the Raman band located at \( \sim 1095 \text{ cm}^{-1} \) depends on the concentration or quantity of cellulose. This is even more obvious when looking at Figure 5.11 which shows typical Raman spectra for BC3 and BC6 networks in the range of 1050 to 1150 cm\(^{-1}\).

Figure 5.12 reports a typical shift of the Raman band initially located at \( \sim 1095 \text{ cm}^{-1} \) towards a lower wavenumber position upon tensile deformation for BC6 networks. Similar shifts towards a lower wavenumber position have also been observed for BC3 networks. This shift is characteristic for cellulosic material deformed in tension and has already been reported previously in the literature and is related to the molecular deformation of the cellulose backbone (Eichhorn et al., 2001; Hsieh et al., 2008; Rusli et al., 2010). The origin of these stress-induced Raman band shifts has already been discussed in Section 2.3.7.

![Figure 5.11 Typical Raman spectra in the range of 1050 to 1150 cm\(^{-1}\) for BC3 and BC6 networks.](image)

\[ \text{Figure 5.11 Typical Raman spectra in the range of 1050 to 1150 cm}^{-1} \text{ for BC3 and BC6 networks.} \]
Figure 5.12 A typical shift towards a lower wavenumber of the Raman band initially located at \(\sim 1095 \text{ cm}^{-1}\) upon tensile deformation for a BC6 network.

Figures 5.13 and 5.14 report detailed shifts towards a lower wavenumber of the Raman band initially located at \(\sim 1095 \text{ cm}^{-1}\) for BC3 and BC6 networks respectively as a function of strain and stress. Figure 5.14 suggests that the stress-transfer occurring in BC3 networks is greater than for BC6 networks. BC3 and BC6 networks have respectively Raman band shift rates of -1.3 and -0.9 cm\(^{-1}\) %\(^{-1}\). The higher the Raman band shift rate, the higher the stress-transfer within the network leading to molecular deformation. This is further confirmed in Figure 5.14 where the Raman band shift is plotted as a function of stress. The Raman band shift rate for BC3 and BC6 networks have been found to be respectively -16.5 and -5.7 cm\(^{-1}\) GPa\(^{-1}\).
Figure 5.13 Detailed shifts of the Raman band initially located at \( \sim 1095 \text{ cm}^{-1} \) for BC3 and BC6 networks as a function of strain.

The origin of the higher stress-transfer measured for BC3 networks compared to BC6 networks might be because BC3 networks have a less laminated structure compared to BC6 networks (see Section 5.3.1.5). As mentioned in Section 5.3.1.5, more energy is likely to be dissipated in BC6 networks compared to BC3 networks. This is probably due to the delamination mechanism occurring when BC networks are submitted to external tensile deformation. Consequently the molecular deformation of the top layer (where the laser mainly interacts) is may be more reduced if more BC layers delaminate. One can also observe a sort of plateau for thicker BC6 networks. This may be attributed to BC layer delamination.
5.3.2 Bacterial Cellulose Networks/Polylactide Composites

5.3.2.1 Thermal Behaviour and Morphology of Processed Polylactide

Figure 5.15 reports the thermal behaviour of processed PLA films and BC3/PLA and BC6/PLA composites. As expected for PLA films, a cold-crystallisation at ~132 °C as well as a melting peak at ~171 °C is observed during heating. During cooling no crystallisation peak is observed. This is because PLA like polyethylene terephthalate (PET) is a slow crystallisation polymer. If one wants to observe a crystallisation peak, cooling rates typically lower than 5 °C min$^{-1}$ must be applied (Wang et al., 2005). This shows that it is very likely that the processing conditions, reported in Section 5.2.2.2.1, allowed us to obtain transparent and amorphous PLA films.

---

Figure 5.14 Detailed shifts of the Raman band initially located at ~1095 cm$^{-1}$ for BC3 and BC6 networks as a function of stress.
Figure 5.15 Differential scanning calorimetry curves for polylactide (PLA) and BC/3/PLA and BC6/PLA composites for the second heating cycle and one cooling cycle, where $T_g$ is the glass transition temperature, $T_{cc}$ is the cold-crystallisation temperature and $T_m$ is the melting temperature.

Figure 5.16 reports a typical powder XRD pattern for processed PLA films. A broad peak, which is characteristic of an amorphous polymer, is observed. This shows that the processing condition used in this study allowed the formation of an amorphous PLA films. For BC3/PLA and BC6/PLA composites, a crystallisation peak is observed at $\sim$110 °C. The presence of BC nanofibrils at the interface may be responsible for the development of a transcristalline phase. Such phenomenon has already been observed in polypropylene reinforced with cellulose nanocrystals (Gray 2008). PLA reinforced with wood flour and microcrystalline cellulose has also shown transcrystallinity (Mathew et al., 2006). However the contribution of transcrystallinity on the mechanical reinforcement and on the stress-transfer is not proven.
Figure 5.16 An X-ray powder diffraction pattern for processed PLA films in the range of 10 to 40°.

Figure 5.17 reports the comparison of the crystallisation peaks for BC3/PLA and BC6/PLA. One can clearly see a higher enthalpy of crystallisation for BC3/PLA (~4.0 ± 0.0 J g\(^{-1}\)) compared to BC6/PLA (~1.4 ± 0.2 J g\(^{-1}\)). This can be an indication of a higher interaction surface area between BC3 networks and PLA compared to BC6 networks. This is probably due to the higher total surface area of BC3 networks compared to BC6 networks as shown in Section 5.3.1.2. In addition to this, one can also observe a higher enthalpy of melting and a higher enthalpy of cold-crystallisation for BC3/PLA composites compared to BC6/PLA composites and PLA. This may further indicate that BC3 networks are more favourable to the development of a transcrystalline phase due to their higher total surface area compared to BC6 networks.
**Figure 5.17** Differential scanning calorimetry curves for PLA and BC3/PLA and BC6/PLA composites between 80 and 125 °C of the cooling curve. $T_c$ is the crystallisation temperature. Arrow indicates the position of small crystallisation peak.

It is still uncertain whether transcrystallinity is really present in the composites. Further work would have to image the fibre-resin interface using transmission electron microscopy. Indeed during DSC experiments cooling rates of 10 °C min$^{-1}$ were used. In reality during the processing of the composites using compression moulding, the cooling rate was measured and found to be ~55 °C min$^{-1}$. Consequently at this higher cooling rate it is not certain if a crystallisation peak should be observed.

### 5.3.2.3 Tensile Mechanical Properties

Typical stress-strain curves for BC3/PLA and BC6/PLA composites and PLA are reported in Figure 5.18. It is clear that the stiffness and strength of PLA is
improved by the presence of BC; by 100 % and 350 % respectively for an ~18 % volume fraction of BC fibres. These values could be improved if BC networks were fully impregnated with resin. The mechanical properties of BC3/PLA and BC6/PLA composites and PLA are reported in Table 5.4.

In order to compare the reinforcement efficiency of BC3 and BC6, the mechanical properties were divided by their respective weight fraction introduced in the composites. It was difficult to control the volume fraction, and so by dividing the mechanical properties by this value a normalisation was carried out. This is referred to as e.g. relative Young’s modulus (GPa %$_{\text{BC}}^{-1}$) in Table 5.5.

![Graph](image)

**Figure 5.18** Typical stress-strain curves for bacterial cellulose networks cultured for BC3/PLA and BC6/PLA composites. Volume fractions for BC3/PLA and BC6/PLA are indicated on the graph.
Table 5.4 Summary of the tensile mechanical properties for PLA and BC3/PLA and BC6/PLA composites.

<table>
<thead>
<tr>
<th>Material</th>
<th>Young’s modulus (GPa)</th>
<th>Young’s modulus calculated from the rule of mixture (GPa)</th>
<th>Stress at failure (MPa)</th>
<th>Strain at failure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC3/PLA</td>
<td>2.1 ± 0.2</td>
<td>2.3</td>
<td>46.9 ± 2.7</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>BC6/PLA</td>
<td>4.0 ± 0.4</td>
<td>4.0</td>
<td>115.2 ± 9.8</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>PLA</td>
<td>2.0 ± 0.2</td>
<td>NA</td>
<td>27.7 ± 2.5</td>
<td>23.5 ± 17</td>
</tr>
</tbody>
</table>

Figure 5.19 reports typical specific stress-strain curves of BC3/PLA and BC6/PLA. One can clearly see that for the same volume fraction the reinforcement is more pronounced when PLA is reinforced with BC3 networks. This is very important since culturing the bacterial cellulose for 6 days may not benefit the reinforcement of the composite.

![Typical specific stress-strain curves for BC3/PLA and BC6/PLA composites.](image)
CHAPTER 5

If a culturing time of 3 days is sufficient for the production of high strength BC/PLA composites, then the process of making composites by this route could be more cost effective. At an industrial scale this could help to reduce the time of production and the price of the material. Theoretical Young’s moduli and specific Young’s moduli obtained from the rule of mixtures are reported in Tables 5.4 and 5.5. Experimental values are very close to these theoretical values showing the good interfacial properties between BC networks and PLA.

Table 5.5 Summary of the relative tensile mechanical properties for BC3/PLA and BC6/PLA composites.

<table>
<thead>
<tr>
<th>Material</th>
<th>Relative Young's modulus (GPa %⁻¹)</th>
<th>Relative Young's modulus calculated from the rule of mixture (GPa %⁻¹)</th>
<th>Relative stress at failure (MPa %⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC3/PLA</td>
<td>0.5 ± 0.1</td>
<td>0.5</td>
<td>10.9 ± 1.9</td>
</tr>
<tr>
<td>BC6/PLA</td>
<td>0.2 ± 0.0</td>
<td>0.2</td>
<td>6.4 ± 1.1</td>
</tr>
</tbody>
</table>

5.3.2.4 Tensile Fracture Surface

Figures 5.20 a and 5.20 b report scanning electron microscopy images of the tensile fracture surface for BC3/PLA and BC6/PLA composites. These images show that BC3 and BC6 were not fully impregnated and that laminated composites have been obtained. It is actually very difficult to impregnate nanoporous networks with a high viscosity polymer.
Figure 5.20 Scanning electron microscope images of fracture surfaces for (a) a BC3/PLA composite and (b) a BC6/PLA composite at the same scale.

Also these images reveal a good interaction between the top layers of BC networks and PLA. Furthermore it looks as if delamination is occurring preferably between BC3 and BC6 layers and not at the interface between BC and PLA as shown in Figure 21 (b). This observation is even more evident for BC3/PLA composites as shown in Figure 5.21.

Figure 5.21 Scanning electron microscope images of fracture surfaces for a BC3/PLA composite.
5.3.2.5 Molecular Deformation

Figure 5.22 reports typical Raman spectra for PLA and BC3/PLA and BC6/PLA composites in the range of 300 to 1600 cm$^{-1}$. One can see that the Raman band initially located at ~1095 cm$^{-1}$ belonging to BC3 and BC6 can be detected through the PLA resin.

Figure 5.22 Typical Raman spectra in the range of 300 to 1600 cm$^{-1}$ for PLA, BC3/PLA and BC6/PLA composites with the Raman band initially located at ~1095 cm$^{-1}$ highlighted.

