

Bacterial cellulose:
not just a lightweight food,
but also a fascinating scaffolding material

**Preparation and characterization of unaltered and mechanically reinforced bacterial
cellulose aerogels**

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Abstract

The first part of the present work summarizes the development, properties and applications of aerogels and proposes a definition of the term. Then the three main classes of aerogels based on silica, synthetic polymers and pyrolytically generated carbon are briefly described. The comparatively new class of cellulosic aerogels is finally introduced. To provide a background for the practical work that was conducted, also properties and applications of bacterial cellulose and the technique of drying by means of supercritical CO₂ are described.

The second part concentrates on the practical work conducted during the thesis. Neat bacterial cellulose aerogels were prepared by means of repeated solvent exchange and drying with supercritical CO₂ and subjected to different analyses. Uniaxial compressive tests showed that the material was transversely isotropic which was supported by microscale deformations occurring in frozen water-soaked bacterial cellulose samples. Further structural investigations by SEM revealed discontinuities, whose origin is not yet clarified, and the presence of large voids (tens to hundreds µm in diameter).

After investigations on neat bacterial cellulose aerogels, the material was modified by various compounds in order to create interpenetrating networks as a reinforcement against mechanical loading. Pretests with tetraethyl orthosilicate, soluble glass, dilactide and 2-methoxyhydroquinone indicated the better suitability of carbon-based compounds. Further tests were carried out with terephthalic acid and cellulose acetate. Only cellulose acetate yielded the expected interpenetrating network and effectively reinforced the material.

Porosity measurements were carried out on some samples indicating an extremely broad pore size distribution ranging over several powers of ten from the nm-scale to the µm-scale. However, currently no technique is capable to capture the full pore size distribution.

Keywords: Bacterial cellulose, aerogel, mechanical properties, porosity, reinforcement

Zusammenfassung

Der erste Teil der vorliegenden Arbeit fasst die Entwicklung, Eigenschaften und Anwendungen von Aerogelen zusammen und schlägt eine Definition des Begriffes vor. Zuerst werden anorganische Silizium-basierte Aerogele diskutiert, danach jene aus synthetischen Polymeren und pyrolytisch erhaltenem Kohlenstoff. Schließlich wird die neue Gruppe der cellulosischen Aerogele vorgestellt. Als Unterbau für die durchgeführten praktischen Arbeiten werden auch Eigenschaften und Anwendungen von Bakteriencellulose und die Technik des Trocknens mit überkritischem CO_2 beschrieben.

Der zweite Teil konzentriert sich auf den praktischen Aspekt dieser Arbeit. Aus reiner Bakteriencellulose wurden Aerogele durch Trocknung mit überkritischem CO_2 hergestellt und unterschiedlichen Analysen unterzogen. Einachsige Druckversuche zeigten, dass das Material transvers isotrop war, was durch Mikroskopische Deformationen in gefrorenen wassergetränkten Bakteriencelluloseproben unterstützt wurde. Weitere Strukturuntersuchungen mittels REM deckten Diskontinuitäten auf, deren Ursprung noch ungeklärt ist, und das Vorhandensein großer Hohlräume (mit Durchmessern von einigen Dutzend bis einigen Hundert μm).

Nach Untersuchungen an unbehandelter Bakteriencellulose, wurde das Material mit unterschiedlichen Substanzen modifiziert um interpenetrierende Netzwerke als Verstärkung gegen mechanische Belastungen zu schaffen. Vorversuche mit Tetraethylorthosilicat, Wasserglas, Dilaktid und 2-Methoxyhydrochinon deuteten auf die bessere Eignung von Kohlenstoff-basierten Substanzen hin. Weitere Versuche wurden mit Terephthalsäure und Celluloseacetat durchgeführt. Lediglich Celluloseacetat führte zur Ausbildung des erwarteten interpenetrierenden Netzwerkes und tatsächlich zu einer Verstärkung.

Porositätsmessungen wurden an einigen Proben durchgeführt und deuteten auf eine sehr breite Porengrößenverteilung hin, die über mehrere Zehnerpotenzen vom nm zum μm Bereich reichte. Es gibt derzeit allerdings keine Methode, die in der Lage ist, die volle Porengrößenverteilung zu erfassen.

Schlagwörter: Bakteriencellulose, Aerogel, mechanische Eigenschaften, Porosität, Verstärkung

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Introduction

In nature cellular materials are a common way of reducing weight and amount of bulk material used in a structural part while still maintaining sufficient mechanical properties. The most famous examples are certainly bone and wood. Their outstanding properties have been the focus of many scientific investigations and in particular wood has played an important role in man made structures for a very long time because its advantages were already recognized very early.

In general natural materials have the advantage over engineering materials that they are produced by biochemical processes and grow more or less on their own. Over time the processes yielding natural materials have evolved to a high degree of efficiency and blended into the biological system on earth. The advantages of such an efficient production together with the equilibrated implementation into the whole biosphere are obvious. However, natural materials also feature a high degree of complexity compared to engineering materials. This confronts their implementation into man made structures with considerable problems. Thus thorough investigations not only of these materials' properties but also of their synthesis become necessary.

Aerogels are at the very low end of density of solid materials. Known since their discovery by Kistler (1931), until now mainly silica based aerogels have been in use. However, they are an engineering material. With the appearance of celluloseic aerogels that can be obtained from both plant (Jin et al. (2004), Innerlohinger et al. (2006), Liebner et al. (2008)) and bacterial cellulose (Maeda et al. (2006), Liebner et al. 2010a), not only another organic counterpart to silica aerogels was discovered, but also aerogels derived from natural resources.

There is hardly any information at all on the morphological and mechanical features of cellular solids as obtained by scCO_2 drying of bacterial cellulose lyogels. Therefore the aim of this work thoroughly characterise these properties, as the assessment of the mechanical properties and morphology were expected to allow for a better positioning of bacterial cellulose aerogels in the field of solid materials. The present work is supposed to provide some basic information about bacterial cellulose aerogels so that possible applications may be identified and more specific research can be conducted to design the material for a particular application. Furthermore the work also deals with attempts to reinforce the aerogels in a controlled manner to provide them with a broader basis for applications.

