Production Methods and Characteristics of Bacterial-Cellulose Composites
T.G. CHICIUDEAN

Production Methods and Characteristics of Bacterial Cellulose Composites
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PhD Thesis

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PhD Thesis

Abstract

Present research involves the thorough study of several aspects regarding the design, manufacturing and testing of two composite materials that combine the advantages of bacterial cellulose (BC) as reinforcement material and PVA respectively Epoxy as resins matrix. Tests have been performed on the new composite materials developed in the university laboratory as prototypes, with investigations including standard tensile tests, flexural tests, and water sorption tests. The particularity in this research is that not only the integration of raw BC material within a composite structure is being addressed, but also the production of BC itself. The investigation starts with research on the bacteria employed to produce cellulose: Acetobacter Xylinum. Therefore, the complete research areas covered in this thesis are: Acetobacter Xylinum cellulose production, growth medium optimization in a semi-controlled environment, alkaline treatment of wet BC pellicles, de-hydration of BC pellicles, manufacturing BC based composites using PVA matrix, manufacturing BC based composites using Epoxy matrix, mechanical characterization of BC based composites using PVA or epoxy matrix, water sorption characteristics of the new BC based composites. In brief, two hybrid composite materials have been designed, manufactured and tested. A summary of the material’s most valuable parameters and properties is further presented: the composite with PVA matrix is a light hybrid composite (density around 1.45 g/cm$^3$), having a Young’s modulus of 42 GPa, tensile strength of up to 370 MPa and a diffusion coefficient of $\sim$3.2-3.8·10$^{-7}$ m$^2$/h; the composite with Epoxy matrix is also a light hybrid composite (density around 1.6 g/cm$^3$), with a Young’s modulus of 60 GPa, tensile strength of up to 465 MPa and a diffusion coefficient of $\sim$4·10$^{-8}$ m$^2$/h. These mechanical properties, particularly those exhibited by the BC composite with Epoxy matrix are superior to the ones reported in literature for BC composites and also comparable to the ones of bi-directional glass-fiber reinforced composites (GFC).
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Acknowledgments

The author extends his gratitude and appreciation to all the people who have helped complete the work depicted in this thesis. The effort in the last few years could not have been appropriately performed without direct help, support and understanding from numerous persons who have influenced, guided and stood by the author since the very beginning of this research and up to the final stage of writing down the work, printing and presenting it.

To my Family
Chapter 1

Introduction

This thesis presents a personal point of view on today’s most utilized structural materials (composites) with focus on Bacterial Cellulose Composites (BCC). There are thousands of materials available for use in engineering applications [1]. Often in literature most materials are classified in three main classes based on the atomic bonding forces: metals, ceramics and polymers. Within each of these classifications, materials are reorganized based on their chemical composition, certain chemical characteristics or mechanical properties. In addition to these types, materials can be combined to create a new class of materials referred to as composites (materials).

1.1 Composite materials

The term composite could mean almost anything if taken at face value [2], since at a close examination, all engineering materials are compound of dissimilar subunits. Commonly, a composite is defined as a combination of two or more distinct materials. This combination is engineered in such a way that the constituent materials retain their own distinctive properties and the new created material will inherit the properties as a set, properties that can not be achieved by any of the components acting alone. According to this statement, it can be determined that a wide range of engineering materials fall into this category. For example, concrete is a composite because it is a
mixture of Portland cement and aggregate [2]. Fiberglass sheet is a composite since it is made of a glass fiber as reinforcement structure and a polymer as an imbedding matrix [2, 3]. Wood is a (natural) composite structure made up of: cellulose fibers in combination with hemicelluloses mostly glucomannan/glucuronxylan and lignin [4]. Last but not least, one of the most advanced composite structures acknowledged by science until now is the vertebrate skeleton-based material “the bone”. In the last two cases mentioned above, the structure of the material exceeds the boundary of engineered composites and incorporates other functionalities which are related with living systems. To better distinguish materials that can be classified as composites, the next subdivision is proposed in this thesis: The term Synthetic-Composites (SC) [4] will be used to designate engineered composites having in composition synthetic organic or inorganic compounds and the term natural-bio-composites will include any combination of two or more compounds which occur in biological processes. Finally, any combination of the previous listed types will be referred to as biosynthetic-composites. Within the next section a brief description of these classes and their constituent materials will be presented.

1.1.1 Synthetic composites

Synthetic composites have found practical applications in many areas of human daily life for quite some time. Often, we do not realize that more than 50% of urban environment is made from synthetic composites - especially steel reinforced concrete. The possibility of combining different material systems (nonmetal-metal-ceramic) provides unlimited variation. It will not be possible within the scope of this thesis to be complete and exhaustive in describing all these types of composites, but by classifying these materials based on their matrix, a brief description can be presented.

Most commercially produced composites use a matrix material, which can be a polymer, metal or ceramic material. Based on this fact, synthetic composites can be divided in three sub-groups: SC with polymer matrix, SC with metal matrix and SC with ceramic matrix as one can see in figure 1.1.

Synthetic composites with polymer matrix are often called composites with resin solution. There are many different commercial polymers available depending upon the starting raw ingredients.
The most common polymers are polyester, poly(vinyl ester), epoxy resin, phenol resin, polyimide, polyamide, polypropylene, PEEK, and others. The reinforcement materials are often ceramic fibers (e.g. glass fibers, basalt fibers), natural/synthetic polymer fibers (e.g. flax, cotton or aramid fibers) but also commonly ground minerals in different shapes and forms. For these types of composites, the strength of the final product is greatly dependant on the ratio between the resin and fiber content. Another factor of influence on the strength of the final product is fiber orientation and fiber length. If the material is a fiber-reinforced composite, it can be divided in two main categories referred to as short fiber-reinforced materials and continuous fiber-reinforced materials.

Continuous reinforced materials are often constituted from layered or laminated structures. The woven and continuous fiber styles are typically available in a variety of forms, being pre-impregnated with the given matrix (resin), or dry. Both types can be unidirectional of various widths, plain weave, harness satins, braided, and
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stitched [6]. These types of materials are most commonly used in high-tech applications in aerospace industry or ships and car manufacturing.

Short fiber-reinforced materials are typically employed in compression molding and sheet molding operations. These come in the form of flakes, chips, and random made (which can also be made from a continuous fiber laid in random fashion until the desired thickness of the ply / laminate is achieved) and used on large scale in wood industry.

*Synthetic composites with metal matrix* can be considered the majority of engineering metals. But often in literature a special group of metal composites are referred to as alloys. “An alloy is a partial or complete solid solution of one or more elements united in a metallic matrix” [6]. To avoid confusion, this type of metallic composites will not be discussed in this thesis. However, there are other numerous composite materials similar to *SC with polymer matrix*, having one or more metals as matrix and referred to as Metal Matrix Composites (MMCs). The particularity of these composites is that the matrix is a monolithic material into which the reinforcement is embedded and the matrix is completely continuous. This means that there is a path through the matrix to any point in the material, unlike metal sandwiched composites [6]. In contrast with *SC with polymer matrix*, the reinforcement does not always serve a purely structural task (reinforcing the compound), but it is also used to change physical properties such as wear resistance, friction coefficient, or thermal conductivity. For many researchers, the term metal matrix composite, is often equated with the term light metal matrix composite. Substantial progress in the development of light metal matrix composites has been achieved in recent decades, so that they could be introduced into the most important applications. In traffic engineering, especially in the automotive industry, MMCs have been used commercially in fiber reinforced pistons and aluminum crank cases with strengthened cylinder surfaces as well as particle-strengthened brake disks [5].

*Synthetic composites with ceramic matrix*, also known as Ceramic Matrix Composites (CMCs), combine reinforcing ceramic or metal phases with a ceramic matrix to create materials with new and superior properties. In ceramic matrix composites, the primary goal of the reinforcement is to provide toughness to an otherwise brittle ceramic matrix. These types of materials can be classified based on their ceramic matrices as either oxides or non-oxides. The most common oxide matrices include alumina, silica, mullite, barium aluminosilicate, lithium aluminosilicate and calcium aluminosilicate. In the case of non-oxide ceramics, the more common used materials are SiC, Si₃N₄, boron carbide, and AlN.
1.1.2 Natural bio composites

Natural-bio-composites are materials which occur in biological processes; wood for example is a natural bio composite material. The wood structure is similar to the synthetic composites, containing an orientated hard phase for strength and stiffness, and a softer phase for toughness [7]. Other natural composites include bones, teeth, skin, plant leaves and bird feathers. Basically, any organ part of living organisms is a natural bio composite material having a structural and biological function. These types of materials are in most cases fully optimized to fulfill their functionality so that the complexity of their structure is extremely high (fig. 1.2).

![Diagram of Feather and Bone Structure]

Even more than that, in some cases, the physical properties of these materials exceed the properties of the most advanced synthetic composites. For example, silky threads spun by the spider can be as strong as steel and have been recently found to contain a gel-core encased by a solid structure of aligned molecules. For many scientists these materials are a continue source of inspiration. Nowadays, more than ever, researchers are using these natural systems either as models or raw material to develop low-cost high-performance biosynthetic composites.
1.1.3 Biosynthetic composites

Biosynthetic composites are hybrid materials in which one or more of the compounds are natural bio-composites. Some of the readers associate the term biosynthetic composite with new modern cutting-edge materials and technologies. However, this is partially true; biosynthetic composites were used as construction materials for thousands of years like clay-straw in house construction, wood-bone/wood-stone for weapons and more recently plywood. It is also true that in the modern society, a series of materials, which can be referred to as biosynthetic composites, are used in high-tech automotive and medical applications. These modern biosynthetic composites can be divided in two groups based on their main chemical component as cellulose based biosynthetic composites and non-cellulose based biosynthetic composites.

The majority of cellulose-based biosynthetic composites are compound of natural cellulose fibers. The use of these bio-fiber reinforced composites has nowadays been extended to almost all industry fields: flax fibers are utilized in car industry for disk brakes to replace asbestos fibers; bio-based composites for roof structures from soy oil-based resin and cellulose fibers; bamboo utilized as reinforcement in structural concrete elements etc [10].

The non-cellulose based synthetic composites include primarily calcium-based materials and are especially used in medical applications. However, there are also cellulose-based composites having medical applications like cellulose used as implant material, encapsulation, wound care, cellulose-based hemodialysis membranes [11]. Moreover, cellulose-based composites are of particular interest for researchers because of their low-cost and versatility applications.

Cellulose provides many opportunities to improve the physical properties of a range of composites. Extensive research has been performed in the last years on hybrid cellulose composites and the results varied with cellulose purity and fiber size. Cellulose composites also provide an excellent green alternative material. However, concerns common to such materials include fiber uniformity, aggregation and/or interaction with the polymer component. Anyway, it remains an on-going research for an alternate approach to effective dispersion, fiber uniformity and size to realize the benefits of polymer/cellulose composites.
1.2 Research objective and approach

The research performed in this thesis is focused on a search for an alternate approach in producing cellulose-based composites with high-end fiber uniformity. The main objective of this research is an in-depth investigation on bacterial cellulose as raw material for the production of nano-cellulose based composites.

Bacterial cellulose (BC) is a three-dimensional reticular network of fine fibrils (2-4 nm in diameter) coalesced into a ribbon (100 nm width, 1-9 µm length) one-hundred times smaller than wood fibers. Because of the extensive hydrogen bonding within the reticulated network, it can hold up to 700 times its dry weight in water. The crystallinity index is above 70% and a separate fibril can have a theoretical Young’s modulus of 173 GPa and a tensile strength in the order of 2 GPa [11]. These mechanical properties are based on a pure cellulose fiber and therefore, they are never reached in actual materials that consist of cellulose fibers. These unique properties emphasize the potential of BC as a new material for use in the production of quality paper [12], paint additives, diet and dessert foods, artificial skin [13], wound dressing [14] and ultrafiltration membranes [15]. In addition to these applications, in the recent years, several research groups have taken on the difficult task of quantifying the properties of a series of BC composite materials. The studies revealed a 18-28 GPa Young’s modulus and a tensile strength of 260-425 MPa [11].

Despite the achieved result in terms of strength, there are a number of important issues which were not fully addressed, like alternative resin systems (e.g. Poly(vinyl alcohol) (PVA) or Epoxies) water absorption characteristics and concise alkaline treatment of BC. Therefore the objective of this thesis is:

***

To produce and determine the mechanical properties of a new series of BC based composites having as resin system PVA or Epoxy.

***

The approach

The present thesis describes and proposes an integrated design approach for producing two types of BC based composites. The first investigation is concerned
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with the use of raw-BC material to produce (1) laminar composites with PVA matrix and (2) laminar composites with Epoxy matrix. The approach covers the entire development process and manufacturing life cycle of the investigated composites. Not only the integration of raw BC material within a composite structure is being addressed, but also the production of BC itself. The investigation starts with research on the bacteria employed to produce cellulose: *Acetobacter Xylinum*. The arguments in choosing particularly this type of bacteria will be presented in detail within the next chapter. Therefore, to be complete and exhaustive, the research will address the next main areas:

- *Acetobacter Xylinum* cellulose production, growth medium optimization in a semi-controlled environment;
- Alkaline treatment of wet BC pellicles;
- Dehydration of BC pellicles;
- Manufacturing BC based composites using PVA matrix;
- Manufacturing BC based composites using Epoxy matrix;
- Mechanical characterization of BC based composites using PVA matrix;
- Mechanical characterization of BC based composites using Epoxy matrix;
- Water sorption characteristics of the new BC based composites.

These areas of research will provide a full understanding of the advantages of using polymer/cellulose composites and their technical performance. The presented investigation is rather qualitative than quantitative; no standard materials were derived. The main reason is the low budget that was allocated in this research, approximately 20 K€ *auto-financed* and the infrastructure of “POLITEHNICA” University of Bucharest, Faculty of Applied Chemistry and Materials Science and Technical University Delft, Faculty of Aerospace. However, a patent has been submitted for approval for one of the new-developed BC based composite.

**1.3 Thesis outline**

In this section the outline of the thesis is presented. The layout of the thesis is illustrated in figure 1.3. The work in this thesis is a bottom-up approach, starting with a literature review section followed by a study on BC production and medium
optimization. The paper continues with an overview on BC alkaline treatment and some preliminary studies on manufacturing BC based composites are described. The last three chapters characterize the new BC based composites.

Fig. 1.3 Thesis layout.

**Chapter 2** contains a general introduction on bacterial cellulose and arguments to sustain BC as an alternative high-tech composite. The most relevant applications of BC are briefly presented.

**Chapter 3** The aim of this chapter is to present the advantages of using bacterial cellulose as structural material.

**Chapter 4** In this chapter a quantitative description of existing bacterial-cellulose based composites is generally presented.

**Chapter 5** This chapter presents a set of *A. Xylinum* growing medium and procedures. These are presented in conjunction with a description of two production studies

**Chapter 6** In this chapter the alkaline treatment of wet BC pellicles and the dehydration of BC pellicles are addressed.
Chapter 7 In this chapter some preliminary studies on manufacturing BC based composites are presented. This chapter provides the blue-prints for the development of a series BC based composites.

Chapter 8 This chapter presents a generic view on the plasticity theory. This provides the basis to investigate the mechanical properties of the new BC based composites.

Chapter 9 In this chapter a set of relevant studies are presented and discussed. The mechanical properties of the new BC based composites are the main focus.

Chapter 10 The objective of this chapter is to present a set of water absorption studies and the behavior of the new BC based composites in humid environments.

Chapter 11 This chapter concludes the thesis and a set of recommendations are derived.
Literature


Chapter 2

Cellulose - an alternative for high-tech composites

The use of cellulose fibers in technical composite applications has recently been the subject of interest and intensive research across Europe. Many automotive components are already being produced from natural composites, mainly based on polyester or PP and fibers like flax, hemp or sisal. In addition to the classical applications of cellulose fibers, bacterial cellulose is beginning to be more and more considered as an alternative high strength fiber solution. This chapter presents a general introduction on bacterial cellulose and its applications.

2.1 The history of cellulose

Cellulose is the most common bio-polymer on Earth. It comprises 33% of all biological matter and has an annual production of over 100 billion tons [1]. Cellulose has been used for centuries as a raw material from trees and other plants in various applications, but it was first isolated from plant matter by the French chemist Anselme Payen in 1839 [1]. He reported that the substance had the same composition as starch, but it was different in structure and properties. Today, cellulose is known as a polysaccharide with the formula ($\text{C}_6\text{H}_{10}\text{O}_5)n$, and consisting
of a linear chain of several hundreds to over ten thousand linked glucose units (fig. 1.1).

This material, generally recognized today as cellulose, is produced by plants, bacteria and a single animal species, namely members of the tunicate (“sea squirts”) family (fig. 1.2), which makes use of cellulose nanostructures (skeleton).

It is however commonly extracted from plant material such as wood, flax, hemp, sisal or cotton. In plants, cellulose is found in a composite form composed of polymers of lignin and carbohydrates as hemicelluloses and cellulose which are physically and chemically bound together [2]. The matrix which is lignin in plant matter bonds the cellulose fibers together acting as a resin system. Cellulose is the reinforcement material and hemicellulose acts as an interfacial compatibilizer between cellulose and lignin. Even if cellulose constitutes a significant portion of the material, mechanical characteristics such as strength and stiffness of the plant material are inferior to the properties of pure cellulose.
When comparing natural fibers, it can be observed that mechanical characteristics like tensile strength and Young’s modulus generally go down with lower cellulose content (table 2.1). For high strength materials, it would clearly be preferable to contain as much cellulose in a natural material as possible.

The highest cellulose content in natural fibers from plants is about 82-83%.

<table>
<thead>
<tr>
<th>Types of Fibre</th>
<th>Cellulose (%)</th>
<th>Lignin (%)</th>
<th>Hemicellulose (%)</th>
<th>Pectin (%)</th>
<th>Wax (%)</th>
<th>Tensile strength (MPa)</th>
<th>Young’s modulus (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Coir</td>
<td>36-43</td>
<td>41-45</td>
<td>0.15-0.25</td>
<td>3-4</td>
<td>-</td>
<td>131-175</td>
<td>4-6</td>
</tr>
<tr>
<td>Bast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Jute</td>
<td>61-71.5</td>
<td>12-13</td>
<td>13.6-20.4</td>
<td>0.2</td>
<td>0.5</td>
<td>393-773</td>
<td>13-26.5</td>
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<td>18.6-20.6</td>
<td>2.3</td>
<td>1.7</td>
<td>345-1100</td>
<td>27.6</td>
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<td>3.7-5.7</td>
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<td>0.8</td>
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<td>13.1-16.7</td>
<td>1.9</td>
<td>0.3</td>
<td>400-938</td>
<td>61.4-128</td>
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<tr>
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<td>8.0-11.0</td>
<td>10.0-14.2</td>
<td>10.0</td>
<td>2.0</td>
<td>468-640</td>
<td>9.4-22.0</td>
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<td>PALF</td>
<td>70-82</td>
<td>5-12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>413-1627</td>
<td>34.5-82.51</td>
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<tr>
<td>Seed</td>
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<td></td>
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<tr>
<td>Cotton</td>
<td>82.7</td>
<td>-</td>
<td>5.7</td>
<td>-</td>
<td>0.6</td>
<td>287-800</td>
<td>5.5-12.6</td>
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<tr>
<td>Man-Made</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>E-glass</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2000-3500</td>
<td>70</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>4570</td>
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<tr>
<td>Aramid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3000-3150</td>
<td>63-67</td>
</tr>
<tr>
<td>Carbon</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4000</td>
<td>230-240</td>
</tr>
</tbody>
</table>

Table 2.1 Composition and comparative properties of some natural and man-made fibers [3].

There are, however some different species of bacteria that can produce a pure form of cellulose but *Acetobacter Xylinum* seems to be the only one capable of producing it in industrial amounts. *A. Xylinum* is a gram negative bacterium that is able to produce pure cellulose with the same chemical composition as cellulose produced by plants. In the next sub-chapter the bacteria and the cellulose produced by it will be described in detail.

### 2.2 Introduction to bacterial-cellulose

Bacterial cellulose (BC) was first written about in a scientific paper by Brown [4,5] in 1886. The paper described a fermentative process which formed a gelatinous transparent membrane at the surface of an acetic fermentation. The membrane had
the capability to grow to a thickness of 25mm and proved to be very strong and tough. The membrane, also called pellicle, proved to be cellulose formed by a bacterium. The bacterium was named *Bacterium Xylinum* by Brown, but later it was classified as *Acetobacter Xylinum*.

Later research showed that bacterial cellulose produced by *A. Xylinum* had the same chemical composition as the cellulose produced by plants, but it showed unique and superior physical properties. Until today, bacterial cellulose is the most pure existing natural cellulose. Several bacterium species like *Sarcina, Agrobacterium, Rhizobium, Acetobacter* etc. have also the ability to synthesize cellulose [6] but in small quantities. A classification of the bacteria’s capability to produce cellulose is presented in table 2.2. However, from these bacteria, *A. Xylinum* is the only species of bacteria known to be capable of producing cellulose in commercial quantities. Rows of pores characteristically secrete mini-crystals of glucan chains which then coalesce into microfibrils (fig. 2.3 a). [7,8]. Clusters of microfibrils result in a compound structure known as the ribbon (fig. 2.3 b). The dimensions of the ribbons formed by *A. Xylinum* are in the range of 2-4 nm thickness, 70-80 nm width and 1-9 μm in length and form a reticular structure at macro scale [7, 9, 10]. The pellicle is comprised of randomly oriented ribbons whose structure is stabilized by extensive hydrogen bondings, which explains its water holding capability of up to 700 times its dry weight [11, 12].