Figure 5.23 reports typical Raman spectra for PLA and BC3/PLA and BC6/PLA composites in the range of 1050 to 1150 cm$^{-1}$ and further shows that the Raman band located at ~1095 cm$^{-1}$ can be detected through the PLA resin in BC3/PLA and BC6/PLA composites having respectively BC volume fractions of ~4.3 and 18%. The detection of the Raman band located at ~1095 cm$^{-1}$ in a laminated composite containing 4.3% of BC is however possible while when BC fibrils are dispersed in polymer matrix to form a nanocomposite. It has been reported by Rusli (2011) that
one needs at least a volume fraction of 10 vol.% of cellulose nanowhiskers and if not more to obtain a well defined peak.

Figure 5.23 Typical Raman spectra in the range of 1050 to 1150 cm\(^{-1}\) for PLA, BC3/PLA and BC6/PLA composites.

Figure 5.24 reports typical Raman spectra in the wavenumber range of 1050 to 1150 cm\(^{-1}\) for a PLA strip at 0 and 2.5 % tensile deformation. No shifts are observed meaning that PLA does not contribute to the shift of the Raman band located at \(\sim\)1095 cm\(^{-1}\) belonging to BC. Figure 5.25 reports detailed shifts of the Raman band initially located at \(\sim\)1095 cm\(^{-1}\) for BC3 networks and BC3/PLA composites as a function of strain. The Raman band shift rates for these materials have been respectively found to be \(-1.3\) and \(-2.0\) cm\(^{-1}\) %\(^{-1}\). Figure 5.26 reports similar data for BC6 networks and BC6/PLA composites. Raman band shift rates of \(-0.9\) and \(-1.8\) cm\(^{-1}\) %\(^{-1}\) have been respectively reported. Consequently it seems that stress transfers to the BC networks when embedded in a PLA matrix. The Raman band shift rate with respect to strain for BC3/PLA is higher than for
BC6/PLA composites which indicates a stronger interaction between PLA and the BC3 networks than PLA with the BC6 networks. This is probably due to BC3 networks having higher total available surface area (95 ± 15 g m\(^{-2}\)) for bonding with the resin, compared to BC6 networks (7 ± 2 g m\(^{-2}\)), as reported in Section 5.3.1.2.

![Typical Raman spectra](image)

**Figure 5.24** Typical Raman spectra for processed PLA in the range of 1050 to 1150 cm\(^{-1}\) at 0 and 2.5 % tensile deformation.

Also DSC experiments have shown that BC3/PLA composites have a higher enthalpy of crystallisation (~4.0 J g\(^{-1}\)) compared to BC6/PLA composites (~1.6 J g\(^{-1}\)), which may be indicative of enhanced PLA interaction with BC nanofibrils. It is important to note that sometimes one can observe a sudden decrease of the Raman band shift as highlighted in Figures 5.26 and 5.27. This decrease can be attributed to the interfacial breakdown between the matrix and the reinforcement.

The enhanced interaction between BC3 networks and PLA is even more evident when comparing the Raman band shift rate of BC3/PLA and BC6/PLA composites.
as a function of stress (relative to their respective BC volume fraction). Relative Raman band shift rates of $-31.2 \text{ cm}^{-1} \text{ GPa}^{-1} \%^{-1}$ and $2.5 \text{ cm}^{-1} \text{ GPa}^{-1} \%^{-1}$ have been respectively obtained. These data are reported in Figures 5.27 and 5.28.

**Figure 5.25** Detailed shifts of the Raman band initially located at ~$1095 \text{ cm}^{-1}$ for BC3 and BC3/PLA composites as a function of strain.

**Figure 5.26** Detailed shifts of the Raman band initially located at ~$1095 \text{ cm}^{-1}$ for BC6 and BC6/PLA composites as a function of strain.
Figure 5.27 Detailed shifts of the Raman band initially located at ~1095 cm⁻¹ for BC3 and BC3/PLA composites as a function of stress.

Figure 5.28 Detailed shifts of the Raman band initially located at ~1095 cm⁻¹ for BC6 and BC6/PLA composites as a function of stress.
5.4 Conclusions

In this Chapter, it has been demonstrated that the stress-transfer mechanisms in BC networks and BC/PLA composite materials can be investigated using Raman spectroscopy. The Raman band initially located at ~1095 cm$^{-1}$ belonging to cellulose has been shown to be detectable throughout the PLA matrix. In addition no Raman band from PLA was found to interfere with the Raman band initially located at ~1095 cm$^{-1}$ belonging to cellulose.

The determination of the tensile mechanical properties of the samples has shown that stress-transfer occurs from the PLA matrix to the BC networks. This led to composite materials having improved tensile mechanical properties compared to PLA. BC networks cultured for 6 days have been found to be constituted of more layers compared to BC networks cultured for 3 days. PLA was found to slightly penetrate the upper layer of BC networks. Consequently the stress must be transferred from the PLA matrix to the BC layers in direct contact with the resin. The interaction between PLA and BC networks was found to be greater when using BC networks cultured for 3 days. This result may be relevant for the design of BC/PLA composite materials with enhanced mechanical properties. For example, one may be able to design these composites by using less cellulose for similar mechanical reinforcement. It has been also shown that stress-transfer efficiency in these materials must depend on both the stress-transfer at the interface and within the BC networks themselves.

Experimental data from Raman spectroscopy, tensile testing and differential scanning calorimetry suggest a greater interaction between PLA and BC networks cultured for 3 days compared to BC networks cultured for 6 days. Raman spectroscopy is consequently a powerful tool to investigate stress-transfer
mechanisms in this form of BC/PLA composites. Chapter 7 will show further investigation where both PLA and BC networks have been chemically modified to consolidate BC layers and the BC/PLA interface.

5.5 References


CHAPTER 6
CROSS-LINKING OF BACTERIAL CELLULOSE NETWORKS USING GLYOXALISATION

6.1 Introduction
Bacterial cellulose (BC) networks cultured for 3 days and 6 days have been used to reinforce polylactide (PLA) as reported in Chapter 5. The morphology of the tensile fracture surfaces of composites comprising BC cultured for 3 days and for 6 days have shown that delamination was mainly occurring between BC layers rather than at the BC/PLA interface (see Section 5.3.2.4). Consequently, in order to try to enhance the mechanical properties of BC/PLA composites, it was necessary to consolidate the interface between BC layers before trying to improve the interaction between hydrophilic BC networks and hydrophobic PLA.

Cellulose can be chemically modified owing to hydroxyl moieties positioned along its backbone structure. Chemical reactions such as esterification, polycondensation, etherification, or acetalysation (Klemm et al., 1998; Heinze and Liebert 2001) are possible routes to cellulose chemical modifications. A chemical substance having at least two reactive groups able to react with hydroxyl moieties can be selected and covalent interactions can then be potentially created between BC nanofibrils and weakly linked BC layers. Glyoxal, among other chemical substances, has been reported as a cross-linking agent for cellulose (Klemm et al., 1998). Most cross-linkers used in industry are, however, toxic, oiled-based and not environmentally friendly. Glyoxal is a relatively low toxicity molecule, and has the advantage of being almost fully biodegradable and can be bio-sourced (Miyata et al., 2000; Ramires et al., 2010). Consequently, glyoxal can be referred to as a "bio"
cross-linking agent for cellulose. Glyoxal has been, so far, mainly used industrially to impart durable wet mechanical properties of cotton fabrics (Welch and Forthright Danna 1982).

This Chapter reports on the potential of glyoxal to enhance the stress-transfer and mechanical properties of BC networks in the dry and wet states. Glyoxalised BC networks could be potentially used in composite materials manufacturing and in the biomedical sector as body implants.

6.2 Materials and Methods

6.2.1 Materials and Chemicals

BC networks were prepared as described in Section 3.2.1 with a culturing time of 14 days.

Glyoxal (~40 wt.% in distilled water), aluminum sulfate hexadecahydrate (Al$_2$(SO$_4$)$_3$16H$_2$O, purity \(\geq 98\%)\), lithium chloride (LiCl; \(\geq 99.5\%)\) and \(N,N\)-dimethylacetamide (DMAc; \(\geq 98\%\)) were kindly provided by Sigma-Aldrich (Gillingham, U.K.).

6.2.2 Procedure for Cross-Linking of Bacterial Cellulose Networks

An aqueous commercial solution containing 40 wt.% of glyoxal was diluted into a 5 wt.% glyoxal solution by adding the necessary amount of distilled water. A catalyst referred to as aluminum sulfate hexadecahydrate, was subsequently added to the glyoxal solution with a concentration of 1 g l$^{-1}$. The glyoxal solution was agitated under mechanical stirring for 15 min to complete catalyst dissolution. Ten narrow strips of BC (~30 mm $\times$ 1 mm $\times$ 0.06 mm each), corresponding to a ~1:280
cellulose:glyoxal molar ratio, were used for each glyoxalisation procedure. BC strips were subsequently submerged in the 5 wt.% glyoxal solution for 5 h.

The impregnation procedure was very important since it allowed the glyoxal solution to interpenetrate and adsorb into the layered structure of BC networks. A convection oven set at a temperature of 150 °C was then used to complete the glyoxalisation reaction. Glyoxal-impregnated BC strips were placed in the same oven for 15 min. A washing step was then performed in distilled water at a temperature of ~70 °C for 60 min to purify glyoxalised BC networks from unreacted glyoxal molecules. The samples were finally dried out overnight at 110 °C.

### 6.2.4 Sample Preparation and Experimental Parameters

#### 6.2.4.1 Dissolution Test

Unmodified and glyoxalised BC strips (~2.5 mm ×1 mm ×0.06 mm) were plunged in LiCl/DMAc (8 wt.% LiCl) solution for 48 h in a room where the temperature and the relative humidity were regulated respectively at 23 ± 1 °C and 50 ± 0.5 %. LiCl/DMAc has been reported to completely dissolve cellulose at room temperature (McCormick *et al.*, 1985). Glyoxalised BC networks were weighed before and after immersion in LiCl/DMAc solution using a high precision scale. The excess of LiCl/DMAc was then taken away from the samples using absorbent paper before weighing the samples. The percentage of swelling (%S) was obtained after 48 h using the equation

\[
\%S = \frac{W_f - W_d}{W_d} \times 100
\]  

(6.1)
where \( W_s \) and \( W_d \) are the swollen and dry weights of the glyoxalised BC networks, respectively. Five samples were tested for each material.

### 6.2.4.2 Powder X-ray Diffraction

XRD patterns as well as the crystallinity index, the lateral crystallite size and the lattice spacing of unmodified and glyoxalised BC networks have been calculated as described in Section 5.2.2.1.3.

### 6.2.4.3 Thermogravimetric Analysis

Thermogravimetric analysis of BC networks has been kindly conducted by Dr. Geert Vanden Poel (DSM Resolve, Thermal Analysis Group, Geleen, The Netherlands). This is because the TGA machine at the Materials Science Centre at the University of Manchester was not working for a long period of time.