Theoretical part

Aerogels

Definition

After Kistler produced and reported on aerogels for the first time in 1931 (and in a more comprehensive article in 1932), a considerable amount of scientific literature has been devoted to this material which lead to several definitions of the term "aerogel". Gesser and Goswami (1989) limited the base material to be a colloidal gel, i. e. a mixture of two continuous phases on a microscopical level. Fricke and Emmerling (1998) did not mention this particular structural feature, however restrict aerogels to be "...sol-gel derived nanostructured materials...". While the term nanostructured is applicable to the material discussed in the present work, it is neither sol-gel derived nor colloidal, as will be shown later. Hüsing and Schubert (2000) defined aerogels in a more general way through

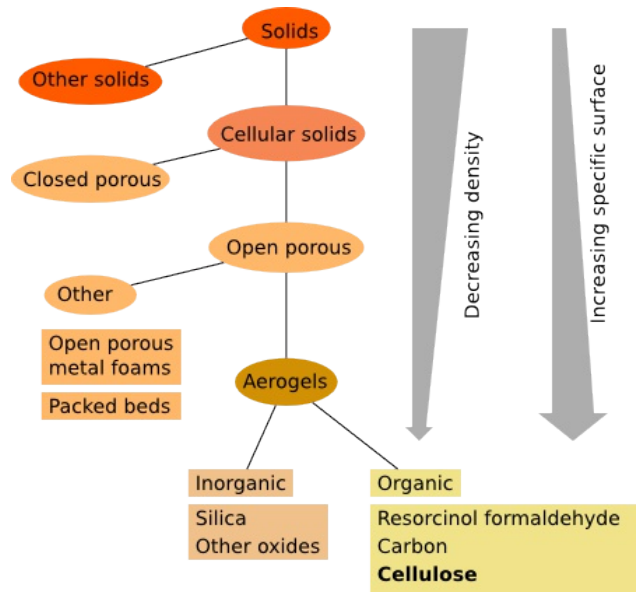


Figure 1: Classification of Aerogels among other solid materials

the production process: "Materials in which the typical pore structure and network are largely maintained when the pore liquid of a gel is replaced by air...", immediately mentioning a somewhat problematic point of their definition: "However, it is not always clear to what extent the structure is maintained." And indeed, the precondition that the structure of the wet gel remains intact throughout the drying process is difficult to maintain because certain structural investigations simply can not be performed on wet gels. This makes the validation of the latter definition impossible in many cases. Kistler (1932) already concluded, that one of the benefits of the ability to replace the liquid phase in a gel by a gas, are more means of investigation of the gel structure.

To circumvent the weaknesses of the above mentioned definitions, the author believes that it would be more convenient to define aerogels by properties that most of the published work agrees on. Thus aerogels would be solids that feature very low density, high specific surface area and consist of a coherent open porous network of loosely packed, bonded particles. This definition distinguishes the material from liquid foams (e.

g. whipped cream), packed beds (freshly fallen snow) and open porous metal foams and gives them a particular place in the group of cellular solids (Figure 1).

Worth mentioning at this point is the metaphor to describe the nature of aerogels rather than a definition by Long and Rolison (2007) – they compared aerogels to buildings and look at the material from an architectural point of view. A building consists of a comparably little amount of walls (i. e. solid volume fraction) compared to the volume occupied by rooms (i. e. void volume fraction). Doors and windows make a building open porous and people moving through them are a good metaphor for substances diffusing

through an aerogel. Considering e. g. Fritz Wotruba's Church of the Holy Trinity (Figure 2), even the packed particles constraint seems satisfied. The only difference that remains between buildings and aerogels is the scale; while the appropriate unit for measuring buildings is obviously meters, that for aerogels lies six to nine orders of magnitude lower, namely in the μm to nm scale.



Figure 2: Fritz Wotruba's Church of the Holy Trinity (Anonymous 2005)

This metaphor is not only interesting as a means of explaining the nature of aerogels to the laymen but it can also help to visualize the role of the chemist or materials scientist in relationship to the aerogel. Whoever designs the structure of an aerogel or modifies its surface characteristics, can be considered some kind of architect or civil engineer or interior architect for the material, though with his or her sphere of activity lying in the nanoscale.

It has been the experience of the author that, even though not particularly scientific, the latter description of the term “aerogel” was far more useful on most occasions explaining his field of activity than any of the previous definitions.

Types and applications

While the proposed definition distinguishes aerogels from other materials, it does not cover the fact, the materials class “aerogel” is not homogenous and one can further differentiate at least two types – inorganic and organic, depending on the material the aerogel's framework consists of. The present work deals with aerogels belonging to the

organic group. Nevertheless, their better investigated and comparably widely used inorganic counterparts have to be briefly described as they make up an integral part in aerogel history and are still subject of intensive research aiming at the development of tailored materials for certain applications.

Inorganic aerogels

Kistler's interest in aerogels was supposedly mainly of scientific nature. However, considering the fact that aerogels were highly porous, applications like high performance insulators or adsorbers would seem obvious. It took some time, namely until the 1940s, until Monsanto developed interest in aerogels (Arlon and Ayers 2011) and introduced the commercially available silica aerogel based product Santocel in the 1950s. Santocel was mainly used as a thickening agent and penetrated the insulation market only in little amounts. The product was discontinued due to the introduction of the cheaper fumed silica (Smith et al. 1995).

Thermal insulation properties of silica aerogels were studied early (Fricke et al. 1987). Also, the transparency of monolithic aerogels and translucency of granulates opened interesting architectural possibilities as transparent/ translucent heat insulation. Nevertheless, the use of aerogels for thermal insulation has remained very limited compared to materials like EPS or XPS commonly used in the construction industry. The company Scobalit offers hydrophobized silica aerogel granules for the thermal insulation of buildings under the tradename Nanogel, but the high price of around € 2000 per loose cubic meter obviously limits the application to some very special cases like improving the thermal insulation properties of glass bricks. Another company – Aspen Aerogels – produces a variety of aerogel containing products for industrial, construction and high performance outdoor gear applications.

Obviously, since their introduction, aerogels have never made it to widespread use in everyday objects mainly due to the high price. In special applications, aerogels have played a far more important role. One very interesting use are Cherenkov radiation detectors. Discovered by Pavel Alekseyevich Cherenkov in 1934, Cherenkov radiation has played an important role in astrophysics and high energy physics for the determination of speed and direction of charged particles (Jelley 1955) and more recently in molecular imaging (e. g. Garay 1987, Park et al. 2010). Cherenkov radiation detectors can be constructed from various materials ranging from gasses to liquids and solids. Depending

on the refractive index of the material used, particles of different masses can be observed. Silica aerogels with refractive indices between 1.01 and 1.1 closed a gap between gas and liquid/solid detectors that could only be narrowed by compressed gasses and made less bulky detector designs possible (Carlson 1986).

The most prominent use of silica aerogels was probably NASA's STARDUST mission whose aim was to retrieve intact cometary dust particles from space (Brownlee et al. 1996). Dust particles in space usually travel at very high speeds of several km s^{-1} . If the deceleration is too quick, conversion of kinetic into thermal energy can vaporise or at least alter the chemical structure of the particle in such a way that further analysis is useless. Thus the deceleration process has to happen slowly. Silica aerogels proved to be a suitable material to satisfyingly solve the problem. High velocity impact tests showed that 15 % to 40 % of the original particle mass could be preserved and it was possible to draw conclusions on particle speed from impact track length. Also of great importance is the fact that silica aerogels can be made strong enough to withstand the exertion of traveling to space (Burchell et al. 1998). Considering the recently sparked debate on the theory that the origin of life on earth is actually extraterrestrial (Hoover 2011), silica aerogels may someday play an integral role in resolving this fundamental question.