<table>
<thead>
<tr>
<th>Genus</th>
<th>Cellulose structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acetobacter</em></td>
<td>pellicle composed of ribbons</td>
</tr>
<tr>
<td><em>Achromobacter</em></td>
<td>fibrils</td>
</tr>
<tr>
<td><em>Aerobacter</em></td>
<td>fibrils</td>
</tr>
<tr>
<td><em>Agrobacterium</em></td>
<td>short fibrils</td>
</tr>
<tr>
<td><em>Alcaligenes</em></td>
<td>fibrils</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>no distinct fibrils</td>
</tr>
<tr>
<td><em>Rhizobium</em></td>
<td>short fibrils</td>
</tr>
<tr>
<td><em>Sarcina</em></td>
<td>amorphous cellulose</td>
</tr>
<tr>
<td><em>Zoogloea</em></td>
<td>not well defined</td>
</tr>
</tbody>
</table>

![Figure 2.3 a). Possible model of glucan chain assembly at the bacterial cell wall [7], b).ribbons of bacterial cellulose [8].](image)
The term ‘fibril’ has been used by various researchers to describe the relatively long and very thin ribbons of cellulosic membrane. Separate fibrils can have a theoretical modulus of 173GPa and a strength in the order of 2GPa [10, 14]. These values are based on a pure fiber of cellulose and therefore they are never reached in materials that consist of cellulose fibers. Paper has been made from cellulose extracted from plant pulp for many years, but it has poor strength and stiffness due to the fact that the cellulose fibers are fragmented. The strength of the material is mainly based on hydrogen bonding between hydroxyl-groups of the cellulose molecules [15], rather than on the strong covalent bonding within molecules. With Bacterial Cellulose it is possible to acquire a material that is pure cellulose and consists of long tangled fibrils that are also branched. The branching is a result of the division of bacteria (fig. 2.4).

During the division of the bacteria that is producing a ribbon of cellulose, the ribbon divides along [9]. The strength of bacterial cellulose is therefore more governed by the theoretical strength of cellulose and by the entanglement of the fibers, which leads to mechanical properties that are far superior to other cellulose based materials. Even more, the crystallinity index of the fibers is above 70%. The degree of polymerization is usually between 2000 and 6000, but can reach 16000 to 20000 and the density is 1.6 g/cm³ [13].

### 2.3 Early research on cellulose production by *A. Xylinum*

During the past, one-hundred and twenty-two years that follow the discovery of Brown’s membrane, other research groups have studied the behavior of *A. Xylinum* and the characteristics of cellulose produced by this micro-organism.

In 1931 Tarr et al. [16, 17] have tested the cellulose production by *A. Xylinum* on different substrates including hexoses, pentoses, mannitol, glycerol, fructose,
galactose, glucose methyl hexoses and other carbohydrates. They concluded that glucose and fructose produce the highest yield and also reported that a small amount of ethanol is necessary for cellulose production.

During the years 1946 to 1963, Hestrin’s research group at the Hebrew University in Jerusalem published several papers on the synthesis of bacterial cellulose. Their first publication was a short note [18] describing microscopic examination of bacterial cellulose. Using a dark-field condenser they were able to discern a web of thin, discrete fibrils. A second short note [19] detailed their discovery, stating that a standing culture of *A. Xylinum* would produce a mesh of cellulose at the surface. The product could be made from a variety of substrates and was formed even when the cells did not grow. The authors suggested that further studies on the mechanism of formation could give insight into the process of cellulose formation inside the plants, which they hoped to investigate at a later time.

In one of their later publications, they also investigated the properties that affect the formation of bacterial cellulose and observed gas bubbles being formed within the submerged cellulose mesh prior to surface film formation [20]. They concluded that the gas probably floated the cellulose and bacteria to the surface. The authors further demonstrated the aerobic necessity of the organism and the incapability to form pellicles in a swirled or stirred flask. Agitation did lead to cellulose production, but at a lower rate and in round masses formations that were about 10 mm in diameter. They also formed mutant strains that do not produce cellulose. The research performed by them between 1954 and 1963 is often referred to as the basis of the field. During this period they published four papers:

- First publication gives details about a simplified procedure for quantifying cellulose by conversion to glucose [21]. The traditional method for converting cellulose into glucose for analysis involves acid hydrolysis and can take many hours to complete at the necessary conditions. The authors presented an alternative method involving preliminary acetylolytic degradation. This treatment rapidly solubilizes the cellulose, making the hydrolysis much simple and rapid. The procedure is shown to be more effective for bacterial cellulose as well as for other forms of cellulose through comparison to the more traditional methods.

- Second publication details a method for the preparation of a nearly cellulose-free suspension of freeze-dried cells that polymerizes glucose to cellulose [22]. Using radio-labeled glucose, the authors were able to demonstrate that the major source of carbon for cellulose synthesis comes from glucose. The optimum pH range for cellulose production was
between 5 and 7, and the resulted cellulose was identified as high-molecular weight, native, crystalline (Type 1) cellulose.

- Third publication examines different substrates and inhibitors of cellulose synthesis by *A. Xylinum*. The micro-organism produced cellulose from various carbon sources, including hexoses, three-carbon compounds, and hexonates [23]. Citrate-cycle intermediates were oxidized, but cellulose was not formed, and phosphate esters did not produce cellulose. The authors determined that only a small amount of energy gained from oxidation was used to produce cellulose, accounting for the usually low yields of cellulose on glucose. In addition, cellulose synthesis was blocked by the supplementation of respiration inhibitors, and it was shown that cellulose production could be stopped while respiration continued. The authors concluded that alternative pathways are available for cellulose production and respiration, and that an intracellular hexose phosphate is an intermediate in cellulose production.

- The final publication in the series covers some observations on the reactions that take place in an extract of *A. Xylinum* cell [24]. Although the cell-free extract did not produce cellulose, the authors proposed a scheme of alternative pathways of carbohydrate metabolism, based up on the results of reactions that took place in the cell-free extract. A later paper showed that cellulose was formed from citric-acid cycle intermediates, and that washed-cell suspensions prepared from these cultures transformed these intermediates into cellulose [25].

Following the research of Hebrew University, several research groups consolidated the characterization of cellulose production by *A. Xylinum*. Because of the fact that the present thesis investigates the utility of the cellulose as an end product and not the bacterium itself, this literature overview will not detail further the research on cellulose production by *A. Xylinum*. However, in chapter 5, a growing medium review is presented in addition to three types of cultivation methods.
2.4  Bacterial cellulose application and usage

Bacterial cellulose is a non-toxic natural material which makes it a good candidate for usage in food industry and medical applications. Because of its unique mechanical properties, has found a series of technical application as well. Within the next section are described some of the most relevant applications of bacterial cellulose.

2.4.1  Food industry

Nowadays, the pellicles produced by *A. Xylinum* are used in the Philippines as a dessert food, called ‘Nata de coco’ (fig. 2.5). It is a jelly-like food, produced from the fermentation of mainly coconut water. It is not known since when it has been used in this way but it is highly appreciated for its high dietary fiber, and its low fat and cholesterol content. It was very popular in Japan in the early nineties, but popularity dropped again after a few years. Similar varieties of the product can be found all over the world. In South America, the coconut water is replaced by pineapple juice and the commercial name is "Nata de pina".

BC can serve as a primary material in food industry as a heat-stable suspension agent as well as a filler to reinforce fragile food hydrogels. It can improve the quality of pasty foods by reducing their stickiness; it can be applied to meat
products as a fat substitute and to jam as a non-caloric bulking agent. One of the commercial food stabilizer and thickener on the market is Cellulon® [26].

2.4.2 Medical applications

Most of the current research deals with the use of BC in medical applications such as wound dressings, scaffolding to direct the growth of new tissue or bone or even the replacement of soft tissue [27]. Extensive testing performed by Xylos Corporation [28] has demonstrated that bacterial cellulose is highly biocompatible and due to the fact that it does not originate from human or animal sources, it can be used in medical applications without any risk of disease transmission. BC is used as wound dressing under the name XCell® (fig. 2.6).

Figure. 2.6 XCell® cellulose wound dressing [28].

“XCell provides a new level of convenience and consistency for chronic wound care. As demonstrated clinically, a single dressing can stay on the wound for up to seven days, potentially reducing the time, materials, and costs of frequent dressing changes. Compared to other popular wound dressing types, XCell is the only dressing with significant dual functionality of hydration and absorption. This capability enables XCell dressings to be used throughout the various phases of wound healing”. [28].

A Brazilian company, BioFill Produtos Bioetecnologicos created a new wound healing system based on BC produced by A. Xylinum. Their line of products includes the following: Biofill® and Bioprocess® (used in the therapy of burns, ulcers as temporary artificial skin), and Gengiflex® (applied in treatment of periodontal diseases) [29].

The Swedish company Arterion is testing and commercializing BC for artificial blood vessels [27]. The produced blood vessels can have a diameter of less than five millimeters, which it is currently not possible to be produced from any other
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material (fig. 2.7). In addition to these, the material compliance can be set to mimic human veins and arteries within the range of physiological blood pressure.

Figure. 2.6 Artificial blood vessel from BC [27].

Bacterial cellulose was tested also as a dialysis filter in treatments of renal failure. A thin membrane of bacterial cellulose (BC) obtained from Acetobacter culture was tested for its performance as a dialysis membrane in aqueous systems by [30]. The BC membrane showed superior mechanical strength to that of a dialysis-grade regenerated cellulose membrane, allowing the use of a thinner membrane than the regenerated cellulose. As a result, the BC membrane gave higher permeation rates for poly (ethylene glycols) as probe solutes. The cutoff molecular weight of the original BC membrane, significantly greater than that of regenerated cellulose, could be modified by concentrated alkali treatments of the membrane. Bacterial cellulose membrane can be obtained in sheets having a thickness of 3 μm up to 0.5 mm. Despite the promising results no commercial product is available in this application area at this moment.

2.4.3 Technical applications

It was discovered that the pellicle produced by A. Xylinum could be converted into a film or sheet when dried and shrinkage can be restricted across the surface of the sheet [9]. Sheets prepared from bacterial cellulose can have a thickness from 3μm up to 0.5mm and have relatively good mechanical properties, like a Young’s modulus of >15GPa across the sheet, which is significantly higher than of other polymeric films or paper (<10GPa). The modulus is about one-tenth of the theoretical value of cellulose (173GPa) [14,31], which has currently been found to be the highest Young’s modulus ever known for two-dimensional organic materials.
However, at the moment, bacterial cellulose is appreciated as great value in a series of technical applications:

Sony Corporation and Ajinomoto developed the first audio speaker diaphragms using bacterial cellulose. These are utilized in high end audio headphones which had a starting price of 3000 $. At the moment, the price has dropped and the product became available worldwide. The product features a compressed low thickness (~20 microns) bacterial cellulose diaphragm. Sony has chosen bacterial cellulose because of its unique dimensional stability/rigidity and capacity to give rise to a sound transducer membrane which maintains high sonic velocity over a wide frequency range. Comparing to aluminum or titanium diaphragm, BC diaphragm produces the same sound velocity along with the warm, delicate sound that the paper diaphragm provides. Trebles are sparkling clear, and bass notes are remarkably deep and rich.

In the recent years BC was proposed as a transparent nano-composite support for organic light-emitting diode (OLED). The present OLED displays are supported on foldable plastics which have extremely large coefficient of thermal expansion (CTE), exceeding 200 ppm K\(^{-1}\) which can induce cracks in the manufacturing processes. In order to restrict the thermal expansion, some researchers have proposed the fabrication of transparent nanocomposites made of plastics reinforced with bacterial cellulose (BC) [33]. Because of the low thermal expansion of cellulose nanofibers, BC offers an extremely low coefficient of thermal expansion, an indispensable property for display substrates of only 4–6 ppm K\(^{-1}\) and a transparency of 81.3 % (fig. 2.7).
Despite its low thermal expansion coefficient, no commercial product is available in this application area at this moment.

In fashion industry, bacterial cellulose is regarded as a substitute for leather. BioCouture research project is investigating the use of bacterial-cellulose, grown in laboratories, to produce clothing. Their ultimate goal is to literally grow a dress in a vat of liquid. Some of their creations are presented in figure 2.8.

![BioCouture denim jacket](image)

**Figure 2.8 BioCouture denim jacket.**

Other technical applications of BC, according to various literature sources are: production of quality paper [33], paint additives, cellulose based aerogels [36] etc.
Literature


Production Methods and Characteristics of Bacterial Cellulose Composites


[34] D.C. Johnson and A.R. Winslow, Bacterial cellulose has potential application as new paper coating. Pulp & Paper, pp. 105–107 May 1990

Chapter 3

Advantages and disadvantages of using BC as a structural material

Bacterial cellulose is a versatile form of cellulose. Despite the increasing success and versatility in certain applications, some of its properties can cause catastrophic disadvantages in others. This short chapter outlines the advantages and disadvantages of BC as a structural material based on different literature sources.

3.1 Advantages of BC as a structural material

The major advantage of BC is its biodegradability and environmentally-friendly nature. In the last few years, many technological innovations have been more and more fuelled by the world’s concern with the depletion of natural resources and the impact of technology on the environment and climate change. Ultimately, there is a strong focus on renewable and biodegradable materials [1]. An important issue with most natural materials used nowadays refers to the mechanical properties which are far more inferior to the properties of synthetic materials such as glass, carbon fiber and aramid used in many different applications, because of their high performance and versatility. Bacterial cellulose might be one of the innovative materials capable to combine the renewability and biodegradability of natural
materials with the high mechanical properties of glass fibers. Figure 3.1 illustrates the renewability and biodegradability cycle of composite-x based on BC.

![Renewability and Biodegradability cycle of BC composite-x.](image)

According to various literature sources, bacterial cellulose has been utilized as raw material for composite manufacturing having the following mechanical characteristics:

- In 2004, Wolfgang Gindl reports the result of a set of tensile tests performed on cellulose acetate butyrate composites reinforced with bacterial cellulose [2]. They produced the composite by solvent evaporation casting. Their composite material contained 10% and 32% volume cellulose, and revealed a Young’s modulus of 3.2, respectively 5.8 GPa, and a strength of 52.6 and 128.9 MPa. In their experiment, they also reported an increase in the elastic modulus which occurred when the specimens were cyclic tensile-loaded and unloaded. This effect was credited by the authors on the cellulose fiber’s capability to reorient from their initially random orientation due to straining.

- In 2005, the Research Institute for Sustainable Humanosphere, Kyoto University, Japan published one of the first relevant papers in the field with clear and realistic data regarding the production of a hybrid BC composite [3]. They reported that processed BC sheets were impregnated with
phenol-formaldehyde resin in order to form a laminar composite using 25 sheets. The formed composites were subject to a three-point bending test and tensile test. Results were compared to similar tests having as subject microfibrilated cellulose (MFC) based composites. The authors concluded that the Young’s modulus for the BC-based composite is 28 GPa and 19 GPa for the MFC-based composite and a tensile strength up to 420MPa in the case of the BC-based composite [3]. This higher modulus of BC composites was credited to the fine and uniform 3D structure formed by *A. Xylinum*. This mechanical high performance is directly connected with the bi-dimensionally orientation of the uniform nanoscale network formed by *A. Xylinum*. This was concluded from the fact that MFC-based composites had the same type of fibers in their structure as BC, but the network that was formed by the fiber resulted from a sedimentation effect.

- A more recent publication of Zhijiang Cai 2010 [4] describes the preparation and characterization of new bacterial cellulose/gelatin scaffold for tissue regeneration using bacterial cellulose hydro-gel. Their composite was produced by incorporating gelatin in the bacterial cellulose followed by freeze-drying. They observed that after incorporation of gelatin in the bacterial cellulose, the Young’s modulus of the composite increased from 3.7 GPa to 3.9 GPa, while the tensile strength and strain at break point decreased from 170 MPa (7.5%) to 114 MPa (4%). They performed also a water sorption behavior test which indicated that the water uptake capacity of the composite was only half of that of pure bacterial cellulose. Finally, they concluded their work with a general comparison table presented in table 3.1.

<table>
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<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile strength (MPa)</td>
<td>150–240</td>
<td>[5,6]</td>
<td>170±16</td>
<td>114±11</td>
</tr>
<tr>
<td>Young’s modulus (GPa)</td>
<td>0.6–9.7</td>
<td>[6,7]</td>
<td>3.7±0.8</td>
<td>3.9±0.7</td>
</tr>
<tr>
<td>Thermal degradation temperature (°C)</td>
<td>300</td>
<td>[8]</td>
<td>264</td>
<td>364</td>
</tr>
<tr>
<td>Crystallinity index (%)</td>
<td>60–80</td>
<td>[9]</td>
<td>87</td>
<td>81</td>
</tr>
<tr>
<td>Elongation at break (%)</td>
<td>2.6</td>
<td>[6]</td>
<td>7.5±0.5</td>
<td>4.0±0.3</td>
</tr>
</tbody>
</table>

Other advantages of BC refer to processing properties: the wear on milling tools is almost negligible compared to that on tools used for the fabrication of parts of metal or synthetic composite parts. Compared to glass, it has lower density and favorable energy absorption, good acoustic and thermal properties. It reveals higher toughness than glass, which means it does not splinter like glass fiber. It has
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the potential for one-step manufacturing even of complex construction elements and it has the capability to be made almost completely transparent [1]. Health risks that come with splintering of the material or use of toxic materials are much smaller when working with BC [1].

Production of metals, glass and carbon fibers requires very high production temperatures and therefore a lot of energy, which also dominates the price. For example, a kilogram of glass fibers demands an energy input of 55 MJ to produce, compared to just 17 MJ for a kilogram of flax fibers. Production of BC on a large scale is not yet investigated, but at least these wasteful high energies due to high production temperatures are not needed [1].

One of the most attractive characteristics of BC for researchers is the fact that BC can be produced in predetermined shapes. Because the cellulose is formed on the interface between the air and the culture medium, the shape of the cellulose is directly determined by the shape of the interface medium. White and Brown [10] used a gas permeable mould, submerged in an *A. Xylinum* culture to produce a seamless cellulose glove (fig. 3.2). This method could be utilized to achieve any desired shape.

![Dried cellulose formed into glove shape](image.png)

Figure 3.1. Dried cellulose formed into glove shape [10]
3.2 Disadvantages of BC as structural material

Even though this thesis has been documented on hundreds of articles, while investigating, I have reached to the conclusion that many of the authors have omitted to outline/specify the potential disadvantages of using bacterial cellulose as a structural material. This is mainly due to the fact that the authors have investigated, in most of the cases, the BC for collateral applications and slightly emphasized the potential of BC as a structural material.

When generally referring to a monolithic structural material, the Young’s modulus and ultimate tensile strength are the dominant characteristics used by engineers to evaluate the structural integrity of the final product. In the case of composites, fatigue plays the essential role. When observing the characteristics of bacterial cellulose thoroughly, it can be easily deduced that it has a high affinity for water absorption. This can be a great value in some of the applications (medical), but in structural applications can lead to catastrophic failure. Most of the structural components undergo thermal cycles with temperature variations between +70°C - 50°C. During such a thermal cycle the water retained by the composite, especially with temperatures below -20°C will ultimately alter the internal structure of the material. Despite the previous pointed issues there are not any reported research investigations existing on the effects of water sorption behavior in bacterial cellulose composites. As mentioned in chapter 1, one of the objectives of this thesis/research is to quantify the water absorption effects on the structural properties of bacterial cellulose.

However, some of the other disadvantages associated with bacterial cellulose as a structural material refer to:

- High price (about 100 x more than plant cellulose), mainly because of high priced substrates (sugars) and medium sterilization;
- Low volumetric yields;
- Lack of large scale production capacity;
- Long-time expansion and maintenance of the cell culture for production;
- Large quantities of water used for treatment and large quantities of caustic residual waters resulted after washing the bacterial cellulose.
Literature


Chapter 4

Cellulose in composites

This chapter presents an overview on the methods and procedures employed to increase adhesion of cellulose in composite materials. The chapter starts with a short literature overview on the theory concerning with the structure of porous bodies and continues with describing the topology elements of composite materials. The chapter ends with the description of a set of new-developed high-end cellulose composites.

4.1 Structural properties of porous bodies

As mentioned before, one of the less investigated characteristics of bacterial cellulose composites is the water sorption behavior. Generally, this characteristic can be determined by two factors: first - the cellulose composite has porous structure, second - cellulose can form hydrogen bonding with water. Despite the fact that cellulose is insoluble in water, it can form hydrogen bonds with water. To a certain extent, any material, even monolithic materials can have porous structure and absorb water. To have a better view on the phenomena, the structural properties of porous bodies are described below:

A porous environment is considered to be a solid containing pores. A geometrical definition of the pore concept is quite difficult. Usually, pores are considered hollow spaces distributed inside the solid that can communicate or not with each other, being closed or open between faces. The communicating portion of a pore is
Production Methods and Characteristics of Bacterial Cellulose Composites

commonly referred to as effective pore space or simply effective porosity. This term is largely used in filtering technology where porous bodies can have a high total porosity related to a low effective porosity. Total porosity is always superior to the effective one [1].

General (volume) porosity is defined as the ratio between pore's volume and form volume:

\[ \varphi = \frac{V_{\text{pore}}}{V_f} \]  

(4.1)

Together with volume porosity, there is the concept of surface porosity, defined as the ratio between the pore's ending area existing onto a sectioned surface of the porous form and the area of the sectioned surface:

For a first approximation, the equality \( \varphi = \varphi_s \) is acceptable.

The simplest model of the porous body is a system of short radius and equal-form particles (mono-disperse granular system). Any poly-disperse granular system can be fragmented into mono-disperse granular systems by real or hypothetical granular metric analysis.

Figure 4.1 shows that spherical particles can be arranged in different ways. The limits are a rare layout (a, cubic) and a dense layout (c, hexagonal). Between these two limits there are all real layouts. In the ideal case, if the particles do not form hydrogen bonds with water, the water holding capacity will be directly proportional with the porosity volume.

On the other hand, the mechanical properties of the composites are generically described by the formula 4.2 where the pore's volume can be substituted by the
resin matrix volume. This is valid if we consider that figure 4.1. represents a local cross-section of a composite.

\[ P_c = (P_f, V_f, P_m, V_m) \]  

(4.2)

where:

- \( P_c \) - mechanical properties of the composite;
- \( P_f \) - mechanical properties of the fibers;
- \( V_f \) - volume fraction of the fibers;
- \( P_m \) - mechanical properties of the matrix;
- \( V_m \) - volume fraction of the matrix.