A thermogravimetric analysis equipment (TGA Netzsch TG 209 F1) was used to investigate the thermal degradation behaviour of unmodified and glyoxalised BC networks. The temperature in the furnace was controlled with a precision of ±1 °C. The samples (~5 mg each) were heated up from 25 to 600 °C at a heating rate of 5 °C min\(^{-1}\) under a 30 ml min\(^{-1}\) flow nitrogen purge gas. The first derivative of the loss weight as a function of temperature was used to determine the onset and degradation temperatures. Before performing the TGA analysis, the samples were conditioned under ambient temperature and humidity. Experiments were performed in triplicate for each material and averages and standard deviations are presented.
6.2.4.4 Water/Air Contact Angle

Water/air contact angles measurements were conducted by Dr. Koon-Yang Lee with the kind permission of Prof. Alexander Bismarck (Polymer and Composites Engineering Group, Department of Chemical Engineering, Imperial College London).

A technique referred to as the sessile drop method (DSA 10 Mk 2, Krüss GmbH, Hamburg, D) has been utilised for the determination of advancing $\theta_a$ and receding $\theta_r$ contact angles. This has been done by placing on the surface of unmodified and glyoxalised BC networks, ultra pure water droplets (~20 µl each at a 6.32 µl min$^{-1}$ flow) using a motorised syringe. Images of the droplets have been taken to measure advancing $\theta_a$ and receding $\theta_r$ contact angles using a DSA software version 1.80.1.12. The time to perform a single contact angle was chosen to be ~4 min to limit capillarity and absorption effects owing to the hydrophilic character of BC. Five locations on the sample surface of unmodified and glyoxalised BC networks have been used to repeat experiments. Average and standard deviations data are presented.

6.2.4.5 Streaming Zeta-Potential

Streaming zeta potential measurements were also conducted by Dr. Koon-Yang Lee with the kind permission of Prof. Alexander Bismarck (Polymer and Composites Engineering Group, Department of Chemical Engineering, Imperial College London).

An electrokinetic analyser (EKA, Anton Paar, Graz, Austria) has been utilised to measure the zeta ($\zeta$)-potentials of unmodified and glyoxalised BC networks. The pH-dependent $\zeta$-potentials were measured after time-dependent measurements have
been performed to limit the influence of swelling effects. This has been done after
equilibration in a 1 mM KCl electrolyte solution at 20 °C. The swelling behaviour
of the unmodified and glyoxalised BC networks was determined from the kinetic
parameters $\zeta_0$ (initial $\zeta$-potential) and $\zeta_\infty$ (final $\zeta$-potential). The relative change in
$\zeta$-potential is defined by the equation

$$\Delta \zeta = \frac{\zeta_0 - \zeta_\infty}{\zeta_0}$$

(6.5)

and is proportional to the sorption capacity of natural fibres (Baltazar-y-Jimenez
and Bismarck 2007). The pH of the electrolyte solution was changed using a
titration unit (RTU, Anton Paar, Graz, Austria) to perform pH-dependent $\zeta$-
potential measurements.

6.2.4.6 Relative Water Absorption Capacity

After conditioning unmodified and glyoxalised BC networks (~ 5 mm ×1 mm
×0.06 mm) in deionised water for 7 days at 23 ± 1 °C and at a relative humidity of
50 ± 0.5 %, the samples were weighed after 2, 5, 11, 24, and 48 h using a high
precision scale. The samples were weighted after the excess of water was removed
using absorbent paper. Relative water absorption capacity (%RWAC) was
determined using the equation

$$\%WAC = \frac{W_t - W_{t0}}{W_{t0}} \times 100$$

(6.6)
where $w_{t0}$ and $w_t$ are the weights of the samples before and after water immersion, respectively. Five samples were tested for both unmodified and glyoxalised BC networks.

6.2.4.7 Tensile Tests

The tensile mechanical properties of dry unmodified and glyoxalised BC networks have been determined as described in Section 5.2.2.1.4.

The wet tensile mechanical properties of unmodified and glyoxalised BC networks were determined after submerging the samples in distilled water for 48 h at $23 \pm 1 \, ^\circ\text{C}$. The samples were then removed from the water and secured on paper testing cards using cyanoacrylate glue (Sigma Aldrich, Gillingham, UK).

6.2.4.8 Scanning Electron Microscopy

A scanning electron microscope (Phillips XL-30 FEG-SEM) set with an acceleration voltage of 5 kV was used to observe the morphology of the tensile fracture surfaces of dry and wet unmodified and glyoxalised BC networks. The samples were gold-coated at 40 mA for 2 min prior to imaging.

6.2.4.9 Raman Spectroscopy

A Raman spectrometer (Renishaw system-1000) set in "VN" configuration was used to investigate the stress-transfer mechanisms of unmodified and glyoxalised BC networks. The samples were deformed by using strain increments of 0.025 % and then 0.1 % at a 0.033 mm min$^{-1}$ elongation rate. Strain increment steps of 0.025 % were chosen up to a strain of 0.4 % so that sufficient data could be obtained from brittle glyoxalised BC networks. These increments were also used
for unmodified BC networks for consistency reasons. Experimental tests were repeated twice for unmodified, "thermally treated" and glyoxalised BC networks.

The "thermally treated" samples correspond to unmodified BC networks exposed to the all elements of the glyoxalisation procedure, but without the presence of glyoxal during the impregnation step. The stress-transfer mechanisms in these materials, were also investigated.

The stress-transfer mechanisms of unmodified and glyoxalised materials were also investigated in the wet state after the samples had been submerged in deionised water for 48 h. The samples were subsequently taken away out of the water. Cyanoacrylate glue was then utilised to stick the samples on paper testing cards. Experimental tests were also repeated twice for consistency reasons.

6.3 Results and Discussion

Figure 6.1 illustrates the chemical reaction that can occur between glyoxal and side hydroxyl groups located along the backbone structure of cellulose. Glyoxal molecules can react with cellulose in order to form either or both acetal and hemiacetal linkages between cellulose polymer chains (Schramm and Rinderer 2000). This chemical reaction is referred to as glyoxalisation and has been described in details by Head (1958). The glyoxalisation reaction is facilitated in the presence of catalysts such as aluminium salts (Kullman and Reinilardt 1978; Welch and Forthright Danna 1982; Welch 1983). The use of these catalysts has been, for instance, found to be particularly efficient to cross-link cotton with formaldehydes (Meyer et al., 1976). Acetalysation of cellulose belongs to the family of proton-catalysed formation reactions (Welch 1983).
6.3.1 Dissolution Test

A possible route to verify if cellulose has been successfully cross-linked is to expose samples to a solvent that can dissolve cellulose. This method has been utilised to check if cotton was successfully cross-linked (Reeves et al., 1955). This work by Reeves et al. (1955) reported the uses of cuprammonium hydroxide which can fully dissolve unmodified cellulose.

Consequently, unmodified as well as glyoxalised materials were exposed to another good solvent for cellulose, namely LiCl/DMAc. After 24 hours, unmodified BC networks were entirely dissolved whereas glyoxalised BC networks

Figure 6.1 Cross-linking reaction between cellulose and glyoxal illustrating the formation of hemiacetal and acetal linkages.
were not dissolved and still swollen after a 2 month solvent exposure. The percentage of swelling of glyoxalised BC networks was determined after 48 hours of immersion in the solvent and a value of 30 ± 4 % has been obtained.

6.3.2 Crystallinity and Crystal Morphology

Figure 6.2 reports typical XRD patterns for unmodified and glyoxalised BC networks. The 101, 10 ̅1, and 002 diffraction planes, typical for cellulose, have been observed for both unmodified and glyoxalised BC networks. This demonstrates that glyoxalisation does not modify the crystal structure of cellulose.

![X-ray diffraction patterns](image)

**Figure 6.2** Typical X-ray diffraction patterns for unmodified and glyoxalised BC networks.

Table 6.1 reports crystallinity index, lateral crystal size and lattice spacing for unmodified and glyoxalised BC networks. No significant differences between these materials are noticed. This is due to the high crystallinity index of BC so the number of available hydroxyl groups that can react with glyoxal is limited.
Hydroxyl groups located in crystalline regions have been reported to be unavailable for chemical reaction (Krässig 1993). Consequently glyoxalisation of BC can be classified as a heterogeneous chemical reaction.

**Table 6.1** Crystallinity index (CrI), lateral crystal size (L) and lattice spacing (c) for unmodified and glyoxalised BC networks.

<table>
<thead>
<tr>
<th>Material</th>
<th>CrI (%)</th>
<th>L (002) (Å)</th>
<th>c (002) (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified BC</td>
<td>94.3 ± 1.2</td>
<td>63.0 ± 2.1</td>
<td>7.8 ± 0.0</td>
</tr>
<tr>
<td>Glyoxalised BC</td>
<td>93.9 ± 0.2</td>
<td>63.6 ± 0.5</td>
<td>7.9 ± 0.0</td>
</tr>
</tbody>
</table>

### 6.3.3 Thermal Degradation

Figure 6.3 reports averaged thermal degradation profiles for unmodified and glyoxalised BC networks. The inset to Figure 6.3 shows similar profiles in the temperature range of 25 to 200 °C. A first weight loss is observed and is very likely to be due to water evaporation. This can be observed in both unmodified and glyoxalised BC networks. The weight loss in this region is, however, not significantly different between these materials. This may indicate that the moisture content at ambient conditions, prior to experiments, was similar for all samples. This is important information, since differences in moisture content may influence both stress-transfer and mechanical properties. Consequently if significant differences are observed in terms of stress-transfer and mechanical properties for dry unmodified and glyoxalised BC networks reported in Sections 6.3.7 and 6.3.9, it is not likely to be due to a difference in moisture content.

Table 6.2 reports onset and peak degradation temperatures for unmodified and glyoxalised BC networks. No significant difference between the materials is
reported for peak degradation temperatures. The onset degradation temperature of
glyoxalised networks is however significantly lower than unmodified BC networks.
This result is in agreement with two independent studies (Choi et al., 1999; Lee and
Kim 2004). Therefore, glyoxalisation of BC networks does not dramatically
decrease their thermal stability.

![Graph showing thermal degradation profiles for unmodified and glyoxalised
BC networks. Error bars indicate standard deviations from the mean. The inset
represents the thermal behaviour from 25 °C to 200 °C.]

**Figure 6.3** Averaged thermal degradation profiles for unmodified and glyoxalised
BC networks. Error bars are standard deviations from the mean. The inset
represents the thermal behaviour from 25 °C to 200 °C.

<table>
<thead>
<tr>
<th>Material</th>
<th>Onset degradation temperature (°C)</th>
<th>Peak degradation temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified BC</td>
<td>319 ± 1</td>
<td>335 ± 4</td>
</tr>
<tr>
<td>Glyoxalised BC</td>
<td>299 ± 1</td>
<td>330 ± 3</td>
</tr>
</tbody>
</table>
6.3.4 Wettability

The wettability of unmodified and glyoxalised BC networks has been investigated by measuring water/air contact angles on their surface. This has been performed to verify if glyoxal hydrophobise BC networks. This effect was suspected since independent research has reported that glyoxal can hydrophobise chitosan (Gupta and Jabrail 2006).