A further application which is not only restricted to silica aerogels is catalysis. The special features of sol-gel chemistry along with their large surface area render silica-based aerogels an interesting starting point for catalyst development (Pajonk 1991). Armor et al. (1985) reported on the synthesis of palladium or nickel doped alumina aerogels that performed similarly to commercially available hydrogenation catalysts at that time. The preparation process itself was reported to yield highly dispersed palladium particles on the alumina that needed no further pretreatment. Similar aerogels were used by Piao et al. (2002) to decompose methane to carbon nanotubes. The very recent review by Pierre (2011) clearly demonstrates that this is a field still very actively investigated.

Organic aerogels

Organic aerogels are of particular interest as fascinating properties can be tailored due to the huge variety of low and high molecular organic compounds. Already Kistler (1932) reported on experiments with organic polymers such as cellophane, nitro cellulose etc.

However, only in the nineties a considerable progress was made in the field of organic aerogels due to serious advances in sol-gel technology, instrumental analysis such as NMR spectroscopy, and polymer technology. Pekala et al. (1989) reported on a sol-gel technique affording aerogels from resorcinol and formaldehyde. Later, the same author reported on aerogels synthesized from melamine/formaldehyde (Pekala et al. 1992) and phenol/furfural (Pekala et al. 1995). Another group of chemical compounds – namely polyurethanes – was used by Rigacci et al. (2004) to either obtain aerogels or xerogels with the intended use as superinsulators. The given examples do not cover the full range of possible base chemicals for organic aerogels, but should give an impression of the magnitude of synthesis possibilities of this aerogel cluster. One of the main fields of application is their subsequent conversion to carbon aerogels.

Carbogels – as the name indicates – are aerogels consisting of carbon. According to the principles of polymer technology, high portions of aromatic moieties in the polymer-backbone increase not only the melting and glass transition temperature of polymers but also their ability to resist higher temperature. Furthermore, aromatic groups considerably contribute to the percentage of remaining solid after pyrolysis. Correspondingly, polymers consisting of aromatic groups are typically used as precursor compounds for carbon aerogels. Resorcinol/ formaldehyde aerogels for example have been converted to carbogels by pyrolysis at 600° C to 1200° C (Al-Muhtaseb and Ritter 2003). Similarly, polyurethane based aerogels can be transformed at 600° C – 800° C, though the treatment is obviously harsh, as structural changes on the molecular level lead to macroscopic deformations (Biesmans et al. 1998). Carbogels have properties that make them promising materials mainly for two applications – supercapacitors and hydrogen adsorbers.

Supercapacitors are devices that can store electric energy and close the gap between rechargeable batteries and normal capacitors. Normal accumulators have high energy densities (i. e. can store large amounts of energy per unit mass), but their power densities (i. e. power available in a short time) are very limited. Capacitors on the other hand, can store only very little energy but can release this amount in a very short time. Supercapacitors are able to store considerable amounts of energy that can be released very quickly and stored over a long period of time and over many charging cycles. This property makes them ideal to power up electrical devices or as back-up batteries and to store short energy pulses.

It was shown that resorcinol-formaldehyde based carbogels, combined with KOH or H₂SO₄ solutions or polymeric electrolytes have great potential in the application as supercapacitors (Pröbstle et al. 2002, Fischer et al. 1997, Pröbstle et al. 2003, Burke 2000). A drawback of these systems is, that they can only be used for voltages as low as a few volts as higher voltages would inevitably oxidise the carbon scaffold which in turn would lose its electrochemical properties. Furthermore, porosity is another key issue which has to be carefully adapted for the type of electrolyte used (ionic liquid, polymeric electrolyte etc.). However, particularly the last disadvantage can be compensated by the very high specific surface areas (up to 2600 m² g⁻¹) of activated carbogels (Hanzawa et al. 1996). Today, a variety of carbogel based supercapacitors are already commercially available under the trade name “PowerStor” by Cooper Bussmann (Anonymous 2011)

Furthermore, highly porous carbon can also be used to store hydrogen through adsorption. At room temperature, certain high-carbon porous matrices show very good properties with respect to this application. The advantage of carbon over metal foams is the lower density and thus reduced weight of hydrogen storage tanks. However, for the application as a hydrogen adsorber, the pore size distribution of carbogels is a critical parameter as mainly micropores (<2 nm) are effective for hydrogen storage (Hynek et al. 1996, Dillon and Heben 2001).

The third promising application field for carbogels is – similar to silica aerogels – catalysis. Several ways exist of doping resorcinol-formaldehyde derived carbogel with metal particles and control their textural features and pore structure (Moreno-Castilla and Maldonado-Hódar 2005). Moreno-Castilla and Maldonado-Hódar (2005) pointed out the potential of carbogels in catalysis but also remarked, that still only little experience existed with actual applications. This fact seems to be still valid today, as no recent literature explicitly dealing with carbogel based catalysis was found by the author.

Cellulosic aerogels

Cellulose based aerogels are the most recent members of the aerogel materials class. First synthesized by Jin et al. (2004) from non derivatized cellulose dissolved in calcium thiocyanate tetrahydrate (Ca(SCN)₂•4H₂O) and regenerated in methanol, the most distinct feature of cellulosic aerogels in contrast to their synthetic and inorganic counterparts was already clearly visible at that time. All aerogels obtained up to now

consisted of loosely packed bonded more or less round particles (Figure 3, a and b). In contrast, the structure of cellulosic aerogels is made up of a fibrous network (Figure 3, c and d). A further difference is the preparation process, as it involves the dissolution of an already present solid material (cellulose I) which is then regenerated by an appropriate antisolvent such as water or ethanol. For most of the inorganic and organic aerogels an appropriate non-polymeric precursor compound is dissolved in a suitable solvent and the formation of colloidal particles is initiated by adding a reactant (and catalyst).