A fundamental parameter of the composite’s micromechanics is the representative volume element, which, for a composite with unidirectional fibers, is sketched in the next figure:

![Figure 4.2 Representative volume element within a composite](image)

This volume element represents the smallest portion within the material, where stresses and strains are considered to be macroscopically uniform. Microscopically, these stresses and strains are nonuniform because the material is not homogeneous. For a 3D orientation of the fibers, as it is the case of bacterial cellulose composites, the microscopic representation is more complex. For simplification, it will be easier to consider just two directions and extract an empirical coefficient for third direction. In this case, if the wiring geometry is neglected, then two reference dimensions of the volume element are the distances between the fibers, one for each direction, and the third dimension is imposed by the number of fiber layers in the direction of the material thickness.
The micromechanical analysis of a composite is based on the following simplified hypothesis [2]:

- Lamina is considered macroscopically homogeneous and orthotropic, linearly elastic and without initial internal stresses.
- Fibers are considered homogeneous and isotropic, linearly elastic, having a regular position and being perfectly aligned.
- Matrix is considered homogeneous and isotropic, linearly elastic and having a perfect adherence to the fibers.

Regarding material porosity, it is acknowledged that hollow spaces inside a porous material can be interior (closed) pores, exterior (open at both ends) pores and inter-granular pores or unoccupied spaces. Monofilament fabrics and granular layers of non-porous particles generally have inter-granular spaces. In operations involving flows through porous structures (filtering, capillary pumping etc.), the porosity corresponding to inter-granular spaces and sometimes to open pores presents interest. Pore size distribution is also important, though pore “size” or “diameter” is a simplified notion, because pores are far from having cylindrical form. Most commonly, they have irregular forms with contractions and ramifications. This is why a porous material is preferably characterized by the notion of permeability. But there are cases, especially regarding membranes, that consider as important the size of the pores [1].

This theory stands just in the case when cellulose does not form hydrogen bonds with water or when mechanical properties of the material are evaluated for water free environments. However, in the next part of this chapter the “cellulose- water relation is analyzed”.

### 4.2 Cellulose hydrogen bonding and cross linking

Cellulose has no taste, is odorless, hydrophilic, insoluble in water and most organic solvents, chiral and it is also biodegradable. It can be broken down chemically into its glucose units by treating it with concentrated acids at high temperatures. In pure form, cellulose always absorbs water, which has a plasticizer effect upon it. It is acknowledged that fabrics of cellulosic fibers exhibit good dimensional stability in
the dry state, but can shrink and/or wrinkle when wet. This occurs because, in dry state, the cellulose chains are held together by extensive networks of hydrogen bonds between the hydroxyl groups of adjacent chains in its structure. In other words, the hydrogen bonds form a cross-linked structure. If a stress, such as twisting or folding, is applied to the dry fabric, the hydrogen bond cross-links tend to hold the chains in position and cause the fabric to return to their original position when the deforming stress is removed. However, when the fabric is brought into contact with moisture, water molecules can participate in the hydrogen bonding and penetrate between the cellulose chains, effectively breaking up the cross-linked structure. The water molecules act as a plasticizer for cellulose and the chains may move relative to each other. If the fabric becomes wrinkled in the moist state, the chains move to relieve the strain and there is no effective force to return the fabric to its original shape when the stress is removed. Thus, for cellulosic fabrics to exhibit durable press (also termed permanent press or wrinkle-resistant) characteristics, it is necessary to form cross-links which are not easily broken by water. This is usually done with formaldehyde or formaldehyde derivatives, such as urea-formaldehyde resins.

Several research groups have mentioned that, in case of bacterial cellulose, one of the major problems with producing a composite out of this material is the presence of hydroxyl and other polar groups within its structure. This makes it highly hydrophilic and therefore it doesn’t bond optimally with commonly used resins like epoxy or polyester as they cannot wet the fibers sufficiently. Another problem is the strong crystalline content of cellulose which inhibits the penetration of resin [3, 4].

To overcome these issues, some researchers have modified the cellulose by adding hydrophobic elements to the molecular structure and thereby improving the adhesion to hydrophobic resins. Abdelmouleh [5] describes a method of treating plant cellulose with silanes that react with the OH groups of the cellulose and gives

Figure 4.3: SEM of freshly fracture surfaces of epoxy composites based on long viscose fibres ; a). without treatment, b). after treatments with silanes [5]
it a hydrophobic character. Composites that were made with polyester and epoxy clearly showed an improvement in bonding between the fibers and resin (fig. 4.3). When flexural tests were performed the results revealed an increase in flexural strength of up to 40%. However, the author did not report studies on the composite’s water sorption behavior.

Mohanty [3] describes several methods of treating natural fibers, aiming to improve adhesion characteristics. For example, alkali treatment can remove the hydroxyl group and replaces it with a Na$^+$ ion, which makes the surface rougher and therefore improve the mechanical interlocking of the cellulose with the resin, but also depolymerises the native cellulose. Graft copolymerization was used to graft vinyl monomers onto natural fibers that are compatible with several resin systems. Etherification was done by cyanoethylation and acetylation was used to produce esterified natural fibers which both results in plastic behavior. Isocyanate groups were used to react with the hydroxyl groups in order to form strong covalent bonds and create better compatibility with binder resins. Treatment with polypropylene is believed to increase the surface energy of the fibers and therefore further increase the wettability and interfacial adhesion.

Very good results were obtained also when cellulose was used as matrix. In 2004 Takashi et al, [6] published the Paper “All-Cellulose Composites”, in which they characterize a new type of composite having cellulose as matrix and cellulose fibers as reinforcement fibers. In their studies they used refined ramie as cellulose fibers and for the matrix they used pretreated craft pulp from coniferous trees solved in 8% LiCl. The new composite revealed a tensile strength of up to 480 MPa, this strength being the highest tensile strength ever reported on any cellulose based composite. However, the author did not reported studies on the composite’s water absorption capability.

**Conclusions:**

It can be concluded that cellulose water absorption capability and its cross linking capability are complex issues. Not even in new high-end cellulose composites which utilize plants fibers as row material, the water sorption behavior is not investigated enough. It will be almost impossible to analytically quantify the water absorption capability and mechanical properties of arbitrary cellulose composite without performing in advance tensile and flexural tests in combination with water absorption tests. To account for this, the next chapters will describe the preparation of a set of new bacterial cellulose based composites, their mechanical characteristics and water absorption behavior.
Literature


Chapter 5

Production of Bacterial Cellulose

Until today, there are three known methods to produce bacterial cellulose suitable for industrial application: static culture, submerged culture and rotating disc reactor. Each method is characterized by unique production features but they all share the same growth medium. This chapter describes the growth medium of A. Xylinum and details a series of experiments performed with the aim to establish the optimum growth parameters.

5.1 Growth medium overview

Following the discovery of Brown’s membrane, several researchers have investigated the capability of A. Xylinum to grow in different mediums. Most of the researchers have concluded that a minimum set of conditions are required by the organism to be able to produce cellulose. These conditions are: a liquid environment, a 3 to 6 pH level and a carbon source.

One of the most common utilized growth medium for A. Xylinum is the Schramm-Hestrin [1] medium presented in table 5.1. The initial pH value of the medium is 6.0 and it utilizes Disodium Phosphate as a buffering agent. Many researchers use NaOH or HCl to adjust this value. Another commonly used variant for this medium is when citric acid is substituted with acetic acid.
Table 5.1. Composition of Schramm-Hestrin Medium [1].

<table>
<thead>
<tr>
<th>Components of Medium</th>
<th>Concentration (%, w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>2.0</td>
</tr>
<tr>
<td>Bacto Peptone</td>
<td>0.5</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>0.5</td>
</tr>
<tr>
<td>Disodium Phosphate (anhydrous)</td>
<td>0.27</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>0.115</td>
</tr>
</tbody>
</table>

A more synthetic medium is for example the medium used by S. O. Bae and M. Shoda [2]. This medium uses corn steep liquor-fructose (CSL-Fru)“CSL (Showa Sangyo, Japan) (20 mL) and fructose (40 g) as carbon source. In addition to the carbon source the medium contains the next substances: \( \text{KH}_2\text{PO}_4 \) (1g), \( \text{MgSO}_4\cdot\text{7H}_2\text{O} \) (0.25 g), \( (\text{NH}_4)_2\text{SO}_4 \) (3.3 g), \( \text{FeSO}_4\cdot\text{7H}_2\text{O} \) (3.6 mg), \( \text{CaCl}_2\cdot\text{2H}_2\text{O} \) (14.7 mg), \( \text{NaMoO}_4\cdot\text{2H}_2\text{O} \) (2.42 mg), \( \text{ZnSO}_4\cdot\text{7H}_2\text{O} \) (1.73 mg), \( \text{MnSO}_4\cdot\text{5H}_2\text{O} \) (1.39 mg), \( \text{CuSO}_4\cdot\text{5H}_2\text{O} \) (0.05 mg), and vitamin solution (10 mL). The vitamin solution consisted of (per liter of deionized water) inositol (200 mg), nicotinic acid (40 mg), pyridoxine hydrochloride (40 mg), thiamine hydrochloride (40 mg), D-pantothenic acid calcium (20 mg), riboflavin (20 mg), p-aminobenzoic acid (20 mg), folic acid (0.2 mg), and D-biotin (0.2 mg). The pH of the medium was adjusted to 5.0. An antifoaming agent (Disfoam GD, ihon Yushi, Tokyo, Japan) was used to prevent foam formation on the surface of the medium during cultivation”.

The mediums described previously are synthetic mediums that yield high cellulose production - of up to 6.5 g/L. However, below we propose a more natural and less-expensive growth medium for *A. Xylinum* presented in table 5.2.

Table 5.2. Composition medium proposed in this thesis:

<table>
<thead>
<tr>
<th>Components of Medium</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Honey (g/L)</td>
<td>40</td>
</tr>
<tr>
<td>Vinegar (ml/L)</td>
<td>~ 200</td>
</tr>
<tr>
<td>Yeast (g/L)</td>
<td>15</td>
</tr>
</tbody>
</table>

For this medium, it is recommended to use source water. It is not the optimum medium to yield high production of bacterial cellulose, but because the components of the medium – honey, yeast, vinegar – contain the basic elements required for bacteria growth, the production of cellulose is not very high but
acceptable. Note that honey has a pH between 3.2 and 3.9 depending on the type of honey. The approximate composition of honey is described in table 5.3. In consequence, the concentration of vinegar can be varied or the pH value can be adjusted with NaOH to 4.0. A higher pH value than 4.0 in this medium can produce alcoholic fermentation because the use of yeast enables both the development of *A. Xylinum* and other micro-organisms as well.

Table 5.3. Honey composition:

<table>
<thead>
<tr>
<th>Components</th>
<th>Value per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates (g)</td>
<td>82.12</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>0.2</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.3</td>
</tr>
<tr>
<td>Water (g)</td>
<td>17.10</td>
</tr>
<tr>
<td>Riboflavin (Vit. B2)(mg)</td>
<td>0.038</td>
</tr>
<tr>
<td>Niacin (Vit. B3) (mg)</td>
<td>0.121</td>
</tr>
<tr>
<td>Pantothenic acid (B5) (mg)</td>
<td>0.068</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>0.024</td>
</tr>
<tr>
<td>Folate (Vit. B9) ( μg)</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>0.5</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>6</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>0.42</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>2</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>4</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>52</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>4</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Carbohydrates composition: 48% fructose, 47% glucose and 5% sucrose.

If we comparatively examine the three growth mediums, we observe that the same set of basic elements, existing in Schramm-Hestrin’s medium, S. O. Bae and M. Shoda’s medium and the proposed medium are present in the composition. The key-vitamins, like Vit.B2 and Vit.B3, responsible for inhibition of mutant bacteria, are present in acceptable concentrations in honey. The vinegar in this medium can be substituted with glacial acetic acid for economical reasons. Preliminary experiments showed small differences in cellulose production when glacial acid was utilized. Also honey can be substituted with brown sugar - the composition presented in table 5.4. - or directly with sugar beet
Production Methods and Characteristics of Bacterial Cellulose Composites

juice. When using sugar beet juice, this requires pasteurization in order to avoid medium contamination.

Table 5.4. Brown sugar composition:

<table>
<thead>
<tr>
<th>Components</th>
<th>Value per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates (g)</td>
<td>96.21</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>0.0</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.0</td>
</tr>
<tr>
<td>Water (g)</td>
<td>1.77</td>
</tr>
<tr>
<td>Thiamin (Vit. B1) (mg)</td>
<td>0.008</td>
</tr>
<tr>
<td>Riboflavin (Vit. B2) (mg)</td>
<td>0.007</td>
</tr>
<tr>
<td>Niacin (Vit. B3) (mg)</td>
<td>0.082</td>
</tr>
<tr>
<td>Pantothentic acid (B5) (mg)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>0.026</td>
</tr>
<tr>
<td>Folate (Vit. B9) (μg)</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>0.0</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>85</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>1.91</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>29</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>22</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>346</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>39</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Based on a set of preliminary experiments, we concluded that the most economically efficient production medium consists of: glacial acetic acid, yeast, sugar beet juice, disodium phosphate, riboflavin (Vit.B2) and niacin (Vit.B3). The estimated price of 1 liter of proposed medium is 0.16 euro, excluding the price of water. This medium yields a cellulose production of 3g/L. If peptone is added to the medium, the cellulose production yield increases to 6g/L but the cost of 1 liter of medium becomes 1,168 euro.

The cellulose yield is equally influenced by the medium composition as well as by the cultivation type. The highest yield reported in literature occurs when cellulose is grown in aerated cultivation. However, the cellulose obtained from this type of cultivation is not of interest if used in structural applications. Cellulose can be produced as pellicles suitable for structural applications exclusively in static cultivation and in rotating disk reactor cultivation. The next subchapters describe these two types of cultivation.
5.2 Static culture

In static culture, *A. Xylinum* produces cellulose at the air/liquid interface. Production is inhibited by mixing and aeration. The traditional method of production has been on the surface of static cultures. The method is simple, low-tech, and it is the only widely utilized method of cellulose pellicle production. In static production, an inoculated medium is poured into shallow trays, then covered and allowed to rest for 5 to 20 days until the pellicle nearly fills the tray. The pellicle is removed and washed to eliminate the cells; then it can be processed as desired. During growth, once the pellicle has formed, the cellulose propagates from the surface of the culture. Several researchers have shown that the uppermost layer of the pellicle is the only one growing [3], and the cells that are left further into the pellicle become inactive or die from lack of oxygen. Figure 5.1 shows the pellicle in static tray with the active layer indicated. It is important that nutrients diffuse up to the cells, and oxygen diffuses down. When the pellicle is large, diffusion limitation occur and prevent maximum rate of growth.

![Figure 5.1: Cellulose growing in static culture.](image)

While static production is very simple, it has some drawbacks. Diffusion problems have already been mentioned, but other problems exist as well. The trays, once inoculated, can not be disturbed until harvest time. This makes measurements nearly impossible, since probes can not be introduced into the medium or removed.
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for cleaning, and therefore pH value can not be measured or controlled. This constantly changing environment does not encourage rapid growth, and pH can’t be maintained at optimum levels. Another disadvantage of this system is the inability to access the medium to make changes, such as maintaining a certain substrate concentration or adding a modifying reagent. The last and possibly the greatest drawback to static cultures is scale-up. Scale-up is nearly linear and few economic large scale production units are realized.

To overcome these disadvantages, an improved laboratory system is proposed further. To make static culture economically efficient, we designed a set of new features. The new - developed system utilizes two trays and features a recirculation pump to control the diffusion and evaporation of medium (fig. 5.2.). In the first tray the growth conditions – medium and air - are assured. The first tray was connected to a second tray, which was designed to access the medium during production. In this tray the air was substituted by nitrogen to inhibit the cellulose production.

![Figure 5.2: Controlled static culture.](image)

Using this system, we can maintain constant medium volume and medium circulation. When the pump is operating, a pressure drop can occur. A fixing mesh is used to avoid membrane displacement during medium recirculation. This mesh allows also volume variation in order to increase the medium diffusion within the cellulose pellicle. However, the main disadvantage of this system is that it doubles the quantity of utilized medium.
5.3 Rotating disk reactor

Bacterial cellulose pellicles can be produced in a rotating disk reactor (RDR) [4]. This system consists of a tray with inoculated medium in which a disk assembly is introduced. The disk assembly is made from flat, circular disks mounted on a centered shaft and rotated through and through with a motor. The cells adhere to the surface of the disks, most likely as a result of the extruding fibers, and form a pellicle on the surface of the disks. After 8 to 12 hours, nearly all of the cells are entrained on the disks leaving the medium clear. A schematic drawing of an RDR is shown in Figure 5.3. Solid disks are inferior to perforated or meshed disks, as the holes allow significantly more medium hold-up on the disks, and therefore faster and stronger film formation. Wet cellulose formation per unit area is higher in this system, but the pellicle has twice the water holding capacity of a typical static pellicle. Once dried, the cellulose production per unit area of each method is comparable.

![Figure 5.3: Schematic diagram of a rotating disk reactor.](image)

However, even though this method allows easy access to the medium, it is not suitable in the production of cellulose for structural applications. This is mainly caused by the fact that the disks’ diameter is limited by circumferential speed and gravity.

We can conclude the first part of this chapter by stating that the cellulose production for structural applications can be supported by multiple mediums, but the cultivation type is constrained to static cultivation.
5.4 Experimental data 1

As mentioned before, *A. Xylinum* can produce cellulose on a variety of mediums but growth conditions, like pH value, temperature, oxygen concentration etc. influence the production as well. To identify the optimum conditions for production of bacterial cellulose, a first study was performed. The study targeted the investigation of optimum conditions like optimum pH level, temperature influence on growth, medium height, level of evaporation, air supply in controlled environment etc. The most relevant results are presented below in this section. The driving engine in our studies was the motto “in order to succeed, try harder to fail in small steps”.

5.4.1 Study on optimum pH level

The experiments presented in this section have been conducted at Delft University of Technology. The purpose of this study is to identify the optimum pH level for cellulose production if the medium described below is used.

**Materials:**

Vinegar ≈ 6%, water (Delft tap water), brown sugar, yeast, sodium hydroxide 0.1 N

Table 5.5. Growth medium for experiment 1:

<table>
<thead>
<tr>
<th>Components of Medium</th>
<th>Prob.1</th>
<th>Prob.2</th>
<th>Prob.3</th>
<th>Prob.4</th>
<th>Prob.5</th>
<th>Prob.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown sugar (g/L)</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Yeast (g/L)</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Vinegar (ml/L)</td>
<td>500</td>
<td>333</td>
<td>250</td>
<td>200</td>
<td>166</td>
<td>100</td>
</tr>
<tr>
<td>Water (ml/L)</td>
<td>500</td>
<td>667</td>
<td>750</td>
<td>800</td>
<td>834</td>
<td>900</td>
</tr>
<tr>
<td>pH</td>
<td>3.3</td>
<td>3.6</td>
<td>3.7</td>
<td>4</td>
<td>4.7</td>
<td>5.2</td>
</tr>
</tbody>
</table>

*Cultivation temperature: T=23 °C (±1 °C)*

All probes were equally inoculated with *A. Xylinum* and put in working position. The probes were irradiated during the experiment with a 150 W halogen light from a height of 360 mm. The 6 probes were put in cylindrical glass containers, with a diameter of Ø 67 mm and a height of 60 mm.
Results:

After 80h the membrane formed at the top (see figure 5.4) of the medium was removed, its thickness and mass measured and its structural uniformity analyzed. The results are presented in the table below.

Table 5.6. Results obtained in experiment 1:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prob.1</th>
<th>Prob.2</th>
<th>Prob.3</th>
<th>Prob.4</th>
<th>Prob.5</th>
<th>Prob.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td>20</td>
<td>32</td>
<td>39</td>
<td>45</td>
<td>48</td>
<td>43</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>1.5</td>
<td>2.1</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Structure*</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

* 1 unacceptable, 2 acceptable, 3 good.

Figure 5.5: Cellulose production at different pH levels.
Results:

Probe 1 exhibits high hydration level. The resistance of the membrane is very poor and holes are visible in the structure. In some sections of the liquid media the bacteria grew in isolated colonies (fig. 5.6 – probe 1). Probe 2 exhibits high hydration level; the resistance of the membrane is poor. It is visible that the bacteria grew in isolated colonies (fig. 5.6 – probe 2). Probe 3 exhibits acceptable resistance. It is visible that the bacteria formed similar colonies as probe 2. Probe 4/5/6 shows good resistance. On the surface of probe 4 six bacteria colonies are visible but the defects are insignificant comparing to the overall aspect of the membrane.

Conclusions:

Experimental data show that pH level plays an important role in the production of bacterial cellulose pellicles by A. Xylinum. The major observation is that pH level radically influences the structural quality of bacterial cellulose. The membrane does not behave well under structural loads if the A. Xylinum develops in isolated colonies without forming a homogeneous reticular structure. In relation to medium volume, the cellulose production does not decrease dramatically because of the pH variation, but the resulted cellulose has a very limited technical applicability. However, the A. Xylinum colony formation described previously can be caused also by insufficient medium inoculation or the culture’s incapacity to fully adapt to low values of pH. Note that the inoculum was grown at a 6.0 pH level.
5.4.2 Medium aeration before growth

This chapter describes an experiment performed with the aim to measure to which degree the culture’s incapacity to fully adapt to a prescribed pH value can influence the production of cellulose. The experiments rely on several literature reports describing that, in static culture, the doubling time of *A. Xylinum* growth is in the range of 8 to 10 hours and 4 to 6 hours in agitated/aerated culture [5] or even 2 hours in shaken culture [6]. The same source states that *A. Xylinum* has an ethanol tolerance of up to 10 % and that ethanol does not stimulate the cellulose production but accelerates the doubling time.

Based on this information, we assumed that if the inoculated medium is aerated before static cultivation, it will be capable to enhance the formation of a new generation of bacteria adapted to the new medium pH. To confirm this hypothesis, we conducted a set of experiments in which the medium was aerated with 1.4 L/min of air for 24 hours before static cultivation. Probes were aerated and irradiated for 4 hours with a halogen light of 150 W from a height of 300 mm.

![Fig. 5.7: Photo of cellulose formed after 2 hours in pre-aerated static culture](image)

It was concluded that the medium which was previously aerated, formed in static culture a visible membrane (1-2 mm in thickness) after just three hours (fig. 5.7. the reflection of the light spot is deformed due to the formation of bacterial cellulose). In similar conditions, with no aeration, the pellicle required 24 hours to grow completely.

The experiments performed in the previous sub-chapter were repeated using a 24 hours aeration before static cultivation. The results showed that between pH values
of 3.5 - 4.7, the cellulose production did not vary. Any of these pH values allowed the formation of good quality pellicles.