Measurements revealed that unmodified and glyoxalised BC networks have respectively advancing contact angles of 17.4 ± 1.7° and 29.6 ± 1.8°. A receding contact angle of 9.9 ± 0.6° has been measured for glyoxalised BC networks. It has been, however, impossible to measure the receding angle for unmodified BC networks due to their high hydrophilicity. Results obtained by Gupta et al. (2006) revealed that glyoxal can hydrophobise chitosan to a greater extent than BC networks.

6.3.5 Surface Electrochemistry

Figure 6.4 reports streaming zeta-potentials data against pH for unmodified and glyoxalised BC. For both materials, the streaming zeta-potentials are positive and decrease towards lower streaming zeta-potential values when increasing the pH. This indicates that the surface of both unmodified and glyoxalised BC networks is comprised of acidic moieties. A further indication is the presence of the isoelectric point (iep) at an acidic pH. The rate of change of streaming zeta-potential is even higher for glyoxalised BC networks and even higher negative values are obtained compared to unmodified BC networks. This means that the surface of glyoxalised BC networks contains even more acidic moieties than unmodified BC networks.
This is also supported by the shift of the iep towards a lower pH for glyoxalised BC samples.

Figure 6.4 Streaming zeta-potentials of unmodified and glyoxalised BC networks as a function of pH.

Figure 6.5 reports streaming zeta-potential data as a function of time for unmodified and glyoxalised BC networks. These data were particularly useful to calculate the parameter $\Delta \zeta$ for both materials. High $\Delta \zeta$ values correspond to materials with high relative water absorption capacity. This parameter was found to be 0.255 and 0.184 for respectively unmodified and glyoxalised BC networks. As expected, this means that unmodified BC networks can absorb more water than glyoxalised BC networks due to their relative hydrophilicity.
6.3.6 Relative Water Absorption Capacity

Figure 6.6 reports relative water absorption capacity (RWAC) data against time for unmodified and glyoxalised BC networks. "Relative" water absorption capacity data are reported here because the data are relative to environmental storage conditions of the samples prior to testing. Experimental data suggest that both unmodified and glyoxalised BC networks have been saturated by water after 2 hours of immersion. Their relative water absorption capacity does not significantly change at higher immersion times (5, 12, 24 and 48 hours).

More importantly a significant RWAC difference is noted between unmodified and glyoxalised BC networks. This is probably due to the fact that glyoxalised BC networks swell less due to the presence of chemical cross-links. This result confirms the data obtained from streaming zeta-potential measurements where a higher $\Delta \zeta$ value was obtained for unmodified BC networks. These data are also supported by measurements of the advancing contact angle, which are lower for
unmodified BC networks. Chitosan modified with glyoxal has also shown lower relative water absorption capacity compared to unmodified chitosan (Gupta and Jabrail 2006).

Water absorption is an important issue for cellulose-containing composites (Berglund and Peijs 2010). Consequently by modifying the cellulose reinforcement with glyoxal, one might be able to produce bio-composites with improved resistance to humidity.

![Graph showing relative water absorption against time for unmodified and glyoxalised BC networks.](image)

**Figure 6.6** Relative water absorption against time for unmodified and glyoxalised BC networks.

### 6.3.7 Tensile Mechanical Properties

Figure 6.7 shows typical stress-strain curves for dry and wet unmodified and glyoxalised BC networks. A typical stress-strain curve is also reported for dry "thermally treated" unmodified BC networks. The detailed tensile mechanical properties of all these materials are summarised in Table 6.3. One can observe that Young’s moduli for dry unmodified and glyoxalised BC networks are not
significantly different, with respective values of $10.1 \pm 1.5$ and $10.7 \pm 1.4$ GPa. The stress and strain at failure and work-of-fracture of dry unmodified and glyoxalised materials are however significantly reduced, as shown in Table 6.3. This is probably because glyoxalisation induced the formation of covalent cross-links between BC layers which reduces the BC layer-to-layer mobility and consequently less energy is dissipated during the deformation process.

![Typical stress-strain curves for dry and wet unmodified and glyoxalised BC networks.](image)

**Figure 6.7** Typical stress-strain curves for dry and wet unmodified and glyoxalised BC networks.

In order to investigate the influence of the glyoxalisation treatment on the tensile mechanical properties of dry unmodified BC networks, BC samples have been exposed to the glyoxalisation procedure without using glyoxal. These samples are referred to as dry "thermally treated" BC networks. Data reported in Table 6.3 shows that Young’s modulus is significantly reduced after this treatment has been performed. This significant reduction of Young’s modulus may be due to a reduction of the level of hydrogen bonding between BC nanofibrils. This result
may explain why Young’s modulus of dry glyoxalised BC networks were found not to be significantly higher than Young’s modulus of dry unmodified BC networks. Stress and strain at failure and work-of-fracture are however not significantly altered.

Detailed wet tensile mechanical properties for unmodified and glyoxalised BC networks are also summarised in Table 6.3. Young’s modulus and stress at failure of wet unmodified BC networks are significantly reduced by respectively 438 % and 1570 %. The strain at failure is increased by 261 %. These results can be explained by the presence of water which preferably creates hydrogen bonding between BC nanofibrils. Consequently the intermolecular hydrogen bonding present between BC nanofibrils is disrupted and this may explain the dramatic reduction of both Young’s modulus and stress at failure of BC networks when exposed to water. The glyoxal treatment, however, prevents this loss of tensile mechanical properties by providing water resistant cross-linking. Young’s modulus and stress at failure for wet glyoxalised BC networks are respectively ~3 and 8 times higher than for wet unmodified BC networks. It is very likely that even if the hydrogen bonding between BC fibrils is disrupted, the presence of covalent cross-links helps to maintain the tensile mechanical properties of wet BC networks. The strain at failure and work-of-fracture of wet glyoxalised BC networks is, however, significantly reduced probably due to the presence of cross-links which prevent BC polymer chains from slipping past each other, and so the energy dissipation is reduced as observed for dry glyoxalised BC networks.
Table 6.3 Tensile mechanical properties for dry and wet unmodified and glyoxalised BC networks; E - Young's modulus, \(\sigma_f\) - stress at failure, \(\varepsilon_f\) - strain at failure and \(G\) - work-of-fracture.

<table>
<thead>
<tr>
<th>Material</th>
<th>E (GPa)</th>
<th>(\sigma_f) (MPa)</th>
<th>(\varepsilon_f) (%)</th>
<th>(G) (MJ m(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry unmodified BC</td>
<td>10.1 ± 1.5</td>
<td>165.1 ± 33.9</td>
<td>2.6 ± 0.6</td>
<td>2.5 ± 0.9</td>
</tr>
<tr>
<td>Dry unmodified BC (thermally treated)</td>
<td>7.8 ± 1.1</td>
<td>180.0 ± 24.2</td>
<td>3.6 ± 0.4</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>Dry glyoxalised BC</td>
<td>10.7 ± 1.4</td>
<td>71.0 ± 36.0</td>
<td>0.6 ± 0.3</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Wet unmodified BC</td>
<td>1.9 ± 0.2</td>
<td>9.9 ± 1.5</td>
<td>9.3 ± 1.9</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td>Wet glyoxalised BC</td>
<td>6.0 ± 1.4</td>
<td>76.8 ± 21.2</td>
<td>1.5 ± 0.7</td>
<td>1.3 ± 0.8</td>
</tr>
</tbody>
</table>

6.3.8 Tensile Fracture Surface

Figures 6.8a and 6.8b report scanning electron microscopy images showing the morphology of tensile fracture surfaces for dry unmodified and glyoxalised BC networks. A clear delamination mechanism is observed for dry unmodified BC networks. A brittle tensile fracture surface is, however, observed for dry glyoxalised BC networks. This may indicate the successful covalent coupling of BC layers owing to the glyoxalisation reaction. In addition this may explain the reduction of stress and strain at failure and work of fracture for dry glyoxalised BC networks as shown in Section 6.3.7.

Figure 6.8c reports a scanning electron microscopy image of the morphology of tensile fracture surfaces for unmodified BC networks previously tested in the wet state. During imaging, the samples were however thought to be no longer wet due to the presence of a vacuum in the SEM. This image shows that the sample dimensions are considerably reduced and that a sort of "plasticisation" has occurred. A typical stress-strain curve has been shown in Section 6.3.7 showing that wet unmodified BC networks behave a bit like thermoplastic polymer
materials with an initial elastic behaviour, followed by a yield point, and subsequently plastic deformation until complete sample fracture. Figure 6.8d reports a scanning electron image in the longitudinal direction of wet unmodified BC network samples. This image is a further indication that some sort of "plasticisation" occurs for these samples (see arrows). Figure 6.8e is a scanning electron microscopy image of the tensile fracture surface of wet glyoxalised BC networks. Here, the sample dimensions are preserved. A slight delamination between the BC layers is, however, observed.

![Scanning electron microscope images of fracture surfaces](image)

**Figure 6.8** Scanning electron microscope images of fracture surfaces for dry (a) unmodified and (b) glyoxalised BC networks and for wet (c) unmodified and (e) glyoxalised BC networks. Image (d) presents a longitudinal observation in the tensile direction for wet unmodified BC networks.
6.3.9 Molecular deformation

Figure 6.9 reports typical Raman spectra for unmodified and glyoxalised BC networks. One can observe that there is no difference between these spectra. The signal from glyoxalised BC networks is, however, slightly higher than for unmodified BC networks. This is likely to be due to a thickness effect. The particular glyoxalised BC network chosen to obtain this spectrum was probably slightly thicker than the unmodified BC network used. No change in the shape of the Raman bands may further reveal that the glyoxalisation reaction does only heterogeneously modify the surface of BC nanofibrils. This was also suggested by powder X-ray diffraction data presented in Section 6.3.2.

![Raman spectra](image)

**Figure 6.9** Typical Raman spectra for dry unmodified and glyoxalised BC networks.

It is also important to note the presence of the Raman band located at ~1095 cm\(^{-1}\) for glyoxalised BC networks means that it is then possible to follow the shift towards a lower wavenumber position in these materials, as already described in
Sections 4.3.3 and 5.3.2.5. A typical shift of the Raman band located at \(\sim 1095 \text{ cm}^{-1}\) towards a lower wavenumber is reported in Figures 6.10 and 6.11 for respectively unmodified and glyoxalised BC networks before and after being submitted 0.5\% tensile deformation. The origin of this shift has already been discussed in detail in Sections 2.3.7, 4.3.3 and 5.3.2.5.

![Graph showing Raman shift](image)

**Figure 6.10** A typical shift in the position of the Raman band initially located at \(\sim 1095 \text{ cm}^{-1}\) towards a lower wavenumber position for a dry unmodified BC network.
Figure 6.11 A typical shift in the position of the Raman band initially located at \(~1095\ \text{cm}^{-1}\) towards a lower wavenumber position for a dry glyoxalised BC network.