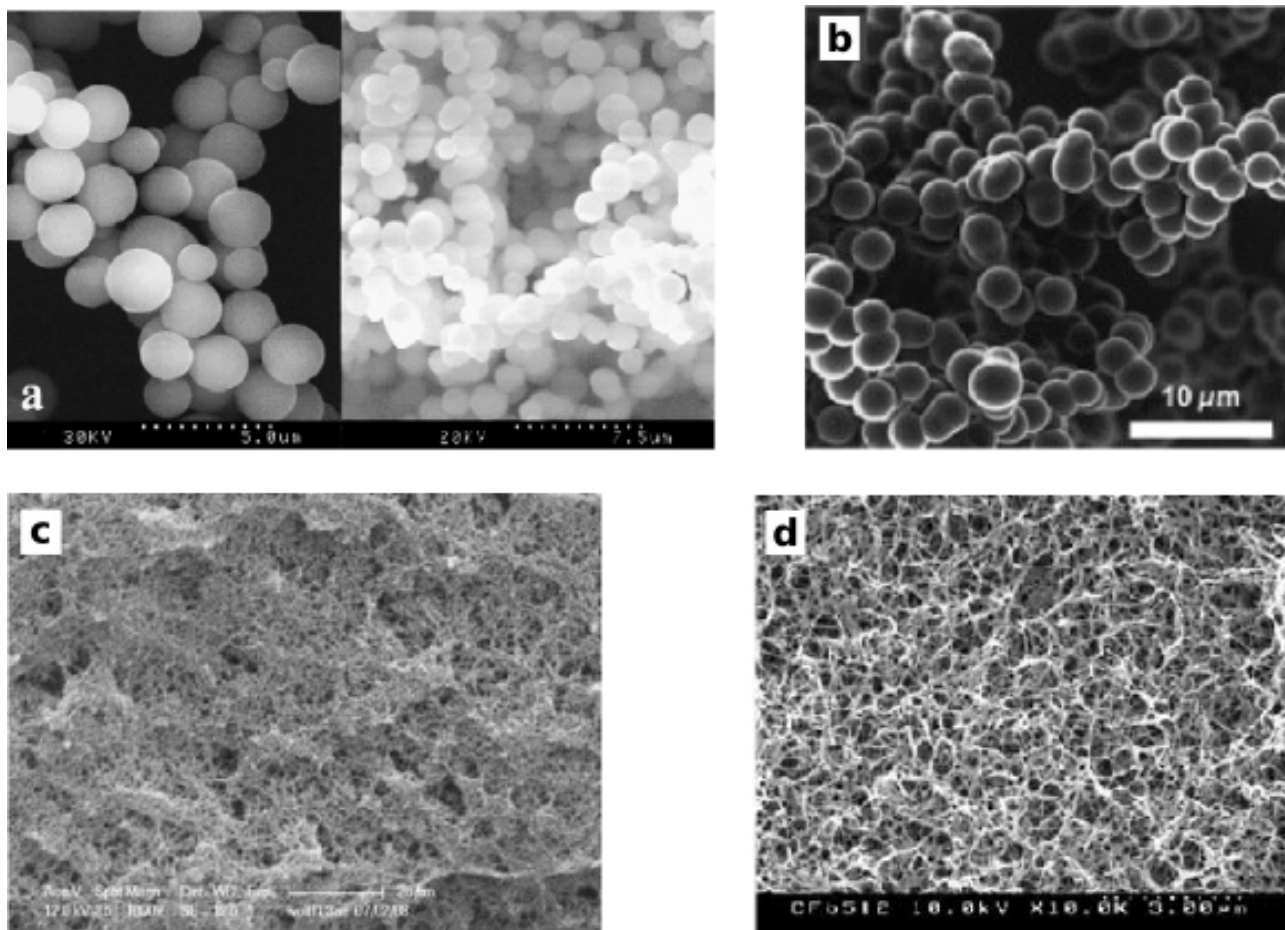


Figure 3: Structures of various aerogels. a: SEM images of silica aerogel (Soleimani Dorcheh and Abbasi 2008), b: SEM image of resorcinol-formaldehyde based aerogel (Al-Muhtaseb et al. 2003), c: cellulosic aerogel from NMMO (Liebner et al. 2009), d: freeze dried cellulosic aerogel (Jin et al. 2004)

Innerlohinger et al. (2006) used N-methylmorpholine N-oxide monohydrate ($\text{NMMO} \cdot \text{H}_2\text{O}$), an environmentally benign direct cellulose solvent used in the Lyocell process by Lenzing AG. Soon afterwards Liebner et al. (2008) also produced cellulosic aerogels via the NMMO route. However, cellulose / NMMO mixtures tend to undergo side reactions under the Lyocell processing conditions between 100°C and 130°C due to the oxidative nature of $\text{NMMO} \cdot \text{H}_2\text{O}$. To reduce the extent of side reactions radical scavengers are commonly used such as propyl gallate. With regard to processing of cellulose derivatives carrying acidic groups via the Lyocell approach, Liebner (2011a) used recently N-

Benzylmorpholine-N-oxide (NBnMO) as an additional stabiliser. The latter had been developed in Rosenau's group as a sacrifice substrate to convert N-hydroxymethylmorpholine-derived carbonium-imminium ions into non-chromophoric, stabilised reaction products. As acidic conditions favor the dehydration of N-hydroxymethylmorpholine to the above-mentioned carbonium-imminium ions, NBnMO is hence an effective means to process acidic cellulose derivatives via the Lyocell process too. Other recent research also investigated ionic liquids as a means of dissolving cellulose (Swatloski et al. 2002) and different groups such as that of Aaltonen and Jauhiainen (2009), or Budtova (Sescousse et al. 2011) managed to produce aerogels via this route.

A somewhat problematic point about some cellulose II aerogels is that depending on the way of preparation, some aerogels can suffer substantial shrinking during drying even if scCO_2 is used (Figure 4), while cellulose I aerogels are at least not subject to this phenomenon to such a large extent. A strong shrinking was also reported by Fischer et al. (2006) for cellulose acetate/ polymethylene polyphenylpolyisocyanate based aerogels and was attributed to the poor compatibility of CO_2 and cellulose which the authors backed up by incompatible Hildebrand solubility parameters. However, this explanation was not further discussed. It has to be considered, that Hildebrand parameters neglect the polar character of cellulose and other solubility measures like Hansen parameters would probably be more appropriate for comparisons. Unfortunately, investigations of this problem were not found by the author.