5.4.3 Study on temperature influence on growth

The experiments presented in this section have been conducted at Delft University of Technology. From previous experiments, we concluded that temperatures below 20 ºC dramatically decrease the production of cellulose by *A. Xylinum*. For this reason, the temperature range in this study was adjusted to temperatures between 20 to 26 ºC.

**Materials:**

Vinegar ≈ 6%, water (Delft tap water), brown sugar, yeast, sodium hydroxide 0.1 N

Table 5.7. Growth medium for experiment 2:

<table>
<thead>
<tr>
<th>Components of Medium</th>
<th>Concentration (/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown sugar (g)</td>
<td>16 g</td>
</tr>
<tr>
<td>Yeast (g)</td>
<td>15g</td>
</tr>
<tr>
<td>Vinegar (ml)</td>
<td>166 ml</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>834 ml</td>
</tr>
<tr>
<td>pH</td>
<td>4.7</td>
</tr>
</tbody>
</table>

**Cultivation conditions:**

The experiment was carried out in two phases. In phase one, we proceeded by setting up 3 cylindrical glass containers with Ø 67 mm in diameter and 60 mm height at 20 ºC and other 3 identical containers at 22 ºC. In phase two, the 6 containers were prepared in the same manner but the cultivation temperature was increased to 24 ºC respectively 26 ºC. Each probe was equally inoculated with *A. Xylinum*.

**Results:**

After 80h the membrane formed at the top of the medium was removed, its thickness and mass measured and structural uniformity analyzed. All the membranes exhibited a homogeneous structure and no relevant differences
between them were identified. Mass and thickness varied with max 1% between the probes. The maximum thickness/mass of the membranes was registered at 24 °C.

**Conclusions:**

Temperature has a major influence on the cellulose production by *A. Xylinum*. Different literature sources claim that at temperatures of 16 °C and 36 °C, the production of cellulose by *A. Xylinum* is inhibited and that the optimum production temperature is between 21-26 °C. The experiment presented above confirmed that *A. Xylinum* has a stable production rate at temperatures of 21-26 °C. However, we observed that when yeast is used to increase production efficiency and the temperature is higher than 24 °C, alcoholic fermentation occurs (fig. 5.8)

![Fig. 5.8: Photo of alcoholic fermentation in bacterial cellulose production](image)

5.4.4 **Medium height optimization**

During previous experiments, it was observed that the medium height in the cultivation recipient influences cellulose production. The first supposition refers to the fact that *A. Xylinum*, during production, decreases the pH value of the medium. After a set of experiments were performed, we concluded that even though it has a reasonable scientific explanation, the height of the medium is not directly connected to the pH value. To investigate further the influence of the medium height on production, a set of empirical experiments were performed. These experiments are described below:
Materials:

Vinegar ≈ 6%, water (Delft tap water), brown sugar, yeast, sodium hydroxide 0.1 N

Table 5.8. Growth medium for experiment 3:

<table>
<thead>
<tr>
<th>Components of Medium</th>
<th>Concentration (/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown sugar (g)</td>
<td>16 g</td>
</tr>
<tr>
<td>Yeast (g)</td>
<td>15 g</td>
</tr>
<tr>
<td>Vinegar (ml)</td>
<td>166 ml</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>834 ml</td>
</tr>
<tr>
<td>pH</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Cultivation conditions:

Seven squared containers with round edges and dimensions of 130 mm width, 130 mm length and a height of 30 mm above the culture medium were used in this experiment. Another squared container, with round edges and dimensions of 200 mm width, 350 mm length has been positioned with a β angle from the horizontal to create a variation of 20 mm to 45 mm in medium height.

Results:

After 80h, membranes were removed from containers, their thickness and mass measured and the structural uniformity analyzed. The membranes from probe 1/2/3/4/5 presented a homogeneous structure and no relevant differences between them were identified. The membranes from probe 6 and 7 presented in their structure transparent spots. The obtained results are presented in table 5.9.

Table 5.9. Results obtained in experiment 3:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nr. of probes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prob.1</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>30</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>1.5</td>
</tr>
<tr>
<td>Structure*</td>
<td>3</td>
</tr>
<tr>
<td>Medium high (mm)</td>
<td>20</td>
</tr>
</tbody>
</table>

* 1 unacceptable, 2 acceptable, 3 good.
The probe positioned with a β angle had a mass of 101g, a thickness of 1.5 mm in the region where the medium was 25 mm in height and ~2 mm where the medium was 45 mm height.

**Conclusions:**

The height of the medium has an influence on the cellulose production but not radically. The data has a qualitative relevance. The medium height is related to the cost. Higher medium column increases the medium volume and consequently the production cost. Based on this fact, a maximum height of 45 mm can be considered optimum.

**5.4.5 Factors of influence on bacterial cellulose growth in static reactors**

In order to optimize the cultivation conditions, a factorial experiment involving four simultaneously analyzed factors (operating temperature, substrate concentration, co-substrate concentration and air specific flow rate) was performed at “Politehnhica” University of Bucharest.

**Experimental set-up:**

A schematic diagram of laboratory set-up used for experimental investigation of cellulose growth in static conditions is presented in figure 5.10.
BC was synthesized in four bioreactors (1), containing culture medium, in form of a pellicle (2) on the air/liquid interface. Bioreactors were fixed in a support (9) and placed in plexiglass enclosure (6) filled with distilled water (7). Temperature, measured by thermometer (8), was maintained at constant value using a heating device (5) equipped with thermostat. Perfect mixing conditions of liquid from enclosure were achieved by submersible pump (4). Atmospheric air fed by compressor (10), whose flow was measured and controlled by device (11), passed through filter (12), active carbon column (13) and silica gel column (14), where it was purified, then it was introduced in bioreactors by an air distributor (3).

**Microorganism and culture conditions:**

A strain of *A. Xylinum* obtained in the Microbiology Laboratory of Chemical Engineering Department at “Politehnica” University of Bucharest was used. For synthesis of cellulose pellicles, a seed culture of *A. Xylinum* was grown in Schramm-Hestrin’s medium in a flask under agitated conditions. After 2 days of preculture, the seed culture was diluted in a 1:10 ratio with prepared culture medium.
Culture medium was composed of fructose (20 g/L, respectively 40 g/L), ethanol (3 cm³/L, respectively 9 cm³/L), citric acid (0.003 g/L), MgSO₄·7H₂O (0.1 g/L), Na₂CO₃·10H₂O (0.1 g/L), CaCl₂·H₂O (0.1 g/L), FeSO₄·7H₂O (0.005 g/L), CoSO₄·H₂O (0.005 g/L), MnSO₄·H₂O (0.005 g/L) and CuSO₄·5H₂O (0.005 g/L). Concentration range for each nutrient was fixed based on literature and own experience. Reagents were dissolved in distilled water, volume was brought up to one liter and pH was adjusted to 5.0 by addition of hydrochloric acid. After this, the culture medium was autoclaved at 121°C for 20 minutes, it was transferred to sterile bioreactors (1) and the batch culture was started.

BC was grown in static conditions for 7 days and then the pellicles produced on the surface of each medium were separated by centrifugation. For removal of microbial product contaminants, the pellicles were washed with water and then boiled in NaOH solution (4%) for 1 hour. The pellicles were then washed with deionised water until a constant and neutral pH value and weighted in wet state. Because of water vaporization in Plexiglas enclosure (6), when temperature and air flow rate were high, it was necessary to add distilled water in bioreactors to maintain a constant level of culture medium.

**Experiment design and statistical analysis:**

Critical cultivation parameters were estimated using a factorial design. Experimental investigation was based on a 24 factorial plan, considering two values (levels) for the process parameters (factors). Four sets of four experiences were performed, each set lasting for 7 days.

Selected factors were operating temperature, fructose concentration, ethanol concentration and air specific flow rate (expressing air flow rate divided by bioreactor transversal surface).

Process responses (dependent variables) were considered BC yield and water specific use, representing cellulose mass, respectively distilled water volume added in each bioreactor, divided by culture medium volume.

**Results and discussion:**

Factors coded values were determined depending on natural values with the following relationships:

\[ x_1 = \frac{T - 30}{5} \]  

(5.1)
Production Methods and Characteristics of Bacterial Cellulose Composites

\[ x_2 = \frac{c_{FR} - 30}{10} \]  
\[ x_3 = \frac{c_{ET} - 6}{3} \]  
\[ x_4 = \frac{G_{air} - 0.42}{0.21} \]

(5.2)  
(5.3)  
(5.4)

Values \( T = 30 \, ^\circ C, \, c_{FR} = 30 \, g/l, \, c_{ET} = 6 \, cm^3/l \) and \( G_{air} = 0.42 \, cm^3/cm^2 \) represent state of process factors within experimental plan centre.

The data concerning performed experiences are presented below.

Table 5.10. Factors and responses values for 24 factorial experiment

<table>
<thead>
<tr>
<th>Exp</th>
<th>Factors natural values</th>
<th>Factors coded values</th>
<th>Responses values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature</td>
<td>Fructose concentration</td>
<td>Ethanol concentration</td>
</tr>
<tr>
<td></td>
<td>( ^\circ C )</td>
<td>( g/l )</td>
<td>( cm^3/l )</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>25</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>25</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>15</td>
<td>25</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>25</td>
<td>20</td>
<td>3</td>
</tr>
</tbody>
</table>
Correlations (5.5) and (5.6) were obtained by processing of data from Table 5.10 on the basis of procedure recommended for a factorial experiment with 2 levels [7]:

\[
y_1 = 11.767 + 0.574 x_1 + 1.167 x_2 - 1.009 x_3 + 0.426 x_4 + 0.592 x_1 x_2 +
+ 0.083 x_1 x_3 + 0.283 x_1 x_4 + 0.193 x_2 x_3 - 0.292 x_2 x_4 + 0.399 x_3 x_4 -
- 0.367 x_1 x_2 x_3 - 0.317 x_1 x_2 x_4 - 0.293 x_1 x_3 x_4 - 0.051 x_2 x_3 x_4 +
+ 0.374 x_1 x_2 x_3 x_4
\] (5.5)

\[
y_2 = 0.629 + 0.129 x_1 + 0.013 x_2 + 0.081 x_3 + 0.358 x_4 + 0.017 x_1 x_2 -
- 0.016 x_1 x_3 + 0.068 x_1 x_4 + 0.013 x_2 x_3 - 0.021 x_2 x_4 + 0.050 x_3 x_4 -
- 0.015 x_1 x_2 x_3 - 0.006 x_1 x_2 x_4 + 0.012 x_1 x_3 x_4 - 0.021 x_2 x_3 x_4 -
- 0.019 x_1 x_2 x_3 x_4
\] (5.6)

Significance of regression coefficients was tested in order to simplify canonical forms of equations (5.5) and (5.6). Thus, from three experiments for 24 plan centre, the following values of BC yield were obtained:

\[
y_1^{01} = 11.3 \text{ g/l}; \ y_1^{02} = 11.8 \text{ g/l}; \ y_1^{03} = 12.3 \text{ g/l}
\]

Square roots of reproducibility variance, \(s_{\beta_j}\), respectively of variance due to regression coefficient \(\beta_j\) \((j = 1...N, N = 16)\), \(s_{\beta_j}\), were obtained with the following correlations [7]:

\[
\bar{y}_1^0 = \frac{3}{3} \sum_{i=1}^{3} \frac{y_1^{0i}}{3} = 11.8
\] (5.7)

\[
s_{\beta} = \sqrt{\frac{\sum_{i=1}^{3} \left(y_1^{0i} - \bar{y}_1^0\right)^2}{2} / 2 = 0.5}
\] (5.8)

\[
s_{\beta_j} = \frac{s_{\beta}}{\sqrt{N}} = \frac{0.5}{\sqrt{16}} = 0.125
\] (5.9)
The results, with respect to the significance of regression coefficients of relationship (5.5) are presented below.

Table 5.11. Significance of regression coefficients of relationship (5.5)

<table>
<thead>
<tr>
<th>j</th>
<th>Regression coefficient</th>
<th>Student variable value</th>
<th>Accepted significance level for $\alpha=0.05$ and $\nu=2$</th>
<th>$t_j - t_{\nu, \alpha/2}$</th>
<th>Verdict</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.767</td>
<td>94.136</td>
<td></td>
<td>$&gt;0$</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.574</td>
<td>4.592</td>
<td></td>
<td>$&gt;0$ rejected</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.167</td>
<td>9.336</td>
<td></td>
<td>$&gt;0$ rejected</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-1.009</td>
<td>8.072</td>
<td></td>
<td>$&gt;0$ rejected</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.426</td>
<td>3.408</td>
<td></td>
<td>$&lt;0$ accepted</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.592</td>
<td>4.736</td>
<td></td>
<td>$&gt;0$ rejected</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.083</td>
<td>0.664</td>
<td></td>
<td>$&lt;0$ accepted</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.283</td>
<td>2.264</td>
<td></td>
<td>$&lt;0$ accepted</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.193</td>
<td>1.544</td>
<td></td>
<td>$&lt;0$ accepted</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-0.292</td>
<td>2.336</td>
<td></td>
<td>$&lt;0$ accepted</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.399</td>
<td>3.192</td>
<td></td>
<td>$&lt;0$ accepted</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>-0.367</td>
<td>2.936</td>
<td></td>
<td>$&lt;0$ accepted</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>-0.317</td>
<td>2.536</td>
<td></td>
<td>$&lt;0$ accepted</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>-0.293</td>
<td>2.344</td>
<td></td>
<td>$&lt;0$ accepted</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>-0.051</td>
<td>0.408</td>
<td></td>
<td>$&lt;0$ accepted</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.374</td>
<td>2.992</td>
<td></td>
<td>$&lt;0$</td>
<td></td>
</tr>
</tbody>
</table>

The results show that only coefficients $\beta_1, \beta_2, \beta_3, \beta_4$ and $\beta_6$ are important, so that correlation (5.5) can be written in simplified form:

$$y_1 = 11.767 + 0.574 x_1 + 1.167 x_2 - 1.009 x_3 + 0.592 x_1 x_2$$

(5.10)
Relationship (5.10) indicates that process response is significantly influenced by factors $x_1$, $x_2$, $x_3$ and interaction between factors $x_1$ and $x_2$. Thus, BC yield has a maximum value at superior levels of $x_1$ (operating temperature) and $x_2$ (fructose concentration) and inferior level of $x_3$ (ethanol concentration). Following the same procedure, relationship (5.6) can be expressed in simplified form:

$$y_2 = 0.629 + 0.129 x_1 + 0.081 x_3 + 0.358 x_4 + 0.068 x_1 x_4 + 0.050 x_3 x_4$$  \hspace{1cm} (5.11)

Correlation (5.11) emphasizes that added water specific use has a maximum value at superior levels of $x_1$ (operating temperature), $x_3$ (ethanol concentration) and $x_4$ (air specific flow rate). Equations (5.10) and (5.11) enable to formulate various optimization problems, for instance to achieve a maximum BC production with a fixed value of water specific use. By minimization of Lagrange function given by the relation (5.12), where $\lambda$ is Lagrange multiplier, the optimal process factors values were obtained. For $\sigma = 0.6$ these optimal values are $x_1 = x_2 = x_3 = x_4 = 0$.

$$L(x_1, x_2, x_3, x_4, \lambda) = y_1(x_1, x_2, x_3, x_4) + \lambda[y_2(x_1, x_2, x_3, x_4) - \sigma]$$  \hspace{1cm} (5.12)

**Conclusions:**

Statistical methods were successfully used in this study to optimize BC synthesis by *Acetobacter Xylinum* in static conditions. A 24 factorial experiment was performed, the selected process factors being operating temperature ($n = 1$), substrate concentration ($n = 2$), co-substrate concentration ($n = 3$) and air specific flow rate ($n = 4$).

BC yield and water specific use were considered as process dependent variables. Quantitative correlations between these variables and process factors were established. The accomplished analysis emphasizes that the most important factors influencing on BC yield are operating temperature and fructose concentration.
5.5 Experimental data 2

The experiment presented in this chapter was performed with the purpose of obtaining uniform bacterial cellulose pellicles for potential use in structural applications. The resulted pellicles were further used to produce/develop two types of PVA cellulose composites, which are presented in chapters 6, 9, 10.

Production of strater culture

The microorganism used in this research (a strain of *A. Xylinum*) was isolated from pineapple origin from Costa Rica. Isolation and purification of the culture was accomplished empirically through repeated plate cultivation on 3.2 pH agar medium. The main composition of the isolation medium was agar-agar (1.6 % w/v), honey (30 g L\(^{-1}\)) (from a local market), peptone (3 g L\(^{-1}\)), yeast extract (3 g L\(^{-1}\)), K\(_2\)HPO\(_4\) (1 g L\(^{-1}\)), adjusted to pH 3.5 with acetic acid [10].

The microorganism was maintained at 4 °C and transferred monthly on an in-house developed serum similar to the inoculation medium. The inoculum was grown in a medium containing: dextrose (20 g L\(^{-1}\)), peptone (5g L\(^{-1}\)), 5 g L\(^{-1}\) yeast extract adjusted to different pH values with acetic acid in static culture. The inoculation was performed in aerated culture for 72 h using a 4 L/min air flow.

Determination of optimum fermentation conditions

Reagents

The chemicals used throughout this experimental investigation were purchased from Carl Roth GmbH + Co. KG. Only poly(vinyl alcohol) (PVA), average molecular weight (Mw) 85,000–124,000 g/mol, 87–89% hydrolyzed was purchased from Sigma–Aldrich and used without further treatment or purification.

Based on previous experimental results, it was established that the factors which influence BC yield are operating temperature and carbon source concentration [9]. In this regard, the fermentation temperature was maintained constant at 24 °C and dextrose concentration at 20 g/L. In order to obtain uniform pellicles, only the initial pH medium and medium heights were considered as dependent parameters of the process. Using the same experimental set-up, several experiments were performed at initial pH values of 2.7, 3.0, 3.5, 4.0, 5.0 and 6.0. For each pH value a static culture was performed using square glass trays with an area of 600 cm\(^2\). The volume was also varied for each pH value from 1 to 6 cm medium height with an increment of 0.5 cm/tray. It was concluded that for BC produced at liquid-air
interface, the production was most favorable at pH 4 and 5 with a medium height of 4.5 cm. Even though the highest BC yield was obtained at pH 4 and 5, a uniform structure was produced at pH 3.5 and 4 in 4.5 cm medium height. These conditions were preferred for the next experiments.

Based on the revealed results, a set of 40 pellicles were produced in a static culture, the experimental parameters being presented in Table 5.12. Previous to static cultivation, the medium was aerated for 24 h in order to speed-up the bacteria’s multiplication rate within the medium. The resulted inoculated medium was qualitatively analyzed using optical microscopy before static cultivation.

Table 5.12. Experimental parameters for BC static culture

<table>
<thead>
<tr>
<th>Medium</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>Growth time (h)</th>
<th>Tray dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose (20g/L)</td>
<td>3.5</td>
<td>24</td>
<td>250</td>
<td>9</td>
</tr>
<tr>
<td>Peptone (5g/L)</td>
<td></td>
<td></td>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td>Yeast Extract (5g/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All the pellicles resulted from the second experiment were used as raw material to produce Bacterial Cellulose PVA composites.

5.6 Experimental data 3

The work in this experiment has been set up with the aim to cross-check the results obtained in previous experiments. With the support of TUDelf University, Faculty of Aerospace Engineering, a master-project was allocated on the subject “Investigation of bacterial cellulose in composites for structural use”. The project was conducted by Tom Dousma under my supervision and Prof. Dr. Ir. M. van Tooren’s supervision. Growth of the bacteria and pellicles was performed in the microbiology laboratory of faculty of Biotechnology, TUDelft with the support of Marijke Luttik and Ton van Maris. In this experiment, the utilized strain of *Acetobacter Xylinum* was ordered from DSMZ. These strains are:

• *Gluconacetobacter xylinus* (Brown 1886) Yamada et al. 1998, DSM 46605, ATCC 14851, IMET 10316, NCIB 8132 (referred to as 46605 strain)

To establish the most suitable high yield growth strains, the bacteria were left to multiply for several days in the prescribed medium by DSMZ: glucose 100g, yeast extract 10g, CaCO$_3$ 20g, distilled water 1000mL, adjust pH to 6.8. The medium with the bacteria was kept in shake flasks at 30°C to optimize the contact with oxygen and stimulate growth and multiplication. The medium was then examined under microscope. The 2004 strain proved to have a satisfactory multiplication rate and reach a considerable biomass, while the 46605 strain was not capable to divide completely after growth, resulting in long strings of connected bacteria. After multiplying for several days in the shaken culture, the medium was subjected to a static cultivation at 30°C to develop the pellicle. The 2004 strain was capable to produce a smooth pellicle, while the 46605 strain, that could not divide properly, did not produce the pellicle. The 2004 strain was utilized further in the experiment.

### Optimizing the medium for pellicle growth

The 2004 strain was delivered with a prescribed medium which is suited for growth and division of the bacterium, but not necessarily for the production of the pellicle. The medium utilized in previous experiments was optimized for an in-house developed *A. Xylinum* strain and proved to be incompatible with the 2004 strain. This was a significant draw-back while experimenting with the 2004 strain. In consequence, it was necessary to redesign an optimum medium for the new strain based on the previous experiments and various literature resources.

Several tests were performed with different media to establish the composition of the medium and growth parameters necessary to obtain uniform high-quality pellicles (table 5.13). Some assumptions and considerations were taken into account in this test:

- The DSMZ medium had high glucose concentration. Since literature sources state that large amounts of glucose lead to pH decrease within the medium due to gluconic acid formation and a medium rich in glucose runs more risk of contamination, the amount of carbohydrate source was limited to a pseudo-standard value used for cultivation of bacteria in the TUDelft Biotechnology laboratory.
Initial tests were performed using honey as carbohydrate source. Despite the fact that honey produced a good yield of cellulose, in order to have a more consistent control on the experiment, this was substituted by synthetic pure glucose and fructose.