Figure 6.12 reports detailed shifts towards a lower wavenumber for the Raman band initially positioned at \(~1095\ \text{cm}^{-1}\) for unmodified BC networks and "thermally treated" unmodified BC networks. One can observe a significant difference between the average gradient of fit obtained for these materials. Gradient of fits of \(-0.9 \pm 0.1\) and \(-0.6 \pm 0.1\ \text{cm}^{-1} \%^{-1}\) have been respectively obtained for unmodified and "thermally treated" unmodified BC networks. These results shows that the molecular deformation is higher for unmodified BC networks compared to "thermally" treated BC networks. These results might be related to a reduction of the level of hydrogen bonding naturally formed between BC nanofibrils due to exposure of the samples to high temperature. This is also supported by higher Young’s modulus data obtained for unmodified BC networks compared to "thermally" treated unmodified BC networks in Section 6.3.7.
Figure 6.12 Detailed typical shifts in the wavenumber position of the Raman band initially located at ~1095 cm\(^{-1}\) for dry unmodified “thermally treated” and unmodified BC networks. Gradient of the linear fits reports both the mean and standard deviations values. Two repeats of each experiment are shown (open and closed symbols).

Figure 6.13 reports detailed shifts towards a lower wavenumber for the Raman band initially positioned at ~1095 cm\(^{-1}\) obtained for dry unmodified and glyoxalised BC networks. The gradients of a linear fit to these data were respectively found to be -0.9 ± 0.0 and -1.6 ± 0.0 cm\(^{-1}\) %\(^{-1}\). Consequently the molecular deformation in glyoxalised BC networks is significantly higher than for unmodified BC networks. This may be due to the presence of chemical cross-links which prevents the delamination of BC layers during tensile deformation as shown by SEM imaging reported in Section 6.3.8. This is also supported by the reduced stress at failure and work of fracture obtained for glyoxalised BC networks compared to unmodified BC networks as reported in Section 6.3.7.
Figure 6.13 Detailed typical shifts in the wavenumber position of the Raman band initially located at ~1095 cm\(^{-1}\) for (a) dry unmodified and glyoxalised BC networks. Gradient of the linear fits reports both the mean and standard deviations values. Two repeats of each experiment are shown (open and closed symbols).

Figure 6.14 reports detailed shifts for wet unmodified and glyoxalised BC networks. A gradient of a linear fit to the data of -1.4 ± 0.1 cm\(^{-1}\) %\(^{-1}\) has been obtained for glyoxalised BC networks. For unmodified BC networks there is, however, no shift. This is probably because the presence of water disrupts the hydrogen bonding present between BC nanofibrils due to competitive hydrogen bonding formation between hydroxyl groups of the polymer and hydrogen bonding formation between hydroxyl groups of cellulose chains and water molecules, as already mentioned in Section 2.1.6. The presence of water has also been found to suppress or "turn-off" the stress-transfer process in cellulose nanowhisker-containing nanocomposites (Rusli et al., 2010). The presence of chemical cross-links may be the reason why one can still observe shifts from glyoxalised BC networks.
networks. Even if the hydrogen bonding is disrupted, the chemical cross-links are still present and prevent slippage between BC nanofibrils. These observations are also supported by tensile mechanical data reported in Section 6.3.7 where Young’s modulus and stress at failure of wet glyoxalised BC networks are significantly higher than for unmodified BC networks.

![Graph showing strain vs. Raman band shift](image)

**Figure 6.14** Detailed typical shifts in the wavenumber position of the Raman band initially located at ~1095 cm\(^{-1}\) for wet unmodified and glyoxalised BC networks. *Gradient of the linear fits reports both the mean and standard deviations values.*

### 6.4 Conclusions

Cross-linking of BC networks using glyoxal was found to be a successful way to consolidate the layered structure of BC networks in both dry and wet states. Determination of the tensile mechanical properties in the dry state revealed that stress and strain at failure and work of fracture of glyoxalised BC networks are significantly reduced compared to unmodified BC networks. Young’s modulus was, however, found not to be significantly changed. As suggested by tensile tests
and Raman spectroscopy experiments performed on "thermally treated" unmodified BC networks, this might be explained by a possible reduction of the level of hydrogen bonding between BC nanofibrils due to exposure to relatively high temperature. Observation of the tensile fracture surface of unmodified and glyoxalised BC networks deformed in the dry state revealed a brittle fracture surface for glyoxlised BC networks. This is indicative of successful covalent coupling between BC layers constituting these BC networks. Delamination between BC layers was, however, observed for unmodified BC networks.

In the wet state, glyoxalisation was found to be particularly useful to maintain the tensile mechanical properties of BC networks when exposed to water. Raman spectroscopy showed that stress-transfer is also preserved thanks to the presence of chemical cross-links. The stress-transfer of wet unmodified BC networks was, however, completely suppressed due to the presence of water.

Future work might consider the use of other cross-linkers such as dialdehyde modified cellulose. More importantly the need to verify the benefit of using cross-linked BC network in a composite material is of interest. This is what it is proposed in Chapter 7.

6.5 References


CHAPTER 7
EFFECT OF GLYOXALISATION AND MALEATION ON THE MECHANICAL PERFORMANCE OF BACTERIAL CELLULOSE/POLYLACTIDE COMPOSITES

7.1 Introduction
As shown in Chapter 5, delamination between BC layers was found to occur predominantly in the networks themselves rather than at the BC/PLA interface. BC networks have therefore been cross-linked using glyoxal as shown in Chapter 6. This modification has been performed to create covalent coupling to consolidate the structure of BC networks. Glyoxalisation of BC networks resulted in a significantly improved stress-transfer in the dry and wet states over unmodified BC networks. Consequently it was interesting to verify if the use of cross-linked BC networks could improve the stress-transfer and the tensile mechanical properties of BC/PLA composites. Glyoxalised BC networks have therefore been used to design composites and some physical properties have been determined and compared to composites prepared using unmodified BC networks.

Polylactide (PLA) and other polymers such as polypropylene or polyethylene, to cite a few examples, can be modified by reactive extrusion using maleic anhydride and dicumyl peroxide. Maleic anhydride (polar monomer) can be grafted along the backbone of a polymer chain to improve its compatibility with other materials such as starch (Zhang and Sun 2004) or silicates (Petersson et al., 2006). This modified PLA is commonly referred to as maleated polylactide (MAPLA). Successful examples of improved compatibility between MAPLA and natural fibres have been reported in the literature (Keener et al., 2004; Chin-San 2009; Nyambo et al.,
Also the modification of BC using maleic anhydride was found to improve its compatibility with PLA (Li et al., 2010).

In this study, maleated polylactide has been prepared and subsequently utilised to design composites using unmodified and glyoxalised BC networks. Both the effect of modification of the matrix and the reinforcement on the mechanical and the stress-transfer properties are consequently reported.

7.2 Materials and Methods

7.2.1 Materials

BC networks were prepared using a culturing time 14 days, as described in Section 3.2.1. The density of these networks was estimated to be ~1.1 g cm$^{-3}$ as shown in Section 5.3.1.1.

PLA L9000 was provided by Biomer (Krailing, Germany). Glyoxal (~40 wt.% in de-ionised water), aluminium sulfate hexadecahydrate ($\text{Al}_2(\text{SO}_4)_3\cdot 16\text{H}_2\text{O}$, purity $\geq 98\%$), maleic anhydride (MA) and dicumyl peroxide (DiP) were all supplied by Sigma-Aldrich (Gillingham, UK).

7.2.2 Sample Preparation and Experimental Parameters

7.2.2.1 Preparation of Glyoxalised Networks

Bacterial cellulose networks were glyoxalised as described in Section 6.2.2.

7.2.2.2 Preparation of Maleated Polylactide

PLA pellets were dried at 40 $^\circ$C for at least 24 h prior to extrusion. Maleated PLA was formulated by adding ~2 wt.% of MA based on the added amount of PLA in the extruder and by adding ~0.5 wt.% of DiP based on the weight of maleic
anhydride. A Haake miniCTW micro compounding (Thermo Scientific) was used to produce MAPLA. PLA was processed for 3 min at 180 °C with a screw speed of 100 rpm.

7.2.2.3 Gel Permeation Chromatography
The GPC measurements were conducted by Dr. Koon-Yang Lee in collaboration with Prof. Alexander Bismarck (Polymer and Composites Engineering Group, Department of Chemical Engineering, Imperial College London).

The measurements were carried out on both processed PLA and MAPLA films using a Polymer Laboratories SEC 50 instrument with two Polymer Laboratories mixed D columns and CHCl$_3$, at a flow rate of 1 ml min$^{-1}$, as the eluent. Narrow molecular weight polystyrene standards (Polymer laboratories, mixed A and B) were used to calibrate the instrument.

7.2.2.4 $^1$H Nuclear Magnetic Resonance
The $^1$H NMR analysis was kindily conducted by Dr. Koon-Yang Lee in collaboration with Prof. Alexander Bismarck (Polymer and Composites Engineering Group, Department of Chemical Engineering, Imperial College London).

The $^1$H NMR analysis was performed using a Bruker AV500 instrument; $^1$H NMR spectra were collected at 500 MHz. Deuterated chloroform (CDCl$_3$) was used as the NMR solvent and reference compound. The concentration of succinyl anhydride groups has been calculated by dividing the total area under the curves (total number of moles of material) by the area of the peaks corresponding to the presence of succinyl anhydride groups.
7.2.2.5 Composite Preparation

BC/PLA, BCG/PLA, BC/MAPLA and BCG/MAPLA composites were prepared as described in Section 3.2.2.2. Their respective BC weight fractions were respectively 15.2 ± 3.2, 13.6 ± 1.2, 15.3 ± 1.2 and 17.0 ± 2.1 wt.%.

7.2.2.6 Thermogravimetric Analysis

The thermal degradation behaviour of PLA and MAPLA films and BC/PLA, BC/MAPLA, BCG/PLA and BCG/MAPLA composites were investigated using thermogravimetric analysis (TGA Q500). The samples (~4 mg) were heated from ~35 to 550 °C at a heating rate of 5 °C min\(^{-1}\) under a flow of 60 ml min\(^{-1}\) nitrogen purge gas. Experiments were repeated three times and averages and their standard deviations are reported. The onset and degradation temperatures were obtained from the first derivative of the weight loss as a function of temperature. The temperature control in the furnace was ± 1 °C.

7.2.2.7 Tensile Tests

The tensile mechanical properties of PLA and MAPLA films and BC/PLA, BC/MAPLA, BCG/PLA and BCG/MAPLA composites were determined as described in Section 5.2.2.1.4.

7.2.2.8 Scanning Electron Microscopy

The morphology of the tensile fracture surfaces of BC/PLA, BC/MAPLA, BCG/PLA and BCG/MAPLA composites were imaged as described in Section 5.2.4.8.
7.2.2.9 Raman Spectroscopy

The molecular deformation of BC/PLA, BC/MAPLA, BCG/PLA and BCG/MAPLA composites was followed as described in Section 5.2.2.1.6.

7.3 Results and Discussion

7.3.1 Molecular Weight of PLA and MAPLA

Figure 7.1 illustrates the chemical reaction that can occur in specific conditions between PLA and maleic anhydride using dicumyl peroxide. This reaction is based on a free radical branching reaction which has been described in detail by Carlson et al. (1998). They subsequently used this maleation reaction procedure to modify PLA with maleic anhydride (MA) by reactive extrusion (Carlson et al., 1999).

\[ \text{Polylactide (PLA)} \rightarrow \text{Dicumyl peroxide (DiP)} \]

\[ 180^\circ \text{C, 3 min} \]

\[ \text{Maleic anhydride (MA)} \]

**Figure 7.1** Chemical reaction occurring during extrusion of PLA in the presence of maleic anhydride and dicumyl peroxide. Reproduced with modifications from Nyambo et al. (2011).