Considerable shrinkage of aerogels from various cellulose sources produced via the NMMO route was also reported by Liebner et al. (2009). The issue of shrinkage is

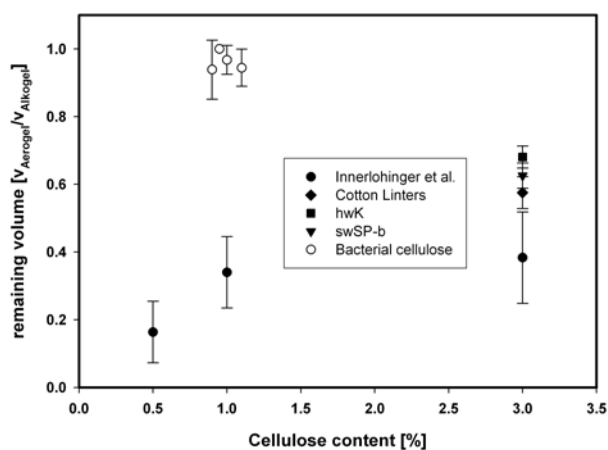


Figure 4: Remaining volume of various cellulosic aerogels after supercritical drying vs. cellulose content (Liebner et al. in preparation)

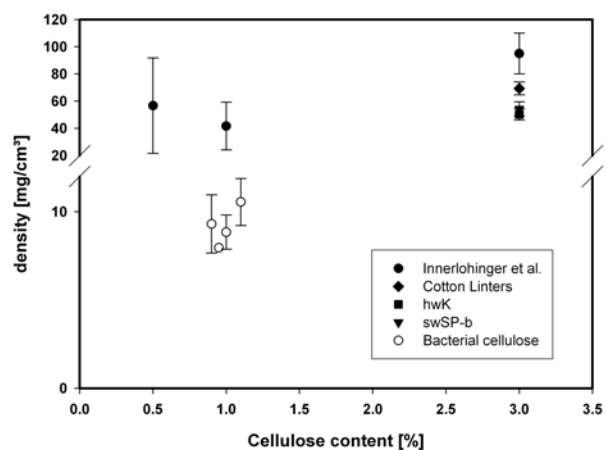


Figure 5: Density of various cellulosic aerogels vs. cellulose content (Liebner et al. in preparation)

particularly troublesome as the reason behind it is still unknown. Interestingly, cellulose based aerogels such as from bacterial cellulose (BC) (Maeda 2006, Liebner 2010) or nano- and micro-fibrillated plant cellulose prepared via mechanical disintegration of native cellulose sometimes followed by an enzymatic treatment (Pääkkö et al. 2008) are not subject to this phenomenon (Figure 4).

Unlike the hitherto reported cellulose II based aerogels, BC aerogels do not require preceding dissolution and subsequent regeneration because the gel structure is already created during the cellulose biosynthesis by bacteria. Considering their very low density of down to 5 mg cm^{-3} (Figure 5) and almost zero shrinkage (Figure 4) which is most remarkable at such low cellulose contents, they represent the youngest and highly intriguing member of the aerogel material class.

Bacterial cellulose

The 1,4- β -glucan cellulose is the most abundant polymer on earth. The biggest part of the annual cellulose production is provided by plants, where cellulose is an integral constituent of the cell wall. Besides plants, also some bacteria (e. g. *Acetobacter xylinum* strains) are capable of generating cellulose as a byproduct of their metabolism (Klemm et al. 1998).

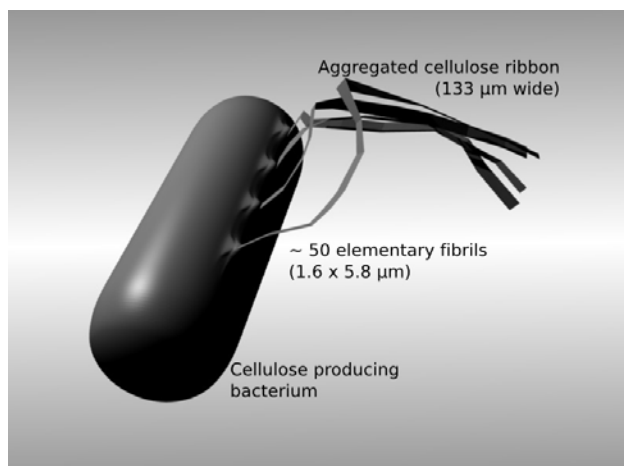


Figure 6: Schematic of cellulose synthesis by *Acetobacter xylinum* (adapted from Brown et al. 1976)

Even though a relatively simple linear polymer, it took about one century of scientific research to elucidate the structure of cellulose and even today basic research is conducted to fully understand the intermolecular interactions and forces and mechanisms leading to cellulose dissolution. Nowadays it is known that cellulose molecules can aggregate to different crystalline domains and amorphous regions. A comprehensive review on the structural investigations on cellulose is given by Zugenmaier (2008). The present work uses bacterial cellulose (BC) as a base material; some particularities of this special kind of cellulose will be discussed in this chapter.

Origin

There is a variety of bacteria that have the ability to produce cellulose as a byproduct of their metabolism including stems of *Pseudomonas*, *Achromobacter*, *Alcaligenes*,

Aerobacter, Rhizobium, Agrobacterium, Azotobacter and Acetobacter (Deinema and Zevenhuizen 1971). Hungund and Gupta (2010) isolated a cellulose producing Enterobacter stem from rotten apple - a result that also impressively demonstrated the abundance of such bacteria in our environment. The comparably recent discovery of Enterobacter by Fujiwara et al. in 2001 is a good indicator that the field of cellulose producing bacteria is yet not fully explored. However, today in bacterial cellulose production for commercial as well as scientific purposes, various types of Acetobacter are used.

In contrast to plant cells, bacteria produce cellulose as an extracellular substance. After a complex biosynthetic pathway (e. g. Ross et al. 1991), cellulosic elementary fibrils are released into the environment through a number of spinnerets lined up along the bacterium and eventually they aggregate to ribbons typical for bacterial cellulose (Figure 6). An interesting aspect of cellulose generation by bacteria is cell division as it contributes to the microstructure of the cellulose pellicle. The cellulose synthesizing sites are also duplicated during cell division (Brown et al. 1976) and when the process is finished, mother and daughter cell are connected to the same cellulose ribbon which contributes to interconnections and entanglement in the framework.

As soon as a suitable cellulose producing stem is isolated, it needs to be given the right conditions in terms of temperature, oxygen supply and nutrients to be able to synthesize cellulose. As a nutrient source, several growth media exist, one that is very common is a mixture introduced by Schramm and Hestrin in 1954 and thus referred to as "Hestrin Schramm growth medium", which consists of 20 g l⁻¹ glucose, 5 g l⁻¹ peptone, 5 g l⁻¹ yeast extract, 1.15 g l⁻¹ citric acid monohydrate and 6.8 g l⁻¹ Na₂HPO₄ · 12 H₂O. But also other growth media exist, including the very easy to prepare Waterman's sucrosebeer solution which is prepared by "adding 40 grams of sucrose to a liter of beer" (Mühlethaler 1949).