Table 5.13. Overview of different media used for pellicle growth

<table>
<thead>
<tr>
<th>Components of Medium</th>
<th>First medium</th>
<th>DSMZ medium</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (g/L)</td>
<td>100</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Fructose (g/L)</td>
<td>25</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptone (g/L)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Yeast-extract (g/L)</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Citric acid (g/L)</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Honey (mL/L)</td>
<td>4</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>CaCO₃ (g/L)</td>
<td>20</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Parameters and results

| Final pH-value | 5.1 | 4.0 | 4.8 | 3.4 | 3.5 | 3.5 |

Results

| First-tray pellicle dry weight | 11.0 | 8.1 | 10.9 | 13.1 | 10.3 | 9.4 |
| Second-tray pellicle dry weight | 9.9  | 10.6 | 9.8  | 11.4 | 10.0 | 10.0 |

- Since fructose - in contrast to glucose - is not processed into an acid, the use of fructose could lead to smaller drops in pH-value. This could have a positive effect in the development of the pellicle.

- In the growth medium of A. Xylinum, peptone and yeast extract are used as nitrate sources. To identify the optimum concentration of peptone/yeast extract, their concentration was varied in the tests.

- The tests were performed in sterile environment in the laboratory using a stable pH value of 7. In some of the tests CaCO₃ was added as a buffer agent in order to maintain the constant value of pH at 7 while in others the buffer was not added to test the effects.

The 2004 strain was cultivated in the DSMZ medium in a shake flask for about 24h and used to inoculate two flasks for test with identical medium. The cultivation was
performed on static medium and left to develop for seven days at a temperature of 30°C. After this period the pellicles that formed were taken out and weighed while still wet. The results are shown in table 5.13.

Conclusion:

Fructose did cause smaller variations in the pH-value, but did not positively affect the pellicle production. The CaCO$_3$ proved to have hardly any effect with the combination of fructose and glucose, but combined with only glucose it negatively affected the growth. Finally, the initial pH of 7 without buffer agent did not influence the production significantly enough to consider a more elaborate investigation on the influence of initial pH. Since the combination of both nitrate sources gave the best results it was decided to continue with medium 4.

Cultivation in large tray

To obtain larger pellicles and be able to control the availability of oxygen, the following tests used a stainless steel tray with a lid, containing a pipe to pump in fresh oxygen (fig. 5.11).

A flow control device was attached and calibrated to replenish the air inside the tray every few hours. The growing of the pellicles proved to be quite difficult and many harvests failed due to fungus formation or others did not form at all. Using the approach “trial and error”, factors were adjusted according to assumptions.
made on contamination occurrence. An overview of the attempts can be found in the master thesis of T.F. Dousma May 2010 TUDelft University.

**Summation of the cultivation method**

The best results were obtained when the medium was aerated for several days using the installation presented in figure 5.12. The cultivation method can be summed in the next four steps:

1). 8 liters of medium were prepared consisting of water with 50g/L glucose, 5g/L peptone, 5g/L yeast, 5g/L CaCO$_3$ and 3 mL of antifoam. The inoculation barrel was filled and the barrel with the medium was sterilized.

2). When the sterilized barrel is cooled to room temperature, a sample of the 2004 strain is injected with a syringe through a small opening in the top of the aeration barrel with caution against contamination. The opening is closed and the air supply is connected to the barrel to start the bubbling of air through the medium.

3). After three to four days, the bacteria have been able to multiply sufficiently and the inoculated medium is poured into a large container without special concern for sterility. Citric acid is used to lower the pH to 4.5 and 10 mL/L of ethanol is added. The medium was poured into the tray and the pellicle left to develop. During the
next ten days, the oxygen was replenished every 2 to 3 days by taking off the lid to check on the progress of growth.

4). After ten days, the pellicle has sufficiently developed to be taken out of the tray. The remaining medium was poured into a large container and replenished with water to 8 liters, along with 10g/L glucose, 2.5g/L peptone, 2.5g/L yeast and 10mL/L ethanol. If pH is lower than 3.5, it can be adjusted to a level between 3.5 and 4.5 with CaCO₃. The medium can be poured back into the tray. This last step can be repeated several times.

**Conclusions:**

The growing of the pellicles proved to be difficult. It is important, however, in further research, to study the optimization of medium and growing process in more detail. During the experiments, a new finding, which was not described previously in literature, was achieved. It was discovered that when sufficient biomass was present when starting a static cultivation, pellicles can form within one day which seemed to prevent contaminations from developing. This finding is important and useful in future research and development of cultivation methods.

*The method was utilized in growing structural-uniform pellicles to be applied in the production of Bacterial Cellulose PVA composites and Bacterial Cellulose Epoxy composites.*
Literature


Chapter 6

Bacterial cellulose treatments

This chapter presents two types of procedures employed in the treatment of bacterial cellulose pellicles. The first procedure was developed at “Politehnica” University of Bucharest to obtain bacterial cellulose sheets to create composites with PVA. The second procedure was developed at Delft University of Technology to obtain cellulose sheets to create composites with different resin systems like PVA and epoxy.

6.1 Alkaline treatment of BC first procedure

When BC pellicles are harvested, apart from cellulose they contain a large amount of water, bacteria and other constituents of the medium (fig. 6.1). Because the objective of this research is to establish the mechanical properties of a set of new composites manufactured from dry sheets of pure BC, the pellicles have to be cleaned and dried to form smooth sheets.

In literature, this process is often addressed to as alkaline treatment of bacterial cellulose pellicles. The process is performed by soaking the pellicles in a NaOH solution. Several concentrations (from 1 to 5%) of NaOH solution have been described in literature but just one from the research studies performed specifically on the best concentration of solution, showed it to be optimum at around 5% [1]. If the NaOH concentration is less than 5%, the bacteria does not saponificate sufficiently, which means they could not be removed from the pellicle and
therefore, contaminated the pellicle while not contributing to the strength. If the solution was stronger, the cellulose has degenerated to such an extent that the strength and stiffness deteriorated and significant shrinkage or curling of the sheets occurred.

This degradation is also influenced by the temperature used within this process [2, 3], typically 80 to 110 °C. It is acknowledged that a cellulose molecule in an alkaline solution is attacked and a reduction of the end-group by the alkali is produced. This reaction is directly proportional to the NaOH concentration and temperature [4].

In an initial stage of experiments we used A.N. Nakogaito’s procedure [3] as a model to process the BC pellicles, which proved to be highly time consuming (~ 2 weeks) since the gel-like pellicles of BC need to be slowly washed in running water. To understand the reason why the pellicles necessitate this slowly treatment, we have to analyze the nature of the forces acting inside the gel.

In general, there are three competing forces acting on a gel polymer network: the rubber elasticity, the polymer-polymer affinity and the hydrogen ion pressure. These forces, collectively called the osmotic pressure, determine the equilibrium state of the gel. The competition between these forces determines the osmotic pressure while the changing balance of these forces produces the volume change. Rubber elasticity tends to shrink the gel under tension and expand it under compression. The elastic force is in equilibrium when the polymer ends are at their root mean square distance. Although the equilibrium volume for elasticity is independent of the external conditions, its force is proportional to the absolute
temperature. As it concerns the BC gel-like pellicles, these forces are acting between cellulose molecules while amplified by the fiber nature of the cellulose. Polymer-polymer affinity depends on the electrical attraction between the polymer and the solvent. An attractive force between the polymer and the solvent causes the absorption of solvent molecules, while repulsive force produces the opposite effect. In the case of BC gel-like pellicles, these forces are absent. This force does not depend on the temperature, but depends on the solvent and volume of the gel. Hydrogen ion pressure is the force exerted by the motion of the hydrogen ions H+ within the gel network. Hydrogen ions enter the gel attracted by the negative charges on the polymer chain, while their random motions tend to expand the gel much as a gas exerts pressure within a contained volume. The hydrogen ion pressure depends on the ionization of the polymer, as well as both temperature and volume. The force is linearly proportional to the absolute temperature and inversely proportional to the square of the volume. In the case of BC gel-like pellicles, these forces are approximately equal with the forces between cellulose and water.

To minimize the washing time, we proceeded by first squeezing out the water, using a uniform vacuum-squeeze method, as presented in figure 6.2. This procedure will cause an increase in hydrogen ion pressure. The procedure takes about 10 min.

![Figure 6.2: Uniform vacuum squeeze of BC pellicles](image)

After uniform vacuum squeeze, the BC thick gel-like pellicles of about 0.2 mm were boiled in 1% (w/w) aqueous solution of NaOH for 20 min in order to remove bacterial cell debris. Then, the pieces were washed in running water for 5 min and again boiled in water for 20 min. The treated pellicles measured approximately 2.5 mm in thickness and were uniform vacuum squeezed again to a thickness of 100 µm. The pH of the squeezed water was 8.0. Pellicles were boiled one more time in water for 30 min and then uniform vacuum squeezed. After this treatment, the pH
of the squeezed water dropped to 7.0 and the process was stopped, accumulating a total treatment time of approximately 2 h. The pellicles tested in this process have been obtained in the experiment described in chapter 5.5.

**Drying the pellicles**

The BC sheets were dried under air flow at 70 °C using the rolling device presented in figure 6.3.

![Figure 6.3: Rolling dryer for bacterial cellulose pellicles](image)

During the drying process, the BC sheets have shrunk to 10% of their initial state. Due to this fact, the BC sheets have to be passed several times between the rolls. While dried, the BC sheets remained attached to the main roll but they were easily detached using acetone [5]. The obtained sheets had approximately 40 μm in thickness and 1.2g/cm³ in density. The entire drying process time lasted for 10 min.

### 6.2 Alkaline treatment of BC second procedure

A second procedure for alkaline treatment of BC was performed in the laboratory of TUDelft using the existing equipment. The process is slightly different from the previous one but shares the same principle. The pellicles obtained using the growth method described in chapter 5.6 were put in a solution of 1M NaOH, placed in a pressure cooker and heated for 30 minutes at a temperature of 110°C to ensure
sterilization. The pressure cooker was used for safety reasons. The solution was left to cool at room temperature and then the pellicle was immersed in fresh water in an Erlenmeyer flask. The Erlenmeyer flask was shaken regularly and water was refreshed every few days until the pH reached a neutral value and pellicle changed color from brownish yellow to milky white, ensuring that the leftovers of the medium and bacteria were removed.

### Storing the pellicles

Because wet pellicles are sensitive to contamination in the wet state if they are not stored properly, ethanol solution 70% concentration was used to prevent development of microorganisms.

### Drying the pellicles

For the drying process it was utilized the Joos laboratory press LAP 100 available in the TUDelft composite laboratory of Aerospace Engineering Faculty. Previous tests with drying pellicles using rollers showed the importance of pressing the pellicles slowly to allow the fibers to shift and rearrange before drying. When pressed too quickly, the fibers cannot shift sufficiently and the pellicles tend to tear.

![Figure 6.4: Removal of water by vacuum with absorbing material.](image)

In order to remove the excess water at low rates, trials were performed by placing the pellicles under a vacuum bag connected to a pump. The vacuum pump produces a pressure of about 50kPa, which is lower than the lowest limit of the Joss mechanical press. The relatively low pressure can press out the majority of water
during 10 to 15 minutes, allowing the fibers to rearrange without risk of pellicle tearing. To reduce the processing time, several layers were stacked. When pressure is applied to this stack, the jelly-like pellicles tend to shift uncontrollably and after water is pressed out, the pellicles are difficult to separate. In order to prevent these issues, the pellicles were stacked between sheets of Teflon cloth. This allows the water to pass through, but prevents the pellicles to stick to each other.

The first tests with vacuum pressing were done by adding absorbent material underneath and on top of the stack to absorb the excess water (fig. 6.4), but the distortion of this material led to irregularities in the sheets. To prevent this issue, the absorbent material was removed and the water excess was collected in a reservoir (fig. 6.5). The pellicles resulted in smooth sheets from which more than 95% of the water was removed [6].

The squeezed pellicles can now be mechanically pressed to press out or evaporate the last moisture more rapidly. Since the vacuum-pressed pellicles are much thinner but still jelly-like, they are fragile and difficult to handle. The pellicles were therefore not taken out of the Teflon layers, but the entire stack was put in the mechanical press, also ensuring that the pellicles would not stick to the press. From literature, it is acknowledged that within reasonable bounds, the cellulose does not suffer any damage in strength or stiffness, when different pressures are applied [38]. Practice proved that pressing the pellicles to a pressure of 0.3MPa and heating them to 110°C for 10 minutes followed by cooling to 40°C before releasing the pressure, results in dry and smooth sheets. It was essential to let the press cool down before opening it, since dry pellicles still deform due to thermal contraction. Trials were performed with stacks of four layer pellicles/Teflon.
Literature


Chapter 7

Preliminary study for obtaining bacterial cellulose composites

This chapter presents a preliminary study on developing a set of new types of bacterial cellulose composites. The study is qualitative; it addresses just the conception phase of the materials. This study was performed at the beginning of the PhD research program and constitutes the basic possible tracks to be followed in this research. The created materials were not subjected to any mechanical tests.

7.1 Glass fiber insertion during growth

Materials:

Vinegar ≈ 6%, water (Delft tap water), brown sugar, yeast, sodium hydroxide 0.1 N

Growth medium:

Brown sugar 16 g, Yeast 15g, Vinegar 166 ml, Water 834 ml, pH 4.7, in this medium the pH value was adjusted with NaOH.
Production Methods and Characteristics of Bacterial Cellulose Composites

**Cultivation conditions and processing**

After 40 hours of static cultivation conditions, at the top of the membrane were placed mono-filaments of glass fiber with 14-25 μm in diameter. Every 24 hours, the procedure was repeated for 3 times. After 130 hours the membranes were removed, the thickness was measured and structural uniformity analyzed. A treatment with 0.1N sodium hydroxide was performed in order to remove the bacterial cell debris at 80 °C for 3 hours. The purified membrane was then dried at room temperature and the edges were fixed on a squared frame to avoid corrugation. Figure 7.1 shows the obtained composite in both hydro and dry stage.

![Figure 7.1: Hydro and dried bacterial cellulose composite with glass fibers.](image)

**Results:**

Glass fibers mono-filaments were entirely incorporated in the membrane’ structure during growth with a 0.5 mm distance between glass fibers layers in hydro-stage. In drained stage, the membrane measured a thickness of 0.1 mm in regions with glass fiber and 20 μm in native regions. The composite obtained revealed very good flexibility but at pulling, a delamination effect occurred between the bacterial cellulose and the glass fibers. *The bacterial cellulose can not adhere to glass fibers.* The forces that act between bacterial cellulose and glass fibers are a result of cellulose contraction from hydro-stage to drained stage. From this experiment we demonstrated that a composite having bacterial cellulose and glass fibers in its structure is unsuitable for technical application. In order to keep the structure integrity a binding agent has to be added, like epoxy resin, PVA, PVC etc.
7.2 Carbon fiber insertion during growth

This experiment intended to demonstrate that objects with a higher thickness than 1 mm can be inserted in the thin structure of bacterial cellulose sheets. This experiment was based on the same approach like the previous one but, instead of using a mono-filament glass fiber, were used a set of carbon fibers with a thickness higher than 1.5 mm. The resulted membrane had a thickness of 3.5 mm, with the inserted set of carbon fibers in the structure (fig. 7.2).

Results:

Surprisingly, in this case, delaminating effects were not visible. This was attributed to the fact that the fiber surface area is insignificant and the properties of bacterial cellulose were governing the physical properties of the system. However, in this particular case the resulted product can not be considered a true composite.
Production Methods and Characteristics of Bacterial Cellulose Composites

material but more or less a structure composed from cellulose membrane and carbon fiber structure.
The results of this experiment were used to prove the feasibility of a project proposal for the Design Synthesis Exercises at TU Delft University, in which a group of 10 students were asked to design and build a new type of wings for Delfly using nano bio-cellulose produced by *A. Xylinum*. However, the project was not applied forward in the exercise.

### 7.3 Bio-cellulose composites with Epoxy

In this set of experiments we used dried bacterial cellulose sheets obtained in the procedure described in chapters 5.5 and 6.1. Dried bacterial cellulose sheets were impregnated by hand lay-up with epoxy resin in layers of: probe 1 three sheets, probe 2 eight sheets and probe 3 sixteen sheets. During impregnation of probe 1, we observed that it was difficult to avoid air insertion in a normal sheet by sheet hand lay-up. Probes 2 and 3 were hand lay-up by submerging the sheets in epoxy resin, assembled together inside the resin container and then squeezed. The impregnated probes were put in metal dies, treated with PVA and pressed at 10 MPa for 24 hours.

![Figure 7.3: Epoxy Bacterial cellulose composite](image)

*Results:*

A new composite material containing bacterial cellulose and epoxy resin was developed. The new material exhibits high rigidity and good resistance at pulling.
Its mechanical properties have not been investigated. At breaking on bending, the material shows locally delamination effects while it is visible that the epoxy resin does not wet enough the bacterial cellulose sheets.

**Conclusions:**

The new developed composite reveals promising properties in this stage, but mechanical analysis is required further to quantify and validate its properties. Delamination effect can ultimately be a severe defect of the material. While a set of experiments proved that epoxy resin isn’t able to penetrate a dried membrane of bacterial cellulose, we can uphold the hypothesis that epoxy resin can be used to bind the bacterial cellulose sheets between each other, but can not act as bonding agent between the nano fibers. After measuring the probes, we deducted that the mass of bacterial cellulose used was 5-10% from the total mass of the composite. To establish the optimal proportion between bacterial cellulose and epoxy resin investigations are presented in chapter 9.

### 7.4 Bio-cellulose composites with PVA

Based on results of the previous experiment, we attempted to find another type of polymer to be used in the conception of new bacterial cellulose composites. SEM analysis showed that bacterial cellulose is formed of a series of sheets (fig. 7.4.). Based on this analysis, we credited that delamination occurs between internal sheets and not necessarily at the interface between resin and bacterial sheet.

![Figure 7.4: SEM of bacterial cellulose sheet structure](image-url)
The new polymer should be able to penetrate between the nano cellulose fibers. For this reason it must be soluble in water or alcohol. One of the polymers that can satisfy these requirements is polyvinyl alcohol (PVA). It is a polymer synthesized in 1924 [1] with excellent film forming, emulsifying, and adhesive properties. It is also resistant to oil, grease and solvent. The most usual formula used today for PVA is written as a 1,3-glycol structure [1, 2] and it is commonly used in PVA/cellulose blends, in which the final product has a density of 1,297 up to 1,505 g/cm$^3$[2, 3]. It is odorless and nontoxic.

Using PVA we attempted to create a set of new composites. Three of them will shortly be described below.

**PVA bacterial cellulose laminate composite using dried BC sheets.**

In the conception of this new type of composite we used dried bacterial cellulose sheets obtained by the procedure described in chapters 5.5 and 6.1. Dried sheets were impregnated by hand lay-up with PVA in layers of about sixteen sheets.

**Results:**

The resulted material exhibits good flexibility and pulling resistance. Under microscopic investigation the new material presented irregular internal structure (fig. 7.5.).

![Figure 7.5: Microscopy 500x of PVA bacterial cellulose laminate composite using dried BC sheets](image-url)
Since the composite presents wrinkled bacterial cellulose sheets, this defect can reduce the pulling resistance of the composite. The portion of PVA is over estimated. The thickness of the PVA film existing between the cellulose sheets is too high comparatively with cellulose sheets thickness.

**PVA bacterial cellulose laminate composite using hydro-cellulose.**

In the conception of this composite, to ensure the penetration of PVA between the nano fibers of cellulose, we used bacterial cellulose membrane in hydro-stage. After treatment with NaOH, bacterial cellulose was submerged in a PVA solution for 24 hours. Impregnated bacterial cellulose was then packed in sets of 16 sheets and dried at room temperature for several days. When examined, the resulted composite showed high rigidity and good pulling characteristics. If submerged in water, it decreases the rigidity but the pulling characteristics increase. When examined under microscope 500X magnification rate, the composite exhibits the aspect of a monolithic material. The bacterial cellulose sheets can not be distinguished.

**PVA Bacterial cellulose composite using shivered cellulose.**

To produce this composite, bacterial cellulose treated with NaOH, was shivered in small pieces having a “diameter” of 1 mm. Shivered bacterial cellulose was submerged in PVA solution for 3 hours. Impregnated bacterial cellulose was 90% drained until a paste was obtained, then placed in a form and dried. At normal examination, the resulted composite shows identical characteristics with the PVA bacterial cellulose laminate composite made with hydro-cellulose. If a foaming agent like ammonium bicarbonate is added in the paste composition, the composite can be formed as foam.

**Conclusions:**

The new series of developed composites showed that bacterial cellulose can be used to produce structural materials. Epoxy bacterial cellulose based composites and PVA bacterial cellulose based composites proved to be good candidates as materials for structural applications. However, this chapter presented just the preliminary work performed for the conception of these new materials. In chapter 9, these experiments are reprocessed and revised to enable and support the creation of well-defined and deep-investigated bacterial cellulose based composite and PVA bacterial cellulose based composite. Basically, more than 250 experiment attempts were performed within this research study.
Production Methods and Characteristics of Bacterial Cellulose Composites

Literature


Chapter 8

Mechanical properties of BC composites

When investigating mechanical properties of a material, it is important - in order to choose the most suitable method for testing - to predict the material’s ultimate behavior. To predict the mechanical behavior of pure BC and of BC in a composite structure, the properties of polymers and cellulose in particular were reviewed. Because tensile testing is a very common test method for materials and BC properties described in literature are obtained mostly based on this type of test, it was the first test method to consider. To validate the results, flexural testing was considered as a second option for testing.

8.1 Tensile test

To assess the mechanical properties of a material, the most common method is by testing the material using a tensile test. This test provides the stress-strain behavior of materials, which is one of the most important characteristics. In the tensile test, a specimen is subjected to a continually increasing uniaxial tensile force while simultaneous observations are made on elongation of the specimen.

"The tensile test [is] very easily and quickly performed but it is not possible to do much with its results, because one does not know what they really mean. They are
the outcome of a number of very complicated physical processes... The extension of a piece of metal [is] in a sense, more complicated than the working of a pocket watch and to hope to derive information about its mechanism from two or three data derived from measurement during the tensile test [is] perhaps as optimistic as would be an attempt to learn about the working of a pocket watch by determining its compressive strength. E.Orowan (1944) [1][2]

Despite these caveats, the tension test remains the most fundamental type of mechanical test applied to determine the material properties of metals, composites and other solids. To perform a tensile test a specimen must be prepared. Test specimens of homogenous materials usually have the dog-bone shape (fig. 8.1) to make sure the clamping is done at a stronger part of the specimen and that failure will occur in the middle part, not caused by clamping influences.