Table 7.1 reports the number-average \( (M_n) \) and the weight-average \( (M_w) \) molecular weight as well as the degree of polydispersity \( (DP) \) of PLA and MAPLA. One can see that after reactive extrusion of PLA with MA and DiP, \( M_n \) and \( M_w \) are respectively reduced by approximately half. DP is consequently increased which is related to a change of PLA chain size distribution. These changes in \( M_n, M_w \) and
DP are due to chemical and thermal degradation reactions occurring due to the presence of free radicals that break the PLA chains (Nyambo et al., 2011). This reduction of molecular weight during the maleation reaction of PLA has also been reported by Carlson et al. (1999). These reactions are also revealed by a colour change of PLA. PLA films were white and transparent, whereas MAPLA films exhibit a very slightly yellowish/brownish colour as shown in Figure 7.2. BC/PLA composites had the same appearance as the image shown in Figure 5.3 for a BC6/PLA composite. A very slightly yellowish/brownish colour was also observed for BCG/PLA, BC/MAPLA and BCG/MAPLA due to glyoxalisation and maleation reactions.

![Image of transparent PLA and MAPLA films.](image)

**Figure 7.2** An image of transparent PLA and MAPLA films.
**Table 7.1** Gel permeation chromatography measurements performed on PLA and MAPLA films. $M_n$ is the number-average molecular weight, $M_w$ is the weight-average molecular weight and PD is the polydispersity.

<table>
<thead>
<tr>
<th>Material</th>
<th>$M_n$ (g mol$^{-1}$)</th>
<th>$M_w$ (g mol$^{-1}$)</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>160 000</td>
<td>212 000</td>
<td>1.3</td>
</tr>
<tr>
<td>MAPLA</td>
<td>73 000</td>
<td>122 000</td>
<td>1.7</td>
</tr>
</tbody>
</table>

7.3.2 Determination of the Amount of Grafted Maleic Anhydride

Figure 7.3 reports liquid-state NMR traces obtained from PLA and MAPLA films previously dissolved in CDCl$_3$ prior to performing the analysis. The NMR profile corresponding to MAPLA exhibits a peak located at ~2.7 ppm. This peak is however missing for PLA.

![NMR spectra](image)

**Figure 7.3** Typical $^1$H NMR spectra for PLA and MAPLA films.
The peak located at ~2.7 ppm for MAPLA has been reported in the literature to correspond to the presence of succinyl anhydride groups (Thompson et al., 1998 and 1999; Vicente et al., 2011). The grafting efficiency of maleic anhydride on PLA was found to be ~1.6 mol % which corresponds to ~2.2 wt.%. The presence of this peak located at ~2.7 ppm demonstrates that some maleic anhydride monomers have been successfully grafted on PLA chains. The presence of other peaks located at ~7 and 6.5 ppm correspond to the presence of remaining unreacted maleic anhydride (Pretsch et al., 2000).

7.3.3 Thermal Behaviour

Figure 7.4 reports thermal degradation profiles for PLA, MAPLA, BC/PLA, BCG/PLA, BC/MAPLA and BCG/MAPLA materials.

![Figure 7.4](image-url)

*Figure 7.4 Typical thermal degradation profiles for PLA, MAPLA, BC/PLA, BCG/PLA, BC/MAPLA and BCG/MAPLA materials.*
A significant difference between the onset degradation temperatures is noted between PLA and MAPLA as shown in Table 2. This probably due to the lower molecular weight of MAPLA compared to PLA as reported in Section 7.3.1.

**Table 7.2 Thermal properties for PLA, MAPLA, BC/PLA, BCG/PLA, BC/MPLA, BCG/MAPLA materials. Error bars are standard deviations from the mean for 3 samples.**

<table>
<thead>
<tr>
<th>Material</th>
<th>Onset degradation temperature (°C)</th>
<th>Peak degradation temperature (°C)</th>
<th>Percentage residual mass at 500°C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>317 ± 1</td>
<td>346 ± 2</td>
<td>-0.2 ± 0.1</td>
</tr>
<tr>
<td>MAPLA</td>
<td>313 ± 2</td>
<td>345 ± 1</td>
<td>-0.1 ± 0.1</td>
</tr>
<tr>
<td>BC/PLA</td>
<td>309 ± 5</td>
<td>343 ± 8</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>BCG/PLA</td>
<td>309 ± 4</td>
<td>345 ± 4</td>
<td>3.5 ± 0.8</td>
</tr>
<tr>
<td>BC/MAPLA</td>
<td>303 ± 1</td>
<td>336 ± 1</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>BCG/MAPLA</td>
<td>292 ± 1</td>
<td>326 ± 2</td>
<td>2.7 ± 0.3</td>
</tr>
</tbody>
</table>

The composites did not show significant differences in terms of onset and peak degradation temperatures except for BC/MAPLA and BCG/MAPLA. These composites have shown significantly lower onset and peak degradation temperatures probably due to the lower onset degradation temperature of MAPLA compared to PLA. In addition, the thermal properties of BCG/MAPLA composites are significantly lower compared to BC/MAPLA composites. This is probably due to the lower onset and peak degradation temperatures presented in Section 6.3.3 for glyoxalised BC networks compared to unmodified BC networks.

The percentage residual mass measured at a temperature of 500 °C was found to be significantly higher for all composites compared to PLA and MAPLA. This is due to the presence of BC networks, which increases the thermal stability of PLA.
and MAPLA in the temperature range of ~350 °C to at least 550 °C. This can be explained by the higher thermal stability of unmodified and glyoxalised BC networks are reported in Section 6.3.3 compared to PLA and MPLA.

### 7.3.4 Mechanical Properties

Figure 7.5 reports typical stress-strain curves for PLA and MAPLA films and BC/PLA, BCG/PLA, BC/MAPLA and BCG/MAPLA composites. The detailed mechanical properties data are reported in Table 7.3.

Young’s modulus and strain at failure of PLA films are significantly reduced after its modification with MA. This is possibly due to the reduction of the molecular weight of MAPLA, as measured using GPC. Stress at failure is however significantly increased. Consequently it is important to normalise Young’s moduli of the composites by dividing by the value obtained for the respective matrix material.

![Figure 7.5 Typical stress-strain curves for PLA, MAPLA, BC/PLA, BCG/PLA, BC/MAPLA, BCG/MAPLA materials.](image-url)
If now Young’s moduli of the composites are compared, one can see that Young’s moduli of BC/PLA, BCG/PLA and BC/MAPLA are not significantly different. These values are lower than the Young’s moduli predicted from the rule of mixtures. Young’s modulus of BCG/MAPLA is however significantly increased and closer to the predicted Young’s modulus. Also the BCG/MAPLA composites exhibited a higher relative modulus ($E_c/E_m$), which is indicative of a better interface. The origin of higher Young’s modulus for BCG/MAPLA composites might come from reduced mobility both at the BCG/MAPLA and BCG/BCG interface. This reduced mobility in BCG/MAPLA composites is further supported by work of fracture values for this sample, which are the lowest of all composites. Inspection of the tensile fracture surface will give further information about deformation mechanisms occurring in these composites.

**Table 7.3** Tensile mechanical properties for PLA, MAPLA, BC/PLA, BCG/PLA, BC/MAPLA and BCG/MAPLA materials; $E$ is Young’s modulus, $E_m$ is Young’s modulus of the matrix, $E_c$ is Young’s modulus of the composites, $E_{predicted}$ is the predicted Young’s modulus calculated from the rule of mixture, $\sigma_f$ is the stress at failure, $\varepsilon_f$ is the strain at failure and $G$ is the work-of-fracture.

<table>
<thead>
<tr>
<th>Material (BC vol.%)</th>
<th>$E$ (GPa)</th>
<th>$E_c/E_m$</th>
<th>$E_{predicted}$ (GPa)</th>
<th>$\sigma_f$ (MPa)</th>
<th>$\varepsilon_f$ (%)</th>
<th>$G$ (MJ m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>2.0 ± 0.2</td>
<td>1</td>
<td>NA</td>
<td>27.7 ± 2.5</td>
<td>23.5 ± 17</td>
<td>-----</td>
</tr>
<tr>
<td>MAPLA</td>
<td>1.6 ± 0.2</td>
<td>1</td>
<td>NA</td>
<td>33.9 ± 3.4</td>
<td>1.6 ± 0.2</td>
<td>-----</td>
</tr>
<tr>
<td>BC/PLA (13.8 ± 3.2)</td>
<td>2.7 ± 0.3</td>
<td>1.4</td>
<td>3.1</td>
<td>65.9 ± 2.3</td>
<td>3.0 ± 0.5</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>BCG/PLA (12.3 ± 1.2)</td>
<td>2.5 ± 0.1</td>
<td>1.3</td>
<td>3.1</td>
<td>55.9 ± 4.5</td>
<td>2.4 ± 0.2</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>BC/MAPLA (13.9 ± 1.2)</td>
<td>2.7 ± 0.2</td>
<td>1.7</td>
<td>3.1</td>
<td>32.2 ± 5.4</td>
<td>1.2 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>BCG/MAPLA (15.5 ± 2.1)</td>
<td>3.2 ± 0.2</td>
<td>2.0</td>
<td>3.3</td>
<td>39.0 ± 9.3</td>
<td>1.4 ± 0.2</td>
<td>0.1 ± 0.1</td>
</tr>
</tbody>
</table>
7.3.5 Micromechanical Modelling

By using the experimental Young’s modulus and stress at failure values obtained for PLA, BC3/PLA and BC6/PLA in Section 5.3.2.3 and for BC/PLA composites reported in Section 7.3.2, the influence of BC volume fraction on both Young’s modulus and the stress at failure of composites was investigated. Experimental data have been fitted using theoretical models that have been used previously in the literature to predict Young’s modulus and stress at failure of composites and nanocomposites. The "Rule of mixtures" as well as the Halpin-Tsai and Tsai-Pagano models have been used to predict Young’s modulus of BC/PLA composites. Pukanszky’s model was used to predict their stress at failure.

The rule of mixtures can be expressed using the equations

\[ E_c = E_m V_n + E_n (1-V_n) \]  \hspace{1cm} (7.1)

\[ \frac{1}{E_c} = \frac{V_n}{E_n} + \frac{(1-V_n)}{E_m} \]  \hspace{1cm} (7.2)

where \( E_c, E_m \) and \( E_n \) are respectively Young’s modulus of the composite, the matrix and BC networks and \( V_n \) is the volume fraction of BC. Equations 7.1 and 7.2 are the Voigt and Reuss models respectively. Voigt’s model assumes that strain is uniform whereas Reuss’ model assumes a uniform stress.