The fermentation itself can take place in different types of fermenters. The most straightforward construction is static cultivation involving a vessel filled with an adequate growth medium (Figure 7). The medium is inoculated with bacteria which, after a lag phase, begin to produce cellulose. A problem with static cultivation is that the possible maximum thickness of the cellulose pellicle is limited by the fact that the aerobic bacteria reside on top of it and the growth medium has to diffuse through an increasingly thick barrier of cellulose. To overcome this problem, agitated fermenters can

be used providing better contact with oxygen and growth medium. However, the oxygen abundance leads to increased survival of non cellulose producing mutants (Cel-) (Valla and Kjosbakken 1982). Also, shear forces caused by agitation influence the product on a microstructural level, by reducing the content of α type crystallites and yields lower E moduli for sheets pressed from this product, though a higher water holding capacity compared to static cultivation was determined (Kunihiko et al. 1998). Similar results with respect to structural properties were obtained for cellulose produced in rotary disk fermenters (Figure 8) by Krystynowicz et al (2002), but the generation of Cel- mutants could be inhibited by adding ethanol. In more recent approaches, the continuous production of BC is investigated, for example in the horizontal lift reactor (HoLiR) concept. This device slowly pulls a BC sheet off a basin with a growth medium allowing to produce BC sheets of variable length and adjustable thickness (Kralisch et al. 2010). Generally, the bacteria type will determine the best fermenter/growth medium combination. The bacteria merely produce cellulose as a byproduct that helps them to survive in certain conditions. *Acetobacter xylinum*, on which the presented results

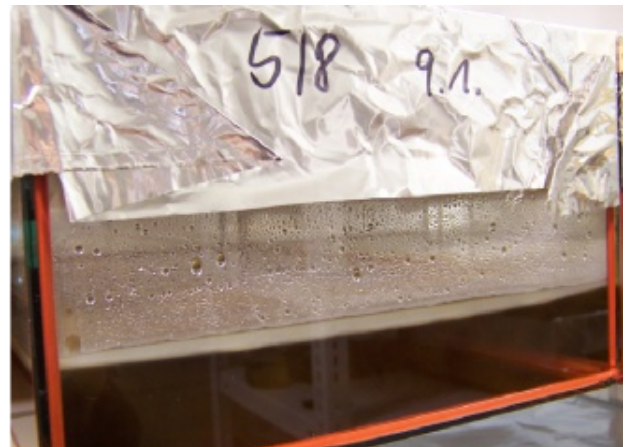
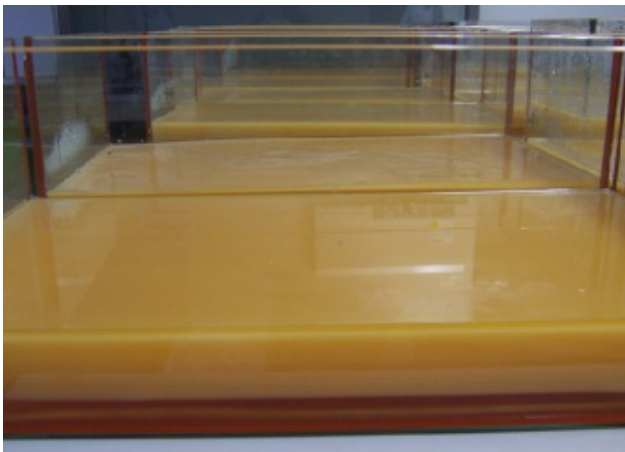


Figure 7: Static cultivation vessels for bacterial cellulose (images kindly provided by fzmb Bad Langensalza).

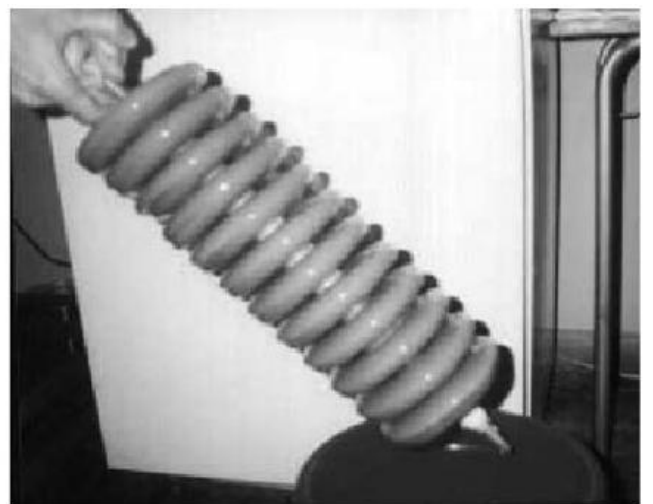
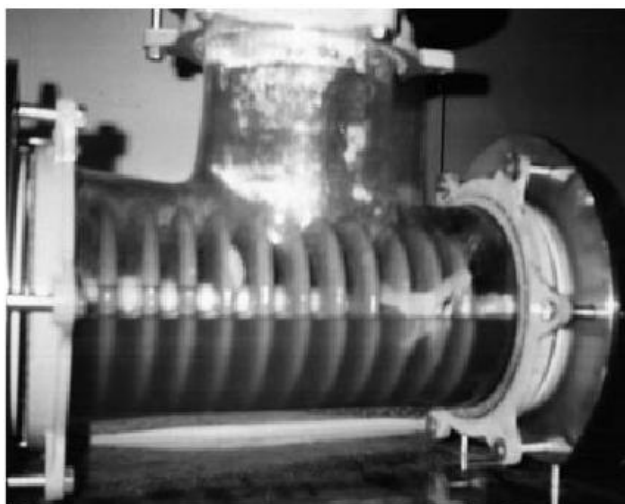


Figure 8: BC cultivation in a rotary disk fermenter (left) and BC on discs after a growing period of 7 days (right) (Krystynowicz et al. 2002)

concentrate, is believed to produce a cellulose pellicle that keeps it floating on the surface for maintaining sufficient oxygen supply. Other bacteria like *Agrobacterium tumefaciens* that are plant pathogens use cellulose for better attachment to plants, similar to the symbiotic *Rhizobium* spp. Another kind - *Sarcina ventriculi* - aggregates to packets and protects itself with a cellulose hull (Ross et al. 1991). The stimulus to produce cellulose comes from the environment and has to be simulated for each of the different strains to achieve satisfying results. Increased occurrence of *Acetobacter xylinum* Cel- mutants in environment with excess oxygen underlines the necessity of the above-said. Other bacteria may behave slightly differently or require completely different conditions.

Properties

Chemically, BC is identical with its plant-produced counterpart. What makes BC a particular kind of cellulose are structural properties and its purity when compared to chemically obtained pulp. Unlike plant-derived cellulose, BC is not compounded with lignin and hemicelluloses, thus no pulping procedure is necessary to obtain cellulose. The growth medium and bacterial residues can be removed by treating with hot aqueous sodium hydroxide and subsequent thoroughly rinsing with water (Hestrin and Schramm 1954). Furthermore, BC fibrils are remarkable when it comes to crystallinity. With a crystallinity index between 65 % and 79 %, BC features higher crystallinities than all plant celluloses (cotton linters: 65 %) and regenerated celluloses (Lyocell: 35 %) (Zugenmaier 2008). The degree of polymerization (DP) of BC seems not particularly high (4000 - 6000) compared to e. g. cotton (up to 15000) or wood (up to 10000). But procedures like pulping or bleaching that need to be applied to other celluloses are not required with bacterial cellulose, thus the DP can be preserved. In contrast, the DP of various pulps drops as low as to several hundreds during the treatment (ibid.).