![Figure 8.1: 3D model and dimensions of a tensile test specimen according to EN61-90 standard](image)

The specimen can then be clamped in a testing machine (fig. 8.1). This machine has one stationary grip and one movable grip that can move with a constant rate of crosshead displacement. Usually, the specimen is pulled until failure occurs.

**Stress-Strain Diagrams**
The immediate result of a tension test is a relation between the axial force and either the change in length (elongation) of a gage portion of the specimen or a representative value of longitudinal strain as measured by one or more strain gages. This relation is usually changed to one between the stress $\sigma$ defined by relation (1) (where $F$ is the force and $A$ the cross-sectional area) and the strain $\varepsilon$ defined by relation (2) (where $l$ is the elongation and $l_0$ is the gage length or strain-gage output), and is plotted as the stress-strain diagram.

$$\sigma = \frac{F}{A} \quad (1) \quad \varepsilon = \frac{l}{l_0} \quad (2)$$

Parameters that remain constant in the course of a test include the temperature and the rate of loading or of elongation. If significant changes in length and area are attained, then it is important to specify whether the area used in calculating the stress is the original area $A_0$ (nominal or “engineering” stress, here denoted simply $\sigma_e$) or the current area $A$ (true or Cauchy stress, $\sigma_t$) - in other words, whether the Lagrangian or the Eulerian definition is used - and whether the strain plotted is the change in length $\sigma_t$ divided by the original length $l_0$ (conventional or “engineering” strain, $\varepsilon_e$) or the natural logarithm of the ratio of the current length ($l = l_0 + \Delta l$) to $l_0$ (logarithmic or natural strain, $\varepsilon_l$) [2]. Examples of stress-strain diagram, both as $\sigma e$ versus $\varepsilon e$ and as $\sigma t$ versus $\varepsilon l$, are shown in fig.8.2.

It is clear that the Cauchy stress, since it does not depend on the initial configuration, reflects the actual state in the specimen better than the nominal stress, and while both definitions of strain involve the initial length, the rates (time...
derivatives) of conventional and logarithmic strain are respectively \( \dot{l}/l_0 \) and \( \dot{l}/l \), so that it is the latter that is independent of initial configuration. But the use of the logarithmic strain in large-deformation problems with rotating principal strain axes may lead to erroneous results.

If we look more closely to a stress-strain diagram, two deformation regions can be easily distinguished (fig. 8.2.).

![Stress-strain diagram](image)

Figure 8.3: Stress-strain diagram for different types of materials

In the elastic region, stress is linearly proportional to strain. When the load exceeds a value corresponding to the yield strength, the specimen undergoes gross plastic deformation. It is permanently deformed if the load is released to zero. The stress to produce continued plastic deformation increases with increasing plastic strain,
i.e., the metal strain-hardens. The volume of the specimen remains constant during plastic deformation and as the specimen elongates, it decreases uniformly along the gage length in cross-sectional area. But not all the materials present elastic and plastic deformation regions; for example ceramic materials deform just elastically and some polymers just plastically (fig. 8.3).

**Elastic deformation**

The stress-strain diagram is characterized by a range of stresses, extending from zero to a limiting stress (say $\sigma_0$) in which the stress is proportional to strain (the corresponding strains are normally so small that it does not matter which definitions of stress and strain are used); $\sigma_0$ is called the proportional limit. Also, it is found that the same proportionality is obtained when stress is decreased, so that the material in this range is linearly elastic, described by the uniaxial Hooke’s law, $\sigma = E\epsilon$. The range of stress-strain proportionality is also essentially the elastic range, and the proportional limit is also the elastic limit.

When the specimen is stressed slightly past the elastic limit and the stress is then reduced to zero, the strain attained at the end of the process is, as a rule, different from zero. The material has acquired a permanent strain. Rate effects, which are more or less present in all solids, can distort the results. The “standard solid” model of viscoelasticity, predicts that in a test carried out at a constant rate of stressing or of straining, the stress-strain diagram will be curved, but no permanent strain will be present after stress removal; the complete loading-unloading diagram presents a hysteresis loop. The curvature depends on the test rate; it is negligible if the time taken for the test is either very long or very short compared with the characteristic time $t$ of the model.

Even in the absence of significant rate effects, it is not always easy to determine an accurate value for the elastic or proportional limit. Some materials, such as soft copper, present stress-strain curves that contain no discernible straight portions. For design purposes, it has become conventional to define as the “yield strength” of such materials the value of the stress that produces a specified value of the “offset” or conventional permanent strain, obtained by drawing through a given point on the stress-strain curve a straight line whose slope is the elastic modulus $E$ (in a material such as soft copper, this would be the slope of the stress-strain curve at the origin). Typically used values of the offset are 0.1%, 0.2% and 0.5%. When this definition is used, it is necessary to specify the offset, and thus we would speak of “0.2% offset yield strength.”
**Ultimate tensile strength**

The maximum value of nominal stress attained in a tensile test is called the ultimate tensile strength or simply the tensile strength. When the specimen is extended beyond the strain corresponding to this stress, its weakest portion begins to elongate — and therefore also to thin — faster than the remainder, and so a neck will form. Further elongation and thinning of the neck — or necking — proceeds at decreasing load, until fracture. In ceramic materials the ultimate tensile strength is equal to the fracture tensile strength.

### 8.2 Possible issues with testing BC sheets

BC is a very brittle material which means that if irregularities occur at, for example, the edges of a test specimen, stress will concentrate in that area during a tensile test (fig. 8.4) and a crack will be initiated, causing the specimen to fail almost instantaneously far below its ultimate strength.

![Figure 8.4: Stress concentrations due to irregularities in the specimen](image)

Since preparation of the specimens from the thin fragile sheets of BC will never be perfect, the results of tensile tests will be significantly influenced by this mechanism.
One way to get more reliable results is to test a composite of BC. The resin system can bridge the gaps between irregularities to some extent to avoid premature brittle fracture. The composite therefore can deform to larger strains and take up more energy before failing. The Cook-Gordon mechanism [4, 5] describes the strengthening of brittle materials. It states that if the ratio of the adhesive strength of the interface to the overall cohesive strength of the solid is in the right range, a large increase in strength and toughness of otherwise brittle solids may result. Another advantage of using a composite is that the irregularities of a single sheet are averaged out because of the multiple layers.

### 8.3 Flexural test

With brittle materials it is customary to use a bending test or flexural test, which avoids the problem of stress concentrations because of irregularities at the edges of the test specimen [3, 6]. A piece of profile or sheet material is suspended over two points and loaded in the middle to create a combination of compressive and tensile forces (fig. 8.5).

![Figure 8.5: Forces involved in a flexural test [6].](image)

A single sheet of BC cannot be tested this way, since it is too thin to support even its own weight in the dimensions needed for a proper test. To perform this test a thicker composite is needed.
Flexural properties are expressed and calculated in terms of the stress and strain that occur at the upper and lower surface of the specimen. The stress-strain curves acquired with this test are very similar to the tensile test results. Advantages of the flexural test over the tensile test is that specimens are easier to prepare and easier to align. The specimen does not have to be clamped tightly, which prevents stress concentrations at the clamping points. In comparison to the tensile test, it is easier to measure small strains, because the actual deformations are sufficiently large to be measured accurately. Usually, a tensile testing machine can also be used to perform the flexural test. The flexural modulus is a measure of the stiffness during the first part of the flexural test and is in most cases similar to the Young’s modulus.
Literature


Production Methods and Characteristics of Bacterial Cellulose Composites
Chapter 9

Mechanical properties of the new bacterial cellulose composites

This chapter presents the development of a series of new bacterial cellulose based composites and their mechanical properties. A clear distinction is made between materials created in collaboration with and materials created at “Politehnica” University of Bucharest. However, mechanical characterization of the materials and SEM analysis were performed at Delft University of Technology.

9.1 PVA bacterial cellulose based composites

As standard reference for preparation of test samples we used standard ASTM D3039/D3039M-00. Details regarding the test equipment can be found in appendix A. The material presented in this subchapter was subjected to a Patent proposal. For proprietary reasons, the drying procedure of the composites is not presented in detail. However, in order not to lose the scientific character of the material description, a set of basic drying parameters are presented.
9.1.1 Determining the properties of pure cellulose

For this test, a total of three samples were created from a special BC sheet using the growth medium presented in chapter 5.5. A single BC pellicle measuring a thickness of 15 mm before alkaline treatment was cultivated for 21 days. After alkaline treatment and drying using the procedure described in chapter 6.1, the BC sheet measured approximately 100 µm in thickness. The test samples were first immersed in water for 30 min and cut into specific dimensions: length 150 mm, width 19 mm. The specimens were further fixed in the grips and dried under a 10 N tensile load force.

**Results:**

During the tensile tests, only one of the submitted samples had a relevant failure character according to standard D3039/D3039M-00 “AGM(1)”. The other test samples failed by long splitting close to grip. A stress-strain diagram for the relevant sample is presented in figure 9.1.

![Figure 9.1: Pure bacterial cellulose stress-strain diagram](image-url)
The ultimate tensile strength of the BC sheets was significantly high 559 MPa. This high tensile strength is credited to the extremely fine and pure cellulose nano fibers. The material exhibited a brittle behavior and the failure occurred due to fiber internal shear and compression forces.

9.1.2 Determining the properties of PVA bacterial cellulose based composites

Preparation of BC composites

Two types of BC composites were developed in this research using PVA as matrix and dried BC sheets, respectively hydro BC pellicles as reinforced fibers. In the case of the composite with dried BC sheets as reinforced fibers, about six BC sheets were immersed in a solution containing 8% (w/w) PVA*, 50% (w/w) ethanol and 42% (w/w) water for 30 min. Impregnated sheets were taken out from the solution and laminated by hand. During lamination, one of the sheet edges was clamped to the lamination board with tape to constrain the sheets not to shift as presented in figure 9.2. The laminated composite was pressed by hand several times to eliminate air and wrinkles, then ethanol and water was left to evaporate at room temperature for 6 h. The resulted composite was then uniform vacuum squeezed to 0.3 mm thickness and a set of smaller probes were cut off in pieces of different sizes: (1a) 9 x 150 mm, (2a) 7.2 x 150 mm, (3a) 10 x 150 mm, (4a) 9.3 x 150 mm, (5a) 6.5 x 150 mm, (6a) 6.7 x 150 mm. Finally, the probes were dried at room temperature for 48 h. The dried probe measured 0.24 mm in thickness.

* molecular weight $M_w=61.000$ and a degree of hydrolysis of 98%
The second composite material was made using non-dried alkaline treated pelllices of BC with 0.2 mm thickness. The pelllices were immersed in a solution of 8% PVA*, 50% ethanol and 42% water for 24 h. Impregnated BC pelllices were stacked together by hand lay-up in layers of about six BC pelllices. To avoid air insertion during lay-up, the BC pelllices were laid-up by submerging the sheets in PVA solution. The resulted probes were uniform vacuum squeezed till they measured 0.8 mm in thickness and dried at room temperature for 48 h. The dried probe measured 0.4 mm in thickness. The probe was cut into smaller pieces with the fallowing dimensions: (1b) 9 x 150 mm, (2b) 19 x 150 mm and (3b) 19x 150 mm.

**Tensile test**

The samples were subjected to tensile tests using the Zwick 1455 machine at a strain rate of 1 mm/min over a spam of 75 mm. As guideline, we used the standard test method for tensile properties of polymers matrix composites materials D 3039/D 3039M-07. The only deviation from the standard was the use of paper tabs for the sample grip area (fig. 9.3).

![Figure 9.3: Rectangular specimen with paper tabs](image)

**Results and discussions**

Figure 9.4 presents the stress-strain behavior of the composite obtained using dried BC sheets, together with the general effect of water sorption.

* molecular weight $M_w=61,000$ and a degree of hydrolysis of 98%
Figure 9.4 BC-dry stress-strain diagram for samples 1a, 2a, 3a, 4a, 5a and 6a. The probes 5a and 6a are measured after water absorption [8].

Figure 9.5 shows the consistency of the results considering the fact that the second composite, made with non-dried BC pellicles, exhibits with 60% less fiber than the one with dry BC sheets.

Figure 9.5: BC-hydro stress-strain diagram for the composites BC-PVA 1b, 2b and 3b [8].
In the diagram presented in figure 9.4, σMax was calculated for sample (5a) and (6a), by omitting the thickness increase as a result of water sorption. It was noticed that the material suffers a plasticizing effect which decreases its mechanical properties in addition to thickness increase. Even though the material behaves well in strength, a more visible effect of plasticization is noticed in its elongation which directly affects Young’s modulus, as shown in figure 9.6.

The data confirms the fact that water is a plasticizer for the composite, but does not affect the fiber strength at this water sorption level. Strength is attributed to the fact that the material, at this value of absorbed water, does not form high extents of hydrogen bonds with the water which means that the material is not in the saturation phase of water sorption. In order to verify this assumption, a set of water absorption experiments were also performed. These experiments are described in chapter 10. Material elongation can be attributed to the fact that water existing in the material matrix allows the cellulose fibers to slide over each other. As described frequently by many authors [1, 2], BC is a random in-the-plane orientation of nanofibrils as it can be seen in figure 9.7 a,b. It was also described as a 3D interwoven network of nanofibrils, “extremely fine, pure and dimensionally uniform” [3]. But a more close examination of a delaminated fracture - see figure 9.7a revealed that BC is far from dimensional uniformity. The fibrils resemble to tree branches in which a thicker fibril is orientated in-plane from which several thinner
fibrils emerge with a small offset from in-plane oriented. This structure explains why the BC fracture resembles to a laminated nano composite (fig. 9.7b) and also the creep effect which is amplified by water sorption [4].

Figure 9.7: SME micrographs of BC-dry composite: a) delaminated fracture b) section view.

Conclusions

Alkaline treated BC pellicles were impregnated with PVA to produce high-strength biodegradable composites. Two types of composites were prepared using dried respectively wet bacterial cellulose sheets. The Young’s modulus of the new-developed composite was significantly higher than any reported for similar composites by other authors, about 42 GPa. It was also observed that the material is able to outstand high stresses even in wet conditions or immersed in water for short periods of time. However, next chapter presents a new-developed BC epoxy composite with higher Young’s modulus and tensile strength.
9.2 Bacterial cellulose based composites with different polymer matrix

This subchapter describes a set of experiments performed at TUDelft University with the purpose to investigate the possibility of using cellulose based composites as structural materials for aeronautical components.

9.2.1 Creating BC-composites using a hydrophilic resin system.

The main two factors in quantifying the success of a resin system are the capability to wet the area of the fibres and the extent of adhesion to the material. Because BC is extremely hydrophilic, it is expected that hydrophobic resin systems such as epoxy will have difficulty penetrating the sheets or wetting the surface of the fibers and will not adhere sufficiently to the material [5, 6]. Therefore, other water soluble resin systems besides PVA were tested in these experiments like methyl cellulose. These resin systems were expected to penetrate the network of fibers sufficiently and adhere properly to the surface.

Attempts with Methyl Cellulose

It was suggested that methyl cellulose, used for wall paper glue, has suitable properties to act as a resin system for structural use. It is also conveniently biodegradable. Experimental attempts were therefore made with this resin system which showed the first major weakness with water soluble systems. It was difficult to ensure the smoothness of the dry BC sheets and to avoid air bubble formation between sheets. Sheets of BC were impregnated with methyl cellulose by using a brush. Impregnated sheets were then stacked together. During this process it was observed that pellicles regain their jelly-like state and become difficult to be stacked in a smooth compact assembly. The pellicle stack was put between two Teflon layers and covered in plastic foil. Using a mechanical press the assembly was pressed to remove excess resin, water and air. When the resulted composite was examined, it was observed that bacterial cellulose sheets slid between each other due to exerted pressure. Based on this test, it was concluded that methyl cellulose is not a suitable resin system to develop bacterial cellulose based composites.
Attempts with PVA

In an attempt to simplify the stacking and removal of excess water, lamination with PVA as resin system was tested. PVA solution is less viscous than methyl cellulose solution which could permit easier stacking of the sheets and removing of excess air, water and resin. A solution of 8% PVA, 50% ethanol and 42% water was used to soak the sheets. It proved easier, but still quite difficult and time consuming to stack the sheets. A stack was made of 8 layers and covered with plastic foil. The stack was pressed and heated to 110°C but after this process, the sheets had shifted again and they were easily peeled off from other layers, proving that an excessive amount of PVA was pressed out along with the water. Pressing with lower pressures and stacking two sheets at a time provided some satisfying results. The two-sheet stack was cut in two pieces, glued with PVA and stacked together again to form a composite of four sheets. When this process was repeated, another problem occurred; the PVA in the top and bottom layers as well as the edges of the product dried out first, enclosing the water in the centre of the product. After releasing the press, the pressurized vapors in the centre expanded, shaping the product in a pillow shape.

From these experiments it was clear that the use of water soluble resin systems caused some problems. The sheets are hard to stack and tend to shift when pressure is applied to remove air and ensure smoothness of layers. Applying the pressure also prohibits water to evaporate from the laminate. To solve the sliding layers issue, the sheets were now separately stuck to a surface with tape before laminating them (fig. 9.2). Next, the stack was pressed at a relatively low pressure <1MPa to remove air and smooth out the layers without removing too much resin and water. The stack was then put in an oven to dry by air at 80°C. Specimens developed with this method proved to delaminate completely during tensile testing, “probably caused by the evaporation of water during the drying, leaving space between the layers”.

Some samples were created by using the method described in chapter 9.1 except for the uniform vacuum squeeze procedure. After stacking the pellicles, these were not machine pressed, but air and wrinkles were pressed out repetitively by hand and the water was left to evaporate at room temperature. When the stack was almost dry, it was once pressed at low pressure to smooth out the layers and subsequently left to dry at room temperature.

Specimens prepared from this composite performed better during tensile testing (see chapter 9.2.3.1) and did not delaminate, but the inside layers proved to be quite irregular when examined using SEM photography (fig. 9.8). These irregularities shift the pellicles locally from the axial direction of the specimen and
Production Methods and Characteristics of Bacterial Cellulose Composites

the favorable in-plane orientation of the fibers in smooth BC-sheets does not contribute entirely to the strength.

Another issue with this method was the long exposure of the drying pellicles to contaminants, which caused fungus development in some cases.

9.2.2 Creating BC-composites using a hydrophobic resin system

Rehydration of the sheets proved to undo the smoothness of the single BC-sheet and the PVA method provided a reasonable composite only when a rigorous method was applied. This proved to be highly time consuming and exploiting. To avoid the issues with water soluble resin systems, a hydrophobic system was used in the form of epoxy resin. Epoxy is well known and frequently used as a successful resin system in fiber based laminates. The risk with epoxy is that it cannot penetrate the sheets properly causing excess epoxy to remain between the sheets and bonding poorly to the cellulose [5].
Attempts with vacuum-pressing

The first attempt to produce an epoxy-BC composite was done by stacking sheets of dry cellulose submerged in epoxy to avoid air in the composite. The stack was then removed from epoxy and pressed using plastic foil and vacuum pressure. The obtained laminate was clearly not smooth and contained pockets of excess epoxy in the structure. During tensile testing some layers completely delaminated. Under optical microscope (fig. 9.9) and SEM (fig. 9.10.1 and 9.10.2) examination, the excess epoxy was clearly visible. The epoxy did manage to penetrate the sheets to such an extent that it adhered to the fibers, visible by the pieces of epoxy showing cellulose fibers attached to it (fig. 9.10.3 and 9.10.4).

![Figure 9.9: Microscopy of Epoxy-BC composite of first attempt a.) 25x, b.) 100x,](image)

Attempts with mechanical pressing

To be able to press out as much excess epoxy as possible, a process similar to the one described in chapter 9.2.1, was used; sheets of cellulose were taped on a surface to prohibit shifting due to pressing. The sheets were then laminated using a brush and the stack was covered in vacuum foil and mechanically pressed with pressures over 1MPa. In this way, excess epoxy was pressed out sufficiently and a proper composite was obtained. The lack of excess epoxy between the smooth layers is visible in figure 9.10.5. A delamination that occurred while preparing a small sample for SEM photography shows fibers torn from the layers (fig. 9.10.6). This means that fibers in the sheet failed before the bonding between fibers of adjacent layers, showing sufficient penetration of the epoxy and proper bonding to
the BC. In figure 9.11 sheets of BC and layers within the sheets are visible at the surface of tensile fracture.

Figure 9.10: SEM Epoxy-BC composite.
Figure 9.10.1: Epoxy-BC composite first attempt (500x), view on surface of failure. Layers of epoxy (a) and BC(b) clearly visible. Layers of BC show the layered structure within the sheets.
Figure 9.10.2: Epoxy-BC composite first attempt (2000x), view on surface of failure. Two sheets of BC (b) with epoxy (a) in between clearly visible. Layers of BC show the layered structure within the sheets.

Figure 9.10.3: Epoxy-BC composite first attempt (2000x), view on surface of failure, showing separated layer of BC (b) from pocket of epoxy (a). Cellulose fibers attached to the epoxy are visible (c).

Figure 9.10.4: Epoxy-BC composite of first attempt (10,000x), view on surface of failure, showing separated layer of BC (b) from pocket of epoxy (a). Cellulose fibers attached to the epoxy are visible (c).

Figure 9.10.5: Epoxy-BC composite made using the mechanical press (200x), view of the side of the specimen, showing the lack of excess epoxy and smoothness of the layers (a). The damage on the right side of the picture is caused by a cut made for observation. The cut also caused some delamination (b), revealing fibers torn from the layers, showing good adhesion between the layers.

Figure 9.10.6: Epoxy-BC composite made using the mechanical press (800x), view of the side of the specimen, showing a delamination caused by a cut made for observation. The layers on both sides (a) are difficult to distinguish because the edge of the specimen is covered with epoxy, (b) is epoxy on the side of the specimen, torn at the delamination, (c) are fibers torn from the layers at the delamination, showing good adhesion between the layers.

After the first attempt, it seemed that the high pressure caused the epoxy to penetrate the sheets better, making the cellulose transparent to some extent in the process. To examine if the pressure had significant effect on the mechanical
properties of the composite, different pressures were applied in the production of the composites.