The Halpin-Tsai model is used to predict the longitudinal (\( E_L \)) and transverse (\( E_T \)) modulus of aligned short fibre composites (Halpin and Kardos 1976). Longitudinal and transverse moduli can be predicted using the equations
Where $E_m$ is Young’s modulus of the matrix and $\zeta$ is a shape parameter of the fibres, $\zeta=(0.5s)^{1.8}$ with $s$ being the aspect ratio (Eichhorn et al., 2010). $\eta_L$ and $\eta_T$ are given by equations:

$$\eta_L = \frac{(E_{fl} / E_m) - 1}{(E_{fl} / E_m) + \zeta}$$  \hspace{1cm} (7.5)

$$\eta_T = \frac{(E_{ft} / E_m) - 1}{(E_{ft} / E_m) + 2}$$  \hspace{1cm} (7.6)

where $E_{fl}$ and $E_{ft}$ are the longitudinal and transverse Young’s modulus of bacterial cellulose nanofibrils and have been reported to be respectively in the range of 79-88 GPa (see Chapter 4) and 11 GPa (Nishino et al., 2002).

Young’s modulus of a 2D in-plane randomly and uniformly distributed fibre composites can be predicted using the Tsai-Pagano model using the equation:

$$E_{random} = (3/8)E_L + (5/8)E_T$$  \hspace{1cm} (7.7)

where $E_{random}$ is the tensile modulus of the randomly oriented fibre. An optimised fitting of the experimental data was found for $s = 6$, $\zeta = 7$, $\eta_L = 0.8$ and $\eta_T = 0.6$. 
Consequently BC/PLA composites would be equivalent to a nanocomposite reinforced with BC nanofibrils having an aspect ratio of 6. This value is low probably because BC nanofibrils were not dispersed in the PLA matrix and so the reinforcement potential of these high aspect ratio fibrils is not fully realised.

A lot of publications mention that BC fibrils have high aspect ratio but there are very few or almost no experimental evidence for this (Kalashnikova et al., 2011). This probably due to the impossibility to determine accurately aspect ratio of BC fibrils in fibrous networks from electron microscopy images (Ogata et al., 2011) due their overlapping and intertwined cellulose nanofibre structure (Jonas and Farah 1998). A range of aspect ratio of ~23-52 for BC nanowhiskers has been reported (Martínez-Sanz et al., 2011). A value of 1000 has been mentioned by Brown (2007) for BC nanofibrils which is much higher than values obtained from cellulose nanowhiskers (Azizi et al., 2005; Yun et al., 2010; Martínez-Sanz et al., 2011; Rusli et al., 2011).

The comparisons between the experimental data of Young’s moduli obtained from tensile tests and predictions from the rule of mixtures and Halpin-Tsai/Tsai-Pagano models are reported in Figure 7.6.

The goodness of fit between experimental data and the theoretical predictions was estimated by calculating the residual sum of squares (RSS) between experimental data and each fit. This allows estimating the discrepancy between experimental data and a theoretical model. The smaller the RSS value, the better the goodness of fit. Voigt’s model fits rather well the experimental data points (RSS = 0.27) compared to Reuss’ model which offers the poorest fitting quality (RSS = 2.94). Consequently the hypothesis of uniform strain is likely to be valid in
this form of laminated composites. In addition the Halpin-Tsai/Tsai-Pagano models offer the same goodness of fit as Voigt’s model (RSS = 0.27).

**Figure 7.6** Experimentally measured tensile modulus for BC/PLA composites compared to theoretically estimations by the rule of mixtures (Voigt and Reuss) and Halpin-Tsai and Tsai-Pagano models.

Stress at failure of the composites, also called sometimes ultimate tensile stress, can be predicted using Pukanszky’s model. Pukanszky’s model assumes that an interphase forms spontaneously in composites and that the ultimate tensile stress is proportional to the volume fraction of the reinforcement phase (Pukánszky 1990; Bilotti *et al.*, 2009). The model is expressed by the following equation

$$\sigma_c = \sigma_m \frac{1-V_f}{1+2.5V_f} \exp(BV_f)$$

(7.8)

or, in terms of relative stress at failure, $\sigma_{rel}$.
\[
\sigma_{rel} = \frac{\sigma_c - 1 + 2.5V_f}{\sigma_m (1 - V_f)} \tag{7.9}
\]

can be rewritten as

\[
\ln(\sigma_{rel}) = \ln \frac{\sigma_c - 1 + 2.5V_f}{\sigma_m (1 - V_f)} = BV_f \tag{7.10}
\]

where \(\sigma_c\) and \(\sigma_{ym}\) are respectively the stress at failure of the composite and the matrix, \(V_f\) is the volume fraction of the filler in the composites and \(B\) is a parameter related to the load carried by the dispersed component and depends on the interaction between the reinforcement and the matrix (Bilotti et al., 2009). \(B\) can be written as

\[
B = (1 + A_f \rho_f l) \ln \frac{\sigma_i}{\sigma_m} \tag{7.11}
\]

where \(A_f\) is the specific surface area of the filler (contact surface), \(\rho_f\) is its density, and \(l\) and \(\sigma_i\) are respectively the thickness and the ultimate tensile stress of the interphase (Pukánszky 1990). An optimised fitting of the experimental data was found for \(B = 9\) which is a way to quantify the strength of the interface. This value is lower than a value of \(B = 14.5\) obtained for sepiolite/polyamide 6 (PA6) nanocomposites (Bilotti et al., 2009) meaning that the interface in sepiolite/PA6 nanocomposites is stronger than BC/PLA composites reported in this thesis.

The comparisons between the experimental data of stress at failure obtained from tensile tests and the prediction from Pukanszky’s model are reported in Figure
7.7. A good agreement between experimental data and Pukanszky’s model is noted (RSS = 0.08).

![Graph of experimental data and Pukanszky's model comparison](image)

**Figure 7.7** The natural logarithm of experimentally measured relative stress at failure for BC/PLA composites compared to a theoretical estimation using Pukanszky’s model.

### 7.3.6 Tensile Fracture Surface

Figures 7.8 reports images showing the morphology of the tensile fracture surface of BC/PLA, BCG/PLA, BC/MAPLA and BCG/MAPLA composites. Figure 7.8a shows that delamination occurs mainly between BC layers rather than at the interface between BC/PLA composites as already observed in Section 5.3.1.5. One can see from Figure 7.8b that delamination of BC layers is largely reduced when BC networks are glyoxalised. Small gaps between BC layers are noted, but that are not visible to the same extent as observed in Figure 6a. In addition to delamination between BC layers, a gap at the BCG/PLA interface is also noted. This is maybe due to the presence of covalent coupling between BC layers; the BCG/PLA...
interface has possibly a lower coupling strength compared to the BCG/BCG interface. This is probably the reason why even if BC is glyoxalised, Young’s modulus of the composite is not increased significantly as reported in Section 7.3.4.

Figure 7.8 Scanning electron microscope images of tensile fracture surfaces for (a) BC/PLA, (b) BCG/PLA, (c) BC/MAPLA and (d) BCG/MAPLA composites.

Figure 7.8c reports the morphology of the tensile fracture surface of BC/MAPLA composites. A large gap between BC layers is noted, whereas the interface appears to remain intact. This consolidation of the interface may be attributed to the better chemical compatibility between BC and MAPLA compared to BC and PLA. This is also confirmed in Figure 7.8d which is an image of the tensile fracture surface of a BCG/MAPLA composite. Only small delaminations occur between BCG layers for this sample. Consequently by both consolidation of BC networks and the
BC/PLA interface, one can considerably reduce the delamination process that may occur in these laminated composites. As shown in Section 7.3.4, both consolidations using glyoxal and modification of PLA with MA result in a significant increase of Young’s modulus.

### 7.3.7 Molecular Deformation

Figure 7.9 reports typical Raman spectra for PLA and MAPLA films and BC/PLA, BCG/PLA, BC/MAPLA and BCG/MAPLA composites where the Raman band positioned at ~1095 cm\(^{-1}\) is highlighted for the composite specimens. As already shown for PLA in Section 4.3.2.5, the Raman band located at ~1095 cm\(^{-1}\) can be detected through the MAPLA resin.

![Figure 7.9](image_url)

**Figure 7.9** Typical Raman spectra in the range of 300 to 1600 cm\(^{-1}\) for PLA, and MAPLA films and BC/PLA, BCG/PLA, BC/MAPLA and BCG/MAPLA composites.

Figure 7.10 reports a typical shift towards a lower wavenumber for a BC/MAPLA composite. Similar shifts have been observed for BC/PLA, BCG/PLA and
BCG/MAPLA composites. The origin of the shift has already been discussed in Section 2.3.7. Similar shifts were also reported in Sections 4.4.3, 5.3.1.6.1 and 6.3.9 for BC networks and composite materials.

**Figure 7.10** A typical shift towards a lower wavenumber of the Raman band initially located at ~1095 cm\(^{-1}\) for a BC/MAPLA composite before and after 0.5 % tensile deformation.

Figure 7.11 shows the detailed shifts towards a lower wavenumber for the Raman band initially positioned at ~1095 cm\(^{-1}\) for BC/PLA and BCG/PLA composites as a function of tensile strain. The data have been fitted using a linear equation. The gradients of these fits were found not to be significantly different for BC/PLA and BCG/PLA composites. Glyoxalisation of BC networks is consequently not sufficient to increase the stress-transfer in these laminated composites. Observation of Figure 7.8b in Section 7.3.6, has shown that delamination occurs mainly at the BCG/PLA interface when BC networks are glyoxalised. The stress-transfer efficiency in BCG/PLA composites seems to be consequently mainly compromised.
at the BCG/PLA interface. This is further supported by Young’s modulus data reported in Section 7.3.2, where no significant difference is observed between BC/PLA and BCG/PLA composites.

**Figure 7.11** Typical shifts in the wavenumber position of the Raman band initially located at ~1095 cm\(^{-1}\) for BC/PLA and BCG/PLA composites under mechanical deformation.

Detailed shifts for BC/PLA composites (sample 1) exhibit a plateau. This can be attributed to delamination occurring between BC layers as they were only occasionally observed for BC networks as reported in Section 5.3.1.6.1. A sudden decrease in the Raman band position is sometimes observed which could be due to BC/PLA interface failure. Detailed shifts for BCG/PLA composites show fewer and smaller plateaus which may indicate that less delamination occurs between BC layers due to their covalent coupling through glyoxalisation. A sudden decrease in the Raman band position is also noted (sample 1).
Consequently it seems that two competitive mechanisms are present during the deformation of these laminated composites: delamination between BC layers and delamination at the BC/PLA interface. Delamination between BC layers seems to be the most significant interfacial failure mechanism for BC/PLA composites. Delamination at the BC/PLA interface however seems most significant for BCG/PLA composites. This statement is also supported by strain at failure and work of fracture data reported for BC/PLA and BCG/PLA composites in Table 7.3. The work of fracture for BCG/PLA composites is significantly lower than for BC/PLA composites. This is likely to be due to less BC layer-to-layer mobility due to glyoxalisation.

These mechanisms are reported in Figure 7.12 and illustrate the possible dominating delamination mechanisms occurring in BC/PLA and BCG/PLA composites when they are submitted to external tensile deformation.

![Figure 7.12 Ideal case deformation mechanisms for BC/PLA and BCG/PLA, laminated composites. Red arrows represent the external forces applied to the composite materials.](image)
The use of glyoxalised BC networks does not significantly change both the stress-transfer and Young’s modulus of BC/PLA composites. Since the glyoxalisation procedure is potentially an additional manufacturing cost, it is perhaps not a justifiable pre-treatment. A matrix material having a better chemical compatibility with cellulose may however justify the need for glyoxalised BC networks for stress-transfer and Young’s modulus improvement.