In addition to parameters like crystallinity and DP, cellulose can occur as an amorphous material and in different crystalline states. Native cellulose usually appears in the thermodynamically not most stable cellulose I state and can be converted to the other modifications by various treatments (Figure 9). The cellulose II structure is typical for regenerated cellulose, but can also be produced by some *Acetobacter xylinum* mutants (Kuga et al. 1993). A further differentiation of cellulose I can be made, when considering the unit cell structure of the crystalline domains. Figure 10 shows the structures of the

monoclinic I β and triclinic I α cellulose crystals (see also Baker et al. 2000). In contrast to cellulose of higher plants, BC and algal celluloses contain a high proportion of I α structures in their crystalline regions (Zugenmaier 2008).

Taking into account the comparably low density of cellulose crystallites (below 1.6 g cm⁻³, Sun 2005), the material features some extraordinary mechanical properties.

Obviously, the direct measurement of mechanical properties on BC fibers is a complicated task. Molecular simulations predict the E modulus to be about 145 GPa which was validated by Raman spectroscopy on tunicate cellulose whiskers (Šturcovà et al. 2005). Measurements by atomic force microscopy also yielded similar results (Iwamoto et al. 2009). The same technique applied to single BC fibers yields lower E moduli of 78 ±17 GPa (Guhados et al. 2005) which takes into account non-crystalline regions.

Higher values (of about 114 GPa) for the same material measured with Raman spectroscopy were reported by Hsie et al. (2008). Even though, significantly lower than the E modulus of pure crystallites, these are still impressive values, when considering that E-glass fibers feature a similar E modulus of 72.5 GPa but their density (2.58 g cm⁻³) is significantly higher (Callister 2001). The random orientation of fibers in a sheet reduces the overall E modulus even further to 28.3 GPa and 33.3 GPa for agitated and static culture, respectively (Kunihiko et al. 1998) - a result also demonstrating the influence of fermentation technique.

Other interesting properties of bacterial cellulose are its water holding capacity and biocompatibility. Kunihiko et al. (1998) reported that BC pellicles from static and

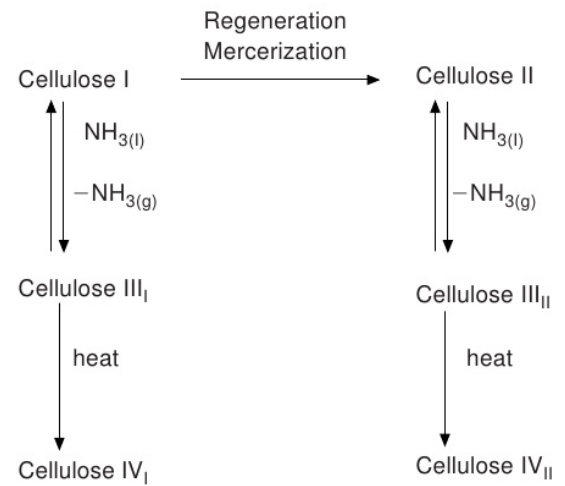


Figure 9: Scheme of the cellulose polymorphism and the interconversion of the different modifications (from: O'Sullivan 1996)

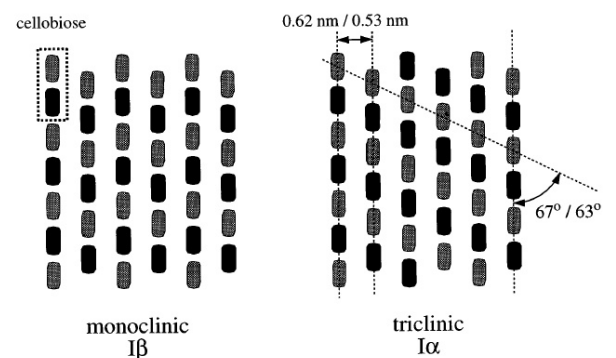


Figure 10: A schematic diagram of the differences between the monoclinic and triclinic forms of cellulose I. Each rectangle represents a single glucose unit, with a pair of glucose units constituting the true crystallographic repeat, cellobiose. In the monoclinic form, the cellobiose units stagger with a shift of a quarter of the c axis period, while the triclinic form exhibits a diagonal shift of the same amount. Two spacings and angles are given, the first referring to the (100) face and the second to the (010) face of the triclinic crystal. (from: Baker et al. 1997)

agitated cultures were able to hold 45 g and 170 g water per gram cellulose, respectively. In vivo biocompatibility tests on rats were carried out by Helenius et al. (2005). The results indicated very good assimilation of BC by the surrounding tissue.

Applications

The most common application of BC is the indigenous dessert nata de coco (Figure 11) produced in the Philippines by fermenting coconut milk in vessels resembling static cultivation fermenters with cellulose producing bacteria (Iguchi et al. 2000). With the increasing investigation of BC properties also new possibilities of BC utilization became available, though still limited to high value products by the comparably high price of the material. Compounded with ordinary cellulose fibers, BC can be used to produce speciality papers with particularly high strength and toughness (Mormino and Bungay 2003). The porous structure of BC allows for impregnation with various compounds and subsequent pressing to sheets. The unnecessary of a defibrillation process preserves the good mechanical properties of the fibers and allows for the production of stronger and stiffer composites than with microfibrillated cellulose (Nakagaito et al. 2005). Gindl and Keckes (2004) also pointed out in their work on cellulose acetate butyrate composites reinforced with BC that the small diameter of cellulosic fibrils - if provided a proper interface to the matrix - can significantly contribute to the overall mechanical properties of the composite. A prominent use of pressed BC sheets are headphone membranes developed by Sony Corporation together with Ajinomoto (US Patent 4742164) making use of the good dynamic mechanical properties of this particular cellulose.

A variety of other applications in composite materials exists, but BC shows also great potential in medical applications. The biocompatibility allows bacterial cellulose to be used as a scaffold for tissues. Svensson et al. (2005) showed the potential of BC as a



Figure 11: Nata de coco (Anonymous 2008)



Figure 12: Microbial cellulose dressing applied on a wounded hand (from: Czaja et al. 2006).

scaffold for cartilage tissue. For this particular application not only biocompatibility plays an important role but also that the material used has similar mechanical properties to the tissue it acts as a scaffold for. Similar results could be obtained for tissue engineered blood vessels by Bäckdahl et al. (2006), a particularly interesting application, since BC can be directly grown in the appropriate tubular shape (Putra et al. 2008). Another promising field of application are wound dressings. BC can be grown in arbitrary shapes and sizes to cover any wound with one single sheet which is particularly an advantage when it comes to burns that can cover large areas (Figure 12). And the high water holding capacity can provide moisture to the wound (Czaja et al. 2006). Since BC does not have any antimicrobial properties per se, it has to be loaded with appropriate agents. The procedure of precipitating silver nanoparticles into bacterial cellulose matrices to produce antimicrobial wound dressings was performed by Maneerung et al. (2008) and described as “surprisingly simple”.