Because of the brittleness of the obtained composites, it proved difficult to cut samples with smooth edges. To ensure smooth edges, the laminated stacks were pressed out to remove excess epoxy first. Stacks were then removed from the press and cut into strips. The strips were again covered in plastic and pressed at 80°C. After 15 minutes, the epoxy hardened sufficiently allowing removal of the composite from the press to be cooled and cured further. Samples of 8 sheets were produced and used in similar tensile tests as performed on the PVA composites (see chapter 9.1.2). Finally, some samples were produced with 29 sheets, pressed at 10 MPa, creating a composite of 0.50 mm thick to be used in bending tests.

**Weight fraction of epoxy and BC in the composite**

Because of the experimental nature of the production of the composites it was not possible to measure the exact amounts of epoxy and BC used for each specimen. To get an idea about the amount of epoxy still left inside the pressed specimens, the weight and volume of specimens was measured. The density and subsequently the weight fraction of epoxy were derived:

\[
\begin{align*}
x_e &= \frac{m_e}{m_{tot}} = \frac{\rho_e \cdot V_e}{\rho_{tot} \cdot V_{tot}} = \frac{\rho_e}{\rho_{tot}} \cdot \left( \frac{1}{V_{BC}/V_e + 1} \right) \\
x_e &= \frac{\rho_e}{\rho_{tot}} \left( \frac{m_{BC} - m_e}{m_e} \rho_{BC} + 1 \right)^{-1} = \frac{\rho_e}{\rho_{tot}} \left( \frac{m_{tot} - m_e}{m_e} \rho_{e} + 1 \right)^{-1} \\
x_e &= \frac{\rho_e}{\rho_{tot}} \left( x_e^{-1} - 1 \right) \rho_{BC} + 1 \left( x_e^{-1} - 1 \right) \rho_{tot} + \rho_{tot} \\
\rho_{tot} (1 - x_e) + \rho_{tot} x_e &= 1 \\
\rho_{tot} (\rho_{BC} - \rho_e) x_e &= 1 - \frac{\rho_{tot} - \rho_{tot} x_e}{\rho_{BC}} = \frac{\rho_{BC} - \rho_e}{\rho_{BC}} \rho_{tot} \\
x_e &= \frac{\rho_{BC} (\rho_{BC} - \rho_e)}{\rho_{tot} (\rho_{BC} - \rho_e)} = \frac{\rho_{BC} - \rho_{tot}}{\rho_{tot}} \rho_{BC} x_e \\
x_e &= \frac{\rho_{BC} x_e (\rho_{BC} x_e - 1)}{\rho_{tot} (\rho_{BC} x_e^2 - 1)} \quad (9.1) \\
(9.2) \\
(9.3) \\
(9.4) \\
(9.5) \\
(9.6) \\
(9.7)\]
Where $\chi_e$ is the weight fraction of epoxy in the composite [-], $m$ is the mass [g], $\rho$ is the density [g/cm$^3$] and $V$ is volume [cm$^3$]. The subscript ‘e’ stands for the epoxy in the composite, ‘BC’ for Bacterial Cellulose and ‘tot’ for the total composite. The density of the epoxy is 1.15 g/cm$^3$ and the density of BC is about 1.6 g/cm$^3$. We can visualize formula 6.1 with the mentioned values in a curve (fig. 9.12).

The measured specimens had a density of approximately 1.45 g/cm$^3$, which shows that the weight fraction of epoxy is approximately between 20 and 30%. Because of the uncertainty in the densities of the BC used and the measurements of the sample’s volume, an accurate value for the fraction could not be established.

**Conclusions**

During the development of a method to produce testable composites, best results were obtained using epoxy as resin-system. Creating composites using hydrophilic resin systems proves to be very difficult if classical vacuum pressing is used. The main issue with water soluble systems was that the dried sheets were hydrated again which turns them back to their jelly-like state. This state makes it hard to stack the sheets smoothly and tends to cause shifting of the sheets when pressure is applied to remove excess water, resin and air while ensuring a smooth layered structure in the cured composite. The pressure also restricts the evaporation of water from the composite, preventing the curing. The only method that resulted in proper bonded sheets with a water-soluble resin system was quite time-consuming and resulted in irregularities in the product.
layers making suboptimal use of the favorable in-plane orientation of the fibers in the smooth BC-sheets. Note: in the drying process of the PVA composites was not used the same method employed in the creation of the composite presented in chapter 9.1.

The use of epoxy as a resin system resulted in a material that is non-biodegradable and non-renewable. Despite this issue, the resulted composite can be used to prove the viability of using BC composites as material for structural use. During the process of developing a technique to produce the epoxy-BC composite it was clear that the excess epoxy was difficult to remove from the laminate. Vacuum pressing proved to be insufficient and pockets of epoxy remained inside the cured composite. The attempt with vacuum pressing did however show that the epoxy is capable of penetrating the BC-sheets sufficiently to bond to the fibers. Using mechanical pressing with pressures over 1MPa it was possible to remove sufficient epoxy and create a composite with smooth, well-bonded layers.

9.2.3 Testing the composite

A considerable number of mechanical tests were performed during this research (more than 100 sample tests). The most conclusive results are presented below.

9.2.3.1 First session of tensile testing

Epoxy-BC pressed at 25MPa

This was the first composite made by mechanically pressing a laminated stack of BC sheets. Eight sheets of BC which were cultivated using the method presented in chapter 5.6 and alkaline treated using the procedure presented in chapter 6.2 were used. Twelve small specimens were cut in pieces with dimensions of approximately 10x40mm, leaving a 20mm span between the paper tabs as presented in figure 9.3.

Epoxy-BC pressed at 10MPa and 20MPa

Based on experience achieved in the first experiment, that is with mechanically pressing the epoxy-BC composite, two new laminates were made using 8 sheets of BC. Two different pressing forces were applied to examine if excess epoxy can be removed with lower pressure and to test the effects on the composite’s properties when exposed to different pressures. To handle the specimens more easily, larger pieces were cut off 16.2x70mm, leaving a span of 50mm between the paper tabs. A
series of twelve specimens resulted, pressed at 10MPa and a series of thirteen specimens pressed at 20MPa.

**PVA-BC dried at room temperature and in the oven**

The production methods of these samples are described previously. Specimens dried at room temperature consisting of about 39 sheets were cut into 4 specimens with dimensions of 16.2x100 mm, leaving 80 mm between the paper tabs. Samples dried in the oven consisted of 8 sheets which were cut into specimens of 16.2x70 mm, leaving a 50 mm between the paper tabs.

**Thickness of the samples**

To calculate the stress and Young’s modulus of the samples it is necessary to know the loaded area of the specimens. Using the microscope available in the laboratory of TUDelft, Faculty of Aerospace Engineering, the thickness of different samples was measured at different points. It was observed that the specimens exhibited thickness variations in the order of magnitude of 0.01mm which is around 5% of the specimen’s thickness. It might be possible to make a more precise estimation of the area of each specimen at the point of failure, but this would be time consuming, since each test specimen should be examined by multiple methods. Because precise results on thickness would not contribute significantly to the conclusions of this research, calculations were made with values obtained using microscope examination. In table 9.1 the measured dimensions of the composites are presented.

Table 9.1. Characteristics of composites used in the first session of tensile tests

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Dimensions (mm)</th>
<th>No. of successful test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Epoxy-BC, pressed at 25MPa</td>
<td>0.16x10x20</td>
<td>12</td>
</tr>
<tr>
<td>2) Epoxy-BC, pressed at 10MPa</td>
<td>0.15x16.2x50</td>
<td>12</td>
</tr>
<tr>
<td>3) Epoxy-BC, pressed at 20MPa</td>
<td>0.16x16.2x50</td>
<td>13</td>
</tr>
<tr>
<td>4) PVA-BC, dried at room temperature</td>
<td>0.22x16.2x80</td>
<td>4</td>
</tr>
<tr>
<td>5) PVA-BC, dried in the oven</td>
<td>0.08x16.2x50</td>
<td>3</td>
</tr>
</tbody>
</table>

**Results**

Some examples of the stress-strain curves are presented in figure 9.14. It is visible that the 10MPa and 20MPa epoxy-BC composites have similar stiffness, but the
10MPa specimens have a higher ultimate strength. The 25MPa has a much lower Young’s modulus comparing to the other epoxy-BC composites. The PVA- dried at room temperature shows ultimate strength similar to the 10MPa epoxy-BC composites, but a lower Young’s modulus. The oven-dried PVA-BC samples failed at a very low ultimate strength and delaminated completely. Because the results obtained with PVA-BC did not exceed the results presented in chapter 9.1, the PVA bacterial cellulose based composite was not considered further for investigation. The spread of ultimate strength and Young’s modulus is shown in figure 9.13.

Figure 9.13: Some examples of stress-strain curves measured on different BC-composites and their Young’s modulus

9.2.3.2 Second session of tensile testing

Using the experience gained in the development of first epoxy-BC composites, new composites could be produced in a more controlled manner in an effort to obtain more reliable test results and make a better comparison between production pressures. The BC used for this test was cultivated using the method presented in chapter 5.6 and alkaline treated using the procedure presented in chapter 6.2.
For this set of tests, samples were cut into pieces before the curing of the resin. In table 9.2 the measured dimensions of the composites are presented:

Table 9.2. Characteristics of composites used in the second session of tensile tests

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Dimensions (mm)</th>
<th>No. of successful test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epoxy-BC, pressed at 10MPa</td>
<td>0.16x15x40</td>
<td>12</td>
</tr>
<tr>
<td>Epoxy-BC, pressed at 15MPa</td>
<td>0.16x15x40</td>
<td>12</td>
</tr>
<tr>
<td>Epoxy-BC, pressed at 250MPa</td>
<td>0.16x15x40</td>
<td>13</td>
</tr>
</tbody>
</table>

**Results**

Some examples of the stress-strain curves are depicted in figure 9.14 [9].

![Stress-strain curves and Young's modulus](image)

Figure 9.14: Some examples of stress-strain curves of BC-Epoxy composites and their Young’s modulus
9.2.3.3 **Flexural tests**

To test the bonding between the epoxy and BC and to obtain better results on the values of mechanical properties, 0.50 mm thick samples, pressed at 10MPa, were used to perform three-point flexural tests (Standard ASTM D790-03). Three-point flexural tests are a more common method of testing brittle materials that fail under relative small strains (less than 5%). However, a disadvantage with this test is that the stresses and strains in the material are relatively uncertain, because the load is applied at the point of bending. Four-point bending avoids this issue by providing a uniform stress between two supports. It is considered a more precise method to provide the mechanical properties of a material. However, research performed on this method of testing has a more quantitative than qualitative nature. The three-point test provided a simple method to perform tests on relatively small specimens, allowing multiple testing with limited material. The material failed at much lower strains than the lower limit of 5% strain for validity of three-point tests and the results could be compared to values from literature [7], performed by the same method.

Using the flexural test, the risk of failure decreases due to stress concentrations at the edges of a sample while showing at the same time if sheets are sufficiently bonded and delamination can be prevented.

![Figure 9.15: Specimen on test bench](image-url)
The test equipment was installed on the same machine used for the tensile tests. The strips were cut in 0.50x10x40 mm pieces and tested until fracture occurred. Stress was calculated using:

\[ \sigma = \frac{3PL}{2bd^2} \]  

(9.8)

Where \( \sigma \) = stress [N/mm\(^2\)]; \( P \) = load [N]; \( L \) = length of span [mm]; \( b \) = width of specimen [mm]; \( d \) = thickness of specimen [mm]. The flexural strength is equal to the maximum strength at the lower surface at the moment of fracture. The strain at the lower surface is calculated using:

\[ \varepsilon = \frac{6Dd}{L^2} \]  

(9.9)

Where \( \varepsilon \) = strain [-]; \( D \) = deflection [mm]; \( L \) = length of span [mm]; \( d \) = thickness of specimen [mm]. The flexural modulus is a measure of the stiffness during the first part of the bending test and in most cases is similar to the Young’s modulus obtained from tensile tests.

**Results:**

In figure 9.16 some examples of the stress-strain curves measured in flexural tests are presented [9].

![Stress-strain curves](image)

Figure 9.16 Stress-strain curves measured on different BC-composites and their Young’s modulus
The most significant difference between the results of flexural test and tensile tests is that the material undergoes plastic deformation just during the flexural test, but the Young’s modulus does not vary so much between the two tests. Moreover, the stress-strain curves in flexural tests resemble to the ones stated in literature [7]. Since flexural test is more common and reliable for brittle materials and deflection can be measured more accurately, it seems that the cause of difference between the two types of tests is mainly due to crack propagation in the tensile test.

It is well know, that the first linear part of the curve during the flexural test is caused by the bending and stretching of the interatomic bonds, following Hooke’s law. Next the curves level off, caused by the uncoiling of the molecules. Finally, the yield point is also the point of failure, because the structure does not allow for much slippage between the molecules. The material therefore cannot strain further plasticly and fails instantly. The yield strength is equal to the ultimate strength. The curves from the tensile test are not linearly at the beginning of the tests because of the use of paper tabs. The tabs are normally used in thin film testing, where the lower Young’s modulus of the films dominates the test results and the influence of the tabs is not visible.

![Figure 9.18: Top end of BC-composite specimen (1), covered by paper tabs (2) in clamps (3) for tensile testing. (a) slipping of the specimen, (b) initial partial gripping of the paper, tearing the surface of the tabs.](image)

During tests it was observed that samples with paper tabs were often not gripped properly and shifted in the clamps (fig. 9.18a). Eventually, the clamps grip and the tensile force can build up, but during the initial gripping, it showed that the paper is gripped on some spots of the surface of the paper before others, distorting and tearing the surface to some extent (fig. 9.18b). These two effects might be the cause of the first non-linear part of the curves.
Literature


Chapter 10

Water sorption characteristics of bacterial cellulose

Typically, biological materials are strongly influenced by moisture content, whereas synthetic materials are significantly affected by temperature change. When increasing the temperature, the majorities of the synthetic polymers undergo secondary transition and then melt [1]. In contrast, most biological materials do not show any melting behavior and undergo rapid thermal degradation. However, a pseudo glass transition can be produced by water sorption, since water can act as a plasticizer for many biopolymers including BC. BC is highly hydrophilic; it has the tendency to take up moisture from the air. Because the moisture leads to an increase in weight and volume, it influences the density of the material when exposed to the environment for a certain amount of time. The volume increase also influences the mechanical properties, since the loaded area of a specimen increases and therefore the stress becomes lower at a constant load. Finally, water sorption is not a linear process and can undergo periodic cycles during the life time of a product, creating an optimal environment for microorganisms to attack and decompose the BC fibers.

In related fields, like wood industry, modeling water transfer in wood, during soaking, has attracted considerable attention and can provide a strong knowledge support in order to investigate water sorption in BC composite materials. From theoretical point of view, the amount of absorbed water in the BC composites is dependent on its density and water diffusivity. The water diffusivity is caused by the
porous structure of the composite in addition to the reactivity of its chemical components and is associated to a general water diffusivity coefficient. From mathematical point of view, the issue of water sorption in wood industry is treated as a diffusive problem based on Fick’s second law of diffusion [2, 3]. Even so, the water transfer is a laborious task, because of the complex associated phenomena which occur during this process. First, wood and BC composites are non–homogenous materials. They are three to two phase systems. In addition, different mechanisms may prevail when water flows into an anisotropic system from different directions [4]. For a given external condition, characterized by a constant water content (as example constant air relative humidity \(\varphi\)) the wood or BC composites (solid phase) acquire water by diffusion until a phases equilibrium is obtained. The equilibrium concentration of water in solids (BC composites) reported to the water concentration in air is named solubility \(S\). It depends on temperature and on the material structure (composition and internal structure of the composite material). A special sorption situation occurs in the case when water is used instead of air with water content. In this case, the solubility is named saturation, \(S_a\), and it is determined by temperature and by the structure of the material.

### 10.1 Theoretical approach

As mentioned before, Fick’s second law can be applied to describe the moisture diffusion process for various materials since it is acknowledged that BC and BC composites can be included in this class of materials. This law can be obtained as a particularization of species conservation (water in the case of BC water sorption) and it has the following mathematical formula:

\[
\frac{\partial C}{\partial t} = \nabla \cdot (D \cdot \nabla C) \tag{10.1}
\]

Where \(D\) is the tensor of diffusion coefficient, \(C = C(t, x)\) is the moisture diffusion, \(t\) is the time and \(x\) is the Cartesian coordinates when \(\nabla\) development is used for this coordinates system; with this coordinates system Fick’s second law becomes:

\[
\frac{\partial C}{\partial t} = \frac{\partial}{\partial x} \left( D_x \frac{\partial C}{\partial x} \right) + \frac{\partial}{\partial y} \left( D_y \frac{\partial C}{\partial y} \right) + \frac{\partial}{\partial z} \left( D_z \frac{\partial C}{\partial z} \right) \tag{10.2}
\]
It can be considered for an isotropic material \((D_x=D_y=D_z=D)\) and Fick’s second law can be rewritten as:

\[
\frac{1}{D} \frac{\partial C}{\partial t} = \frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} + \frac{\partial^2 C}{\partial z^2} \quad (10.3)
\]

The relation between diffusion coefficient and temperature is described by Arrhenius equation,

\[
D = D_0 \exp\left( -\frac{E_D}{RT} \right) \quad (10.4)
\]

Where \(R\) is the universal gas constant, \(E_D\) is the activation energy, and \(D_0\) is the frequent factor of diffusion coefficient.

With respect to solubility or saturation of water fixed by a material and its temperature dependence, the following equilibrium relationships can be applied:

\[
S = \frac{c_s(\varphi, T)}{p(\varphi)} = \frac{c_s(\varphi, T)}{\varphi P_s(T)} = S_0 \exp\left( -\frac{E_s}{RT} \right) \quad (10.5)
\]

\[
S_a = S_{a0} \exp\left( -\frac{E_{sa}}{RT} \right) \quad (10.6)
\]

Where \(T\) is temperature, \(E_s\) and \(E_{sa}\) are specific activation energy, strongly influenced by the solid phase (BC sheets in this case).

If diffusion, in a selected BC composite is studied by weighting the probe’s water content periodically, we have the possibility to obtain, for a fixed experimental temperature, the solubility or saturation when steady state is attained and the main diffusion coefficient of water inside the inspected sample.

### 10.2 Water sorption tests on pure cellulose and epoxy-BC composites

First of all, we show that the experimental water sorption of some BC composites, proposed in this chapter, refers to the case of sample immersion in water. These experimental conditions intend to reproduce the working conditions of these
composites and the manner in which they are affected by rain, downpour, shower, etc.

Sorption tests were performed in the laboratory of “Politehnica” University of Bucharest on two epoxy-BC samples of 8 layers used in the tensile testing and on two epoxy-BC samples of 29 layers used in the flexural testing. Before the tests, the four samples were first put in an oven at 110°C for an hour to remove the water. After removed from the oven, they were immediately weighed to establish the dry weight. The experiment starts by immersing the sample in temperature controlled water, followed then by periodically weighting. To identify the role of the components of the BC epoxy samples in water sorption we use data provided from literature which are presented in figure 10.1[5]

![Figure 10.1: Ratio of mass of water absorbed per mass of Epoxy sample over time (sample thickness 1.8 mm)](image)

The use of bacterial cellulose as material for samples has not been considered because cellulose absorbs water very quickly and it didn’t allow the possibility to measure the mass increase with precision. Therefore, in these experiments the epoxy-BC samples were submerged in water at 50°C and weighed at regular intervals. If it is assumed that epoxy-BC is not an isotropic material, it appears after simple theoretic consideration that the dynamics of saturation curve can be approached with the basic exponential law:

\[ c_s(t) = a(1 - e^{-bt}) \]  

(10.7)
where \( a \) is a constant which describes the equilibrium of saturation and \( b \) is a constant which influences the speed of the saturation and \( t \) is time.

**Results**

![Graph showing ratio of mass of water absorbed per mass of sample over time.](image)

Figure 10.2: Ratio of mass of water absorbed per mass of sample over time. Curves approximated by dashed line.

The results of the sorption tests are plotted in figure 10.2 along with the approximated saturation curves. The epoxy-BC samples display a slower saturation to a lower level. Both values go down with the increase of layers number. As expected, the epoxy in the samples limits the water absorption level with the increasing of layers number of BC and epoxy. In this case it is more difficult for the moisture to penetrate the samples. The comparison of figures 10.1 and 10.2 shows that the BC in BC-epoxy composites are responsible for the level of water saturation, whereas the epoxy is the controller of water input in the composite.

### 10.3 Water sorption tests on PVA bacterial cellulose based composites

A second composite test case for water sorption is the BC-PVA composite. The aim of this study is to test the possibility to use these composites as a flexible material.
where the flexibility is a consequence of water presence inside of the composite. Another scientific interest related to water sorption is the identification of the role of the composite’s components in dynamic investigation. This second set of sorption tests were performed in the Mass Transfer Laboratory of “Politehnica” University of Bucharest on two samples of PVA cellulose based composites, more specifically on samples identical with sample (5b) and (6b) see chapter 9.1. Before the tests, samples were put in an oven at 110°C for an hour to remove the water, then were measured (weighted) and placed in a 1500 ml screw-cap flask containing distilled water.

Experiments were conducted at 25 and 50 °C with different immersion periods: from several minutes to about 250 h. Figure 10.3 shows the water uptake of the BC-dry composite. It was observed that the water uptake increases linearly, then the rate slows down in the saturation phase, and finally leads to a plateau, corresponding to the water uptake in equilibrium. As it was expected, for PVA composites, the level of absorbed water is higher in comparison with pure bio-cellulose. This is mainly caused by the fact that PVA has high affinity to water sorption. However, this doesn’t significantly affect the final strength of the material as it was proved from tests performed in chapter 9.1 (see samples (5b) and (6b)). This is valid when the volume increase of the material is not taken into consideration. With respect to temperature effect on BC-PVA
composites water sorption, figure 9.2 shows that water content at two different temperature levels is higher than the temperature ratio. The next subchapter presents a theoretical approach on the volume increase caused by water sorption and its effects on the mechanical properties.