Figure 7.13 reports detailed typical shifts of the Raman band initially located at \(~1095 \text{ cm}^{-1}\) for BC/MAPLA and BCG/MAPLA composites. The gradients of fit for BC/MAPLA and BCG/PLA composites are respectively \(1.2 \pm 0.3\) and \(1.3 \pm 0.3 \text{ cm}^{-1} \text{%}^{-1}\) which are significantly higher than the values reported in Figure 7.11 for BC/PLA and BCG/PLA composites.

![Figure 7.13](image)

**Figure 7.13** Typical shifts in the wavenumber position of the Raman band initially located at \(~1095 \text{ cm}^{-1}\) for BC/PLA, BCG/PLA, BC/MAPLA and BCG/MAPLA composites.
No significant difference has been however obtained between BC/MAPLA and BCG/PLA composites. It seems that only the modification of PLA with MA is sufficient to increase the molecular deformation at the interface of these materials. This indicates that the BC/MAPLA interface is stronger than the BC/PLA interface. These results might however not be representative of the bulk properties of the composites. The fact that the laser is focused on the top layer of BC networks means that the molecular deformation measured only reflects the molecular mobility at the interface and is not representative of the bulk material. It is also possible that delamination between BC layers may have occurred far away from the BC/MAPLA interface as shown in Figure 7.8c and so the measurement is not sensitive enough to detect this reduction in stress-transfer. The tensile mechanical properties give an indication of the bulk mechanical properties.

Figure 7.14 reports a representation of the ideal delamination mechanisms occurring in BC/MAPLA and BCG/MAPLA.

![Deformation mechanisms](figure)

**Figure 7.14** Deformation mechanisms for BC/MAPLA and BCG/MAPLA composites. Red arrows represent the external forces applied to the composite materials.
CHAPTER 7

7.4 Conclusions

In this Chapter, PLA (polylactide) has been successfully modified using maleic anhydride which was referred to as MAPLA (maleated polylactide). This has been verified by performing liquid NMR experiments and the typical formation of succinyl anhydride groups was proven to occur. GPC measurements have shown a decrease in the molecular weight of PLA after maleation which was related to the poorer mechanical properties of MAPLA compared to PLA films.

MAPLA has then been combined with unmodified and glyoxalised BC networks to produce composites. The mechanical performance of these materials has been compared to composites designed using unmodified PLA. The tensile mechanical properties have been determined and the results revealed that relative Young’s modulus of BC/PLA composites is significantly increased when both BC and PLA are modified with respectively glyoxal and maleic anhydride. Other composites did not show any significant improvement of Young’s modulus. BC/MAPLA and BCG/MAPLA composites were however found to be more brittle than composites designed using unmodified PLA having significantly higher stress and strain at failure and work of fracture. This has been attributed to a reduced mobility of the different layers constituting these composite materials, which is supported by tensile fracture surface images.

The interfaces in BC/PLA, BCG/PLA, BC/MAPLA and BCG/MAPLA composites have been investigated using Raman spectroscopy. The use of maleated PLA was found to increase molecular deformation of the cellulose component. In addition to maleation, the use of glyoxalised BC networks was found not to further significantly increase the stress-transfer. This must be because stress-transfer
measurements performed at the interface may not be fully representative of the bulk mechanical properties.

7.5 References


8.1 Summary

In this study, Raman spectroscopy has been used as the main characterisation tool to probe the stress-transfer properties of BC networks and BC/PLA composites. This has been performed by following the shift towards a lower wavenumber of the Raman band initially positioned at ~1095 cm$^{-1}$ for BC networks and BC/PLA composites submitted to external tensile deformation. These shifts are directly related to the stretching of the cellulose backbone structure.

Chapter 3 revealed that these shifts occur due to the stress-transfer process, and are not due to data scattering. In addition, the exposure of BC networks to the NIR laser was found not to significantly contribute to intensity changes and to the Raman band shift rate of the Raman band initially located at ~1095 cm$^{-1}$.

In Chapter 4, Raman spectroscopy has been used to investigate the micromechanics of BC networks. A determination of Young’s modulus of single BC nanofibrils was proposed. A range of values of 79 – 88 GPa has been obtained, assuming a 2D random orientation distribution to the fibrillar networks. This improved method, over previous estimations (Hsieh et al., 2008), takes into account the influence of the laser and back-scattered light polarisation configuration and both the nanofibril and deformation axis angles. These factors were not taken into account in previous estimations of Young’s modulus of cellulose nanowhiskers (Šturcová et al., 2005; Rusli and Eichhorn 2008) and BC nanofibrils (Hsieh et al., 2008). An even better estimate may be possible by using a single layer of BC to eliminate the influence of BC layer delamination effects on
the estimation of the Raman band shift rate. There is, however, an experimental limitation when using very thin materials. The thinner the BC networks, the lower the intensity of the Raman band located at 1095 cm\(^{-1}\). This leads to inaccurate determination of the wavenumber position of this Raman band and consequently to inaccurate estimation of the Raman band shift rate.

In Chapter 5, the Raman spectroscopic technique has then been used to probe the interface in BC/PLA composites. The culturing time of bacterial cellulose networks has been found to significantly influence the interface in BC/PLA composites. A low culturing time was found to favour the interaction between the upper layer of BC networks and PLA. This has been attributed to the higher total surface area of BC networks cultured for 3 days (~95 m\(^2\) g\(^{-1}\)) compared to BC networks cultured for 6 days (~7 m\(^2\) g\(^{-1}\)). An increased Young’s modulus of the composites was measured after normalising the mechanical properties by their respective volume fraction. Chapter 7 also revealed that the stress is less efficiently transferred from the matrix to the reinforcement in composites prepared using BC networks cultured for 14 days (~0.6 cm\(^{-1}\) %\(^{-1}\)) compared to composites designed using BC networks cultured for 3 days (~2.0 cm\(^{-1}\) %\(^{-1}\)) and 6 days (~1.8 cm\(^{-1}\) %\(^{-1}\)). This confirms that low BC culturing times facilitate the stress-transfer process in this form of composites. It may be worth, however, to study one or two more BC culturing times to confirm this trend. Observation of the tensile fracture surface of composites using scanning electron microscopy revealed that delamination was mainly occurring within the BC networks themselves rather than at the BC/PLA interface. Consequently optimisation of the mechanical properties of this form of composites was still possible through chemical modifications.
In Chapter 6, BC networks have been cross-linked using a dialdehyde commercially called glyoxal. This was carried out in order to reduce the delamination between BC layers seen in Chapter 5. This chemical modification was found to successfully cross-link BC layers constituting the networks. Scanning electron microscopy imaging of the tensile fracture surface of glyoxalised BC networks showed a brittle fracture. This was attributed to the reduced mobility between BC layers due to covalent coupling. Raman spectroscopy revealed that glyoxalisation of BC networks increases their molecular deformation both in the dry and wet states. Exposure of unmodified BC networks was found to "turn-off" the stress-transfer whereas the stress-transfer in glyoxalised BC networks was still present owing the presence of chemical cross-links.

In Chapter 7, glyoxalised BC networks have been used to design composites. Their stress-transfer properties have been determined and compared to composites designed using unmodified BC networks. The stress-transfer in composites designed using glyoxalised BC networks was, however, found not to be significantly improved. This was attributed to a stress-transfer loss at the interface probably due to the higher bonding strength between glyoxalised BC layers compared to the bonding strength of the BC cellulose/PLA interface. This was supported by observation of the tensile fracture surface of the composites using scanning electron microscopy.

Polylactide has been then modified using maleic anhydride using a melt extrusion process. Maleated PLA was subsequently used to design composites using unmodified and glyoxalised BC networks. Raman spectroscopy revealed an improvement of stress-transfer at the interface of composites designed using maleated PLA. This was attributed to the presence of polar succinyl anhydride
groups grafted along the backbone structure of PLA chains. No significant
difference was, however, observed between the stress-transfer properties of
unmodified or glyoxalised BC networks. Tensile mechanical properties of the
composites revealed a significant improvement of the relative Young’s modulus
(relative to Young’s modulus of the matrix) when both glyoxalised BC networks
and maleated polylactide are used to create a composite material. This was
attributed to both reduced interface mobility between glyoxalised BC layers and at
the glyoxalised BC/maleated PLA interface. This was supported by the observation
of the tensile fracture surface of composites using scanning electron microscopy
and by work of fracture data obtained from tensile testing.

To conclude, this study revealed that it is possible to maximise the stress-
transfer and bulk tensile mechanical properties by selecting low culturing BC
networks. Also both cross-linking of these networks and the use of a compatible
matrix is advised. The mass scale production due to low yield and also the cost of
BC is still the main drawback of this source of cellulose compared to other sources.
Further biotechnological research may help to overcome this issue.

Raman spectroscopy was found to be a powerful tool to study interfaces in this
form of composite material. It is, however, important to mention that this tool has
some limitations. One has to use a transparent matrix material to allow a better
detection of the Raman band located at ~1095 cm\(^{-1}\). It is also important to check
that the matrix material does not show any Raman band in the wavenumber range
of 1050 to 1150 cm\(^{-1}\). The concentration of cellulose in the composites is also a
very important factor. The higher the concentration, the higher the intensity of the
Raman band located at 1095 cm\(^{-1}\). This is less an issue when a layer of cellulose is
present in the composite but it is even more important when cellulose nanofibrils
are dispersed in the matrix material. Another important experimental aspect is that Raman spectroscopy only gives information on phenomena occurring at the surface especially if the material is opaque and this has to be taken into consideration when trying to interpret data. Also Raman spectroscopy gives information on both amorphous and crystalline regions and it would be relevant to obtain complementary information by measuring the crystal deformation of BC in these composites using X-ray diffraction from a synchrotron source.

**8.2 Future Work**

The use of another cross-linking agent such as dialdehyde cellulose (Li et al., 2009; Han et al., 2010) may be an alternative to the use of glyoxal. It might even be possible to directly cross-link BC networks by partial oxidisation of BC networks using sodium periodate followed by a typical cross-linking procedure. Several oxidation times could be used to optimise the covalent coupling between BC layers. This would eliminate the time consuming impregnation step and reduce the time of the cross-linking procedure for a reduced cost of production.

The use of a bio-sourced and biodegradable hydrophilic transparent matrix would be also very interesting. It would eliminate the maleation reaction step used to compatibilise hydrophobic PLA with hydrophilic BC. But on the other hand, the composite properties would be likely to be more sensitive to moisture. A cross-linked matrix may, however, help to solve this problem.

As shown in Figure 8.1, it is possible to covalently graft PLA on the surface of cellulose nanowhiskers (CNW) through ring-opening polymerisation (Goffin et al., 2011). This must be applicable to BC networks and may be another route to explore.
Another suggestion for future work would be to perform biodegradability tests to verify if these composites are fully biodegradable. This could be done by following the ASTM standard D5526 – 94(2011)e1 referred to as the "Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under Accelerated Landfill Conditions". Another interesting work would be to study the influence of aging by performing stress-transfer measurements under composting conditions.

8.2 References