10.4 Effects on mechanical properties by change in volume

A simplified relation between the effect of water sorption level and the mechanical properties of the composite was investigated. Since bacterial cellulose is highly hydrophilic, it will immediately start to absorb water from the air/environment when pellicles are dried or composites are cured at high temperature. This water can cause an increase in volume and directly affect the mechanical properties of the composite. To model the effect of water sorption, it was assumed that the composite behaves as a sponge to which a pressure force was applied in order to reduce its thickness. This pressure force is analogous to the pressure applied to remove the water from the BC pellicles. In consequence, it can be assumed that the length and width of a specimen is constant and that the ratio of stress in a saturated specimen and dry specimen can be calculated as follows:

\[
\frac{\sigma_{\text{wet}}}{\sigma_{\text{dry}}} = \frac{A_{\text{wet}}}{A_{\text{dry}}} \approx \frac{d_{\text{dry}}}{d_{\text{wet}} + d_{\text{water}}} = \frac{1}{1 + a} \quad ; \quad a = \frac{d_{\text{water}}}{d_{\text{dry}}} \tag{10.8}
\]

Where \(\sigma\) is the stress [N/mm\(^2\)], \(F\) is the force [N], \(A\) is the loaded area [mm\(^2\)], \(d\) is the sample thickness [mm]. The subscript ‘wet’ indicates the BC with absorbed water, ‘dry’ the dry BC and ‘water’ the absorbed water in the BC. The increase of thickness caused by the water is:

\[
d_{\text{water}} = \frac{V_{\text{water}}}{l \cdot b} = \frac{m_{\text{water}}}{\rho_{\text{water}} \cdot l \cdot b} = \frac{x_{\text{w}} \cdot m_{\text{dry}}}{\rho_{\text{water}} \cdot l \cdot b} \tag{10.9}
\]

Where \(V\) is the volume [mm\(^3\)], \(l\) is the length [mm], \(m\) is the mass [g], \(\rho\) is the density [g/mm\(^3\)] and \(x_{\text{w}}\) is the weight ratio of absorbed water and BC dry weight. This makes the ratio of thicknesses \(<a>\) and ratio of stresses:

\[\]
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\[ a = \frac{x_w \cdot m_{dry} \cdot \rho_{BC} \cdot l \cdot b}{m_{dry} \cdot \rho_{water} \cdot l \cdot b} = \frac{x_w \cdot \rho_{BC}}{\rho_{water}} \approx 1.6 x_w \]  (10.10)

\[ \frac{\sigma_{wet}}{\sigma_{dry}} \approx \frac{1}{1 + 1.6 x_w} \]  (10.11)

Since it was considered that the water sorption does not affect the length or elongation according to the previous assumptions, the ratio of Young’s modulus will become:

\[ \frac{E_{wet}}{E_{dry}} = \frac{\sigma_{wet}}{\sigma_{dry}} \approx \frac{\sigma_{wet}}{\sigma_{dry}} \]  (10.12)

Figure 10.4 Young’s modulus of different BC composites (O - experimentally Observed, E - estimated by mean of relation \( E_{wet}/E_{dry} = \sigma_{wet}/\sigma_{dry} \))

Where \( E \) is the Young’s modulus \([N/m^2]\) and \( \varepsilon \) is the strain. It is clear that this is a simple model, because the water sorption phenomenon is not a linear one. In addition to volume increase, the material undergoes a series of physical and
chemical transformations during water sorption which can alter its mechanical properties. Figure 10.4, as an upgrade of the data given in figure 9.6, shows that the above consideration, relative to the water content effect on mechanical properties of BC composite materials, is correct.

### 10.5 Experimental data processing relative to water diffusion coefficient inside of composites

To determine the diffusion coefficient by using our experiments, we assumed that samples used in these experiments are an infinite plate with a constant thickness. This observation impose to Fick’s law does not consider the diffusion parallel to the surface of the plate. Therefore, inside the sheets there is only one direction for water transport and consequently the water diffusion equation is simplified to:

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}
\]  
(10.13)

If the region \(0<x<l\) is initially at a uniform concentration and the surfaces of the sheet are kept at saturation, we have the following boundary condition:

\[
C(0, x) = C_0(x) = \begin{cases} 
C_s & x = 0, x = l \\
1 & 0 < x < l 
\end{cases}
\]  
(10.14)

Where \(C_0(x)\) is the moisture distribution at the initial time \(t=0\), and \(C_s\) is the saturated moisture content. The boundary condition is a type I diffusion condition with the below expression relative to our case:

\[
C(t, 0) = C(t, l) = C_s (t) = C_s
\]  
(10.15)

This mathematical model of diffusion species transport in a plane sheet with a type I univocity conditions present an analytical solution which can be obtained in various models [6]. Here we consider the separation variables method relative to partly dimensionless state of model obtained by use of dimensionless variable 

\[u(t, x)\], defined below, in the place of \(C(t, x)\).
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\[ u(t, x) = 1 - \frac{C(t, x)}{C_s} \]  \hspace{1cm} (10.16)

With the new variable \( u(t, x) \) the equations 10.14 and 10.15 become:

\[
\begin{align*}
 u(0, x) &= u_0(x) = \begin{cases} 
 0 & x = 0, x = l \\
 1 & 0 < x < l 
\end{cases} \\
 u(t, 0) &= u(t, l) = u_s(t) = 0 \\
 \frac{\partial u(t, x)}{\partial t} &= D \frac{\partial^2 u(t, x)}{\partial x^2}
\end{align*}
\]  \hspace{1cm} (10.17, 10.18)

Because the problem is symmetrical about the central plane of the sheet, it is most convenient to change the domain of \( x \) coordinate from \([0, l]\) to \([-l/2, l/2]\). This change, coupled with the particularization of variables separation method to the model described by equations 10.17 and 10.18, gives the following solution:

\[
 u(t, x) = 4 \sum_{n=0}^{\infty} \frac{(-1)^n}{\pi} \exp \left\{ -\frac{(2n + 1)^2 \pi^2 D}{l^2} t \right\} \cos \left( \frac{(2n + 1)\pi}{l} x \right)
\]  \hspace{1cm} (10.19)

If one wants to express the solution 10.19 in the terms of concentration evolution \( C(t, x) \), the next expression can be obtained:

\[
\frac{C(t, x)}{C_s} = 1 - 4 \sum_{n=0}^{\infty} \frac{(-1)^n}{\pi} \exp \left\{ -\frac{(2n + 1)^2 \pi^2 D}{l^2} t \right\} \cos \left( \frac{(2n + 1)\pi}{l} x \right)
\]  \hspace{1cm} (10.20)

Equation (9.9) describes the relative moisture distribution, which can be obtained directly from water sorption experiments. However, only the change of the overall moisture content can be obtained, so for mean moisture the integration over \( x \) must be done:

\[
\bar{C}(t) = \frac{\int_{-l/2}^{l/2} C(t, x) dx}{C_s} = \int_{-l/2}^{l/2} \left( 1 - 4 \sum_{n=0}^{\infty} \frac{(-1)^n}{\pi} \exp \left\{ -\frac{(2n + 1)^2 \pi^2 D}{l^2} t \right\} \cos \left( \frac{(2n + 1)\pi}{l} x \right) \right) dx \times \int_{-l/2}^{l/2} \cos \left( \frac{(2n + 1)\pi}{l} x \right) dx
\]  \hspace{1cm} (10.21)
When we multiply the elements of ratio \( \frac{C(t)}{C_s} \) with sheet volume, the following relation is obtained for evolution of sheet’s mass during water sorption test:

\[
\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp \left\{ -\frac{(2n+1)^2 \pi^2 D}{l^2} t \right\}
\]

(10.22)

Here, \( M_t \) is the mass of moisture after sorption time \( t \), \( M_\infty \) is the moisture mass corresponding to the sheet saturation.

The above equation (10.22) can be simplified for two cases, one addressing to the initial stage of the sorption when \( Dt/l^2 < 0.05 \) (equation (10.23)) and the second when \( Dt/l^2 > 0.05 \) (equation (10.24)).

\[
\frac{M_t}{M_\infty} = \frac{4}{l} \sqrt{\frac{Dt}{\pi}}
\]

(10.23)

\[
\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \exp \left( -\frac{\pi^2 Dt}{l^2} \right)
\]

(10.24)

For an experiment showing the time evolution of observed \( \frac{M_t}{M_\infty} \) the following procedure for data processing can be used for \( D \) identification:

a) select from experimental data one part and mention \( N_1 \) as number of measured data verifying included in this part;

b) Minimize the functional \( F(D) = \sum_i \left( \left( \frac{M_t}{M_\infty} \right)_i - \left( 1 - \frac{8}{\pi^2} \exp \left( -\frac{\pi^2 Dt}{l^2} \right) \right)_i \right)^2 \);

c) With the established values of \( D \) verify, as example by graphical representation, the agreement between observed and computed \( \frac{M_t}{M_\infty} \) for all \( Dt/l^2 \).

In concretely, data from figure 10.1, 10.2 and 10.3 are summarized in figure 10.5. The evolutions of \( \frac{M_t}{M_\infty} \) were used to identify the diffusion coefficient for water sorption applying the above procedure in several cases: 1) a sheet from classical epoxy (\( M_{t_{ad}} \) vs \( \tau \) in figure 10.5 shows this case from experimental point of view); 2) a sheet of classical epoxy with glass fiber insertion ((\( M_{t_{ad}} \) vs \( \tau \) in figure 10.5 shows this case from experimental point of view); 3) a sheet of classical epoxy with
glass fiber insertion and with an excess of hardening component \((M_{tad3} vs \tau \text{ in figure 10.5 shows this case from experimental point of view})\); 4) a sheet of BC epoxy with 9 sheets of BC in the sandwich structure; 5) a sheet of BC epoxy with 29 sheets of BC in the sandwich structure; 6) a sheet of BC PVA with 9 sheets of BC in the sandwich structure; 7) a sheet of BC PVA with 29 sheets of BC in the sandwich structure.

![Figure 10.5](image)

**Figure 10.5** Dynamics of \(M_t/M_\infty\) versus time for all cases considered for D identification

In table 10.1 we present the values of identified water diffusion coefficient for above considered cases. At the same time with figure 10.6 and 10.7 it is presented a comparison between experimental and computed evolutions of \(M_t/M_\infty\) ratio. For D identification only the experimental data obtained until 125 hours has been used.

<table>
<thead>
<tr>
<th>Case</th>
<th>Material</th>
<th>Material thickness l (m)</th>
<th>D (m²/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sheet from epoxy</td>
<td>1.5 (10^{-3})</td>
<td>3.257 (10^{10})</td>
</tr>
<tr>
<td>2</td>
<td>Sheet as 1 and with glass fibers</td>
<td>1.5 (10^{-3})</td>
<td>6.024 (10^{10})</td>
</tr>
<tr>
<td>3</td>
<td>Sheet as 2 with OMMT</td>
<td>1.5 (10^{-3})</td>
<td>6.748 (10^{10})</td>
</tr>
<tr>
<td>4</td>
<td>Sandwich from BC and epoxy</td>
<td>1.65 (10^{-3})</td>
<td>1.793 (10^{8})</td>
</tr>
<tr>
<td>5</td>
<td>Sandwich as 4 with 29 BC sheets</td>
<td>3.3 (10^{-3})</td>
<td>4.214 (10^{8})</td>
</tr>
<tr>
<td>6</td>
<td>Sandwich BC PVA as 4 50°C</td>
<td>1.65 (10^{-3})</td>
<td>3.802 (10^{7})</td>
</tr>
<tr>
<td>7</td>
<td>Sandwich BC PVA as 4 20°C</td>
<td>1.53 (10^{-3})</td>
<td>3.235 (10^{7})</td>
</tr>
</tbody>
</table>
Figure 10.6  Experimental and computed $\frac{M_t}{M_\infty}$ versus time for BC epoxy sandwich composites

Figure 10.6  Experimental and computed $\frac{M_t}{M_\infty}$ versus time for BC PVA sandwich composites
Production Methods and Characteristics of Bacterial Cellulose Composites

With respect to the reported values of diffusion coefficients from table 10.1 the following observations can be reported:
- the values of water diffusion coefficients in pure epoxy sheet or in epoxy glass fibers composite (from $3 \times 10^{-10}$ $m^2/h$ to $7 \times 10^{-10}$ $m^2/h$) show that they are dense solid materials;
- in sandwich BC-epoxy composites, the sheets strongly increase the water diffusion coefficient comparatively to those of epoxy sheets (it increases from $3 \times 10^{-10}$ $m^2/h$ (epoxy sheet) to $4 \times 10^{-8}$ $m^2/h$); the number of BC sheets in the epoxy BC sandwich increases the water diffusion coefficient;
- in sandwich BC-PVA composites, the diffusion coefficient is higher with one order magnitude with respect to the correspondent BC epoxy composites;
- in sandwich BC-PVA composites, the diffusion coefficient is increased by temperature.

Conclusions

It is clear that the uptake of water will form a problem for BC-composites. The resin system can act as a barrier, but even the epoxy cannot prevent water from getting into the BC. It is observed that the absolute Young’s modulus will be lowered due to the absorbed water as it can be see from figure 10.4. It is possible that the absolute ultimate load of the specimens might show a slight increase due to the increase of toughness, avoiding premature failure due to stress-concentrations. The effect of increasing thickness of the specimen due to the absorbed water will lower the ultimate stress and Young’s modulus significantly. Finally, the uptake of water increases the chance and speed of decay of the material. It is not known how much water was absorbed by the samples used for the mechanical tests, but it is probable that due to the limiting effect of the epoxy on the speed and level of moisture uptake, the samples were relatively dry during testing. When used in structures however, the BC will take up enough moisture over a longer period of time, significantly affecting the mechanical properties. The exact effects could not be studied during this research, but show to be significant and will have to be investigated further. Eventually, the composites will have to be coated or treated sufficiently to limit this uptake of moisture.
Literature


Chapter 11

Conclusion and discussions

The primary goal of this research was to establish if mechanical properties of a BC-composite are sufficient to qualify the material to be further used in structural applications. During the research investigation, epoxy-BC composites were produced and submitted to a series of tests. It revealed that results were comparable with properties of glass-fiber reinforced composites (GFC), see table 11.1 [1]. Moreover, considering values of flexural strength and stiffness obtained from test-specimens, we have reached to the conclusion that BC-composites possesses the necessary properties/characteristics to serve as primary building-material for structural components that are now, for instance, made from typical bi-directional fabric GFC’s.

Table 11.1. Epoxy-BC composites versus glass-fiber composites

<table>
<thead>
<tr>
<th></th>
<th>Epoxy-BC composite</th>
<th>Glass-fiber composite (bi-directional fabric)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultimate strength (flexural) (MPa)</td>
<td>~300-465</td>
<td>~400-600</td>
</tr>
<tr>
<td>Young’s Modulus (flexural) (GPa)</td>
<td>~40-60</td>
<td>~20-25</td>
</tr>
<tr>
<td>Ultimate flexural strain (%)</td>
<td>~1.0-1.5</td>
<td>~1.5-3</td>
</tr>
<tr>
<td>Density of composite (g/cm³)</td>
<td>~1.45</td>
<td>~1.8</td>
</tr>
<tr>
<td>Water absorption after 24h (%)</td>
<td>~0.2-0.3</td>
<td>~0.05-0.07</td>
</tr>
</tbody>
</table>
Production Methods and Characteristics of Bacterial Cellulose Composites

The material reveals ultimate flexural strength very similar to typical values exhibited by GFC’s while the Young’s modulus value of the BC-composite is about twice the size of that exhibited by glass-fiber composites. Moreover, the properties of the BC are omni-directional in-plane, in contrast to the bi-directional fabric GFC’s, which is another valuable characteristic.

During the process of growing the material, producing the composites and testing the mechanical properties of the material, we have reached to several valuable conclusions that could be important for later research. Conclusions and recommendations are found in the separate conclusions-fragment at the end of each chapter, while most important ones will be mentioned here.

Because the bacterial cellulose for pellicle production is grown in a nutrient-rich medium, there is a considerable risk of contamination with other bacteria or fungi. To produce BC reliably and in large quantities, the growing process of the pellicles needs to be done in bio-reactors. It was demonstrated, however, that when the bacteria were sufficiently abundant in the growth-medium from inoculation phase, a pellicle at the surface of the medium was able to form within less than a day. This pellicle seemed to inhibit the development of any other organisms, otherwise harmful to the development of viable pellicles. The most successful harvests during this research were achieved with little caution for contamination, but each time by starting with a medium that was rich enough in bacteria as well as in (sufficient) nutrients. The high concentration of bacteria was obtained by introducing a small amount of bacteria in a closed container with sterile medium and bubbling air through the medium to inhibit the development of the pellicle while stimulate growth and division of the bacteria. This medium with abundant bacteria was then poured in a tray to allow a large pellicle to grow on the surface.

**Obtaining the dried sheets of BC**

An important issue with obtaining BC in smooth dry sheets is to restrict as much as possible the shrinkage in-plane in order to avoid crumpling of the sheets. In research investigations, the most efficient tested way to achieve this was by applying pressure to simultaneously press out water and restrict the sheets’ shrinkage. Anyway, it showed that pressing too strong and too fast did not allow enough time for the fibers to rearrange, causing cracks in the pellicles. A very simple and efficient method to overcome this issue was to cover the pellicles with vacuum foil and use vacuum suction to remove most of the water from the pellicles in a relatively slow and controlled manner. This is followed by pressing under a heated mechanical press which makes the pellicles dry completely. This proved to be the best method/process to obtain very smooth bacterial cellulose sheets from wet pellicles and the less time-consuming (30 minutes).
Producing the composites

The first assumption was that a hydrophobic resin system such as epoxy would be insufficiently capable to bond the hydrophilic layers of BC. It proved however, that using the mechanical press to remove excess epoxy and force the epoxy to penetrate the layers produced a well-bonded composite. As mentioned before, it is important though to eventually develop a composite using a biodegradable and renewable (B&R) resin system. Obvious choices for B&R systems are water soluble resin systems, such as PVA or methyl cellulose, but during the experiments an important flaw of using water soluble systems was discovered. When the sheets are smooth and dry, they are quite easy to handle, laminate, stack and press. However when using a water soluble system the sheets are wetted again, which turns them back to a jelly-like state. In this state it is very difficult to stack the layers smoothly and when pressed too firmly the layers start to shift, altering the laminate. When it is managed to press the layers, the water has difficulty evaporating out of the layers. Even when a composite is successfully produced, the re-hydration of the layers causes them to end up wavy in the composite, making suboptimal use of the favorable characteristics of the smooth BC-sheets. Solutions to this problem would be to use a different technique to produce the BC-composites with water soluble resin systems, for example by rolling (see chapter 9.1.2) or tensioning the BC. An easier way to solve this problem would be to use B&R resin systems that are not solvable in water but can be melted to penetrate and bond the layers or polymerize after lamination like the epoxy resin.

Testing the composites

Because BC is very brittle, it creates difficulties when using tensile testing to obtain mechanical properties. Because of the brittleness, small fractures are introduced at the edges when cutting the test specimen which causes stress concentrations during the tensile testing and ultimately premature failure. Less brittle materials can lower stress concentrations and distribute the stress more evenly over their loaded surface by straining plastically locally. Because BC is less capable of doing this, it is unsure if the tensile force is transferred through the complete area of the specimen or only a part of it, raising the stress in the loaded part considerably. Tests were performed also using a three-point flexural test which is more common for brittle materials. It was necessary however, to create much thicker specimens, because the specimens prepared for tensile testing were too thin to be used, which meant that much more sheet-material was necessary. Avoiding the problems encountered with tensile testing did mean however, that the test results were more reliable. Data provided by these experiments reveals that it is highly recommended to use flexural testing instead of tensile testing to obtain the properties of BC-composites in future research.
Discussion

It must be emphasized that this research has a more qualitative than quantitative character. During this experimental investigation, many assumptions and shortcuts have been made to obtain sufficient material, test specimens and test-data. The values of flexural strength and modulus are therefore only indications of the kind of properties that can be obtained from BC-composites. However, there it seems to be no indication that the values mentioned in table 11.1 could be significantly lower than the actual properties of an epoxy-BC composite. It is likely that by using a more controlled process to create the composites and more accurate methods of measuring the mechanical properties would result in higher rather than lower values.

To eventually create a biodegradable and renewable (B&R) composite, several problems still have to be deeply researched. First of all, some of the composites tested in the research were made with epoxy-resin, which is obviously not biodegradable or renewable. The main drive in commercial success of BC-composites will be that BC is a natural material. Therefore, the success will also depend on whether it is possible to produce a completely B&R composite. This means that it is advisable to develop a composite with a B&R resin system that still possesses similar mechanical properties to those of the epoxy-BC composites, before investing large resources in up-scaling the production of BC.

Another major issue with BC-composites is the tendency to absorb water. It was necessary to investigate this behavior and the effects on the mechanical properties of the material. It can be concluded that BC composites have to be chemically treated or coated to become suitable for use in structural applications.

The most important step in the research of BC-composites would be to make a cost and resource estimation for the production of the material (BC). The methods utilized to obtain material for this research proved to be very slow, laborious and resource intensive and if the cost and speed of producing the raw material cannot be adjusted sufficiently, it will never be able to commercially compete with existing building materials in the car or aerospace-related industry.

Literature

Appendix A

Constituents of medium:

• Yeast extract:
  Carl Roth art.nr. 2363.2
• Pepton:
  Peptone ex soja (animal-free/GMO-free) Carl Roth art.nr. 2832.2
• Anti-foam:
  Antifoam C Emulsion, Sigma Life Science, A8011

Resin systems:

• Epoxy:
  Hexion EPIKOTETM Resin 04908 + EPIKURE Curing Agent 04908
• PVA:
  Polyservice Lossing B-film
• Methyl Cellulose:
  Henkel Perfax Behangplaksel Metyl, normaal en zwaar behang

Production and test equipment:

• Press:
  Joos laboratory press LAP100, maximum pressing force 1000kN
• Materials testing machine:
  Zwick Materials Testing Machine 1455, modernized with the digital Zwick electronics, nominal Load 20kN, 2kN load cell
• Oven:
  Thermo Scientific Heraeus Vacutherm Series model VT 6130 P, 3.0kW
• SEM:
  Jeol JSM-7500F Field Emission Scanning Electron Microscope