Further applications of BC include their use as thickening agents and texturizers for food and paint. It is also used as a dietary additive in food (Vandamme et al. 1997) due to its indigestibility which may boost its use in industrialised countries (nata de coco, e.g.). Ultra-lightweight bacterial cellulose aerogels which can be obtained by scCO_2 -drying of respective alcogels at only 40°C and 80 bar (Liebner et al. 2010a) were recently shown to be promising materials for controlled release applications due to their quantitative rewettability and high dimensional stability throughout the drying process (Haimer 2010).

From alcogel to aerogel

No matter, whether an alcogel is obtained from sol-gel chemistry, a stable dispersion (nanofibrillated plant cellulose) or by biological synthesis as in the case of BC, the most crucial step on the way of becoming an aerogel is the replacement of the antisolvent filling the voids of the solid network structure by a gas. Essentially this means that the antisolvent has to change its aggregate state from liquid to gas. This change can be achieved directly by

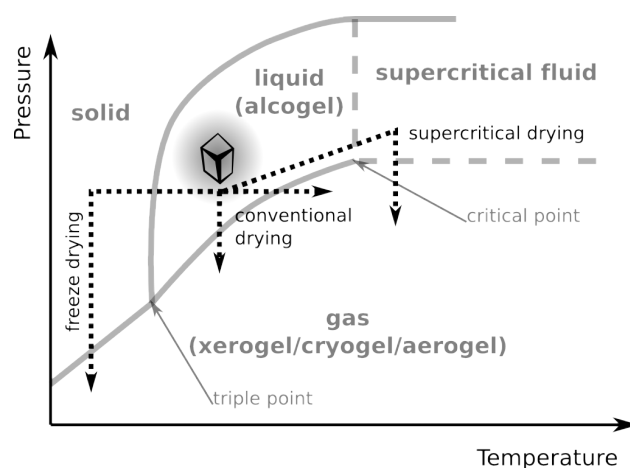


Figure 13: Phase diagram for an arbitrary liquid used as an aerogel solvent and the possible pathways from liquid to gaseous state.



Figure 14: Effects of conventional drying on the shape of bacterial cellulose blocks. Right: swollen BC gel. Left: BC gel after drying for several days at ambient conditions.

evaporation or by taking “detours” via the solid or supercritical states. Figure 13 depicts these three possibilities whose advantages and disadvantages will be discussed in this chapter.

The very first experiments, as reported by Kistler (1931), showed that conventional drying by evaporation of the solvent led to

“shrinkage so great that the effect upon the structure may be profound”. And the effect is indeed profound, when considering the deformation the gel undergoes upon conventional drying under ambient conditions (see Figure 14).

Evaporation of a wetting solvent from the gel creates a liquid/gas phase boundary inside the gel's pores with capillary forces acting on the pores at an angle depending on the wetting properties of the system (Figure 15). Most physics textbooks only analyze the vertical component of the capillary forces to explain why a wetting liquid rises in a capillary. The horizontal components are often neglected because the walls of the capillary are considered strong enough to withstand them. However, in the case of aerogels, the “capillary walls” (i. e. the solid fraction of the gel) are very thin and the horizontal components of the capillary forces are sufficient to deform them irreversibly. The end product of a gel dried by solvent evaporation is referred to as xerogel and is in the case of BC a strongly deformed version of the respective alcogel's shape (Figure 14). Thus other drying techniques have to be considered in order to maintain the dimensional features of the alcogel.

Kistler (1931) avoided the removal of the solvent by evaporation and converted it from liquid to gas by taking the detour via its supercritical state. While this transition still features big changes of density, no phase boundaries are present, thus no capillary forces can develop and the gel's structure can be preserved. However, this process exposes the gel to comparably harsh conditions particularly in terms of temperature – the critical point of ethanol is at 241° C and 63 bar. Such conditions are known to lead to unwanted side reactions during the drying of silica aerogels (Estella et al. 2007).

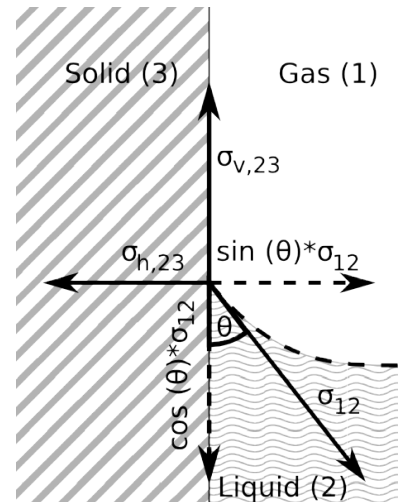


Figure 15: Stresses caused inside a capillary by a liquid (stress between gas and solid is not considered).

Another drawback are dangers coming purely from the fact of using hot pressurized flammable liquids like ethanol. Water as an alternative would be comparably safe, however even higher pressures and temperatures (374° C and 220 bar) are needed to reach the critical point. The high corrosivity of supercritical water (Shaw et al. 1991) is another factor that would considerably increase the costs of an apparatus capable of working with this medium.

Another possibility that avoids a liquid/gas phase boundary is freeze drying, where the solvent is frozen and then transported out of the gel via sublimation. This process is interesting as it requires comparably simple equipment and allows to influence the structure of the finished product by drying parameters like freezing speed and temperature (Jin et al. 2004). Though, while the freeze-drying technique elegantly avoids capillary forces, it also introduces the problem of the liquid to solid phase change of the solvent. Typically, a solvent undergoes volumetric change during solidification and exerts stress on the material surrounding it. Hüsing and Schubert (2005) concluded that crystallisation of the solvent could damage gel networks and a solvent with a low thermal expansion coefficient and high sublimation pressure should be used to maintain the shape of the gel throughout the drying procedure.

More recent research also investigates the possibilities of ambient pressure drying aiming at preserving the gel structure not by avoiding liquid/gas phase boundaries but by strengthening the structure itself so that it can withstand the forces acting on it. Another approach is to change the contact angle between solvent and gel bulk material to directly affect capillary forces (e. g. Hærid et al. 1994).

For the present investigations another drying technique was chosen, namely extraction of the solvent with supercritical CO₂. This method – even though not the simplest one – features rather moderate conditions and yields fully rewettable aerogels with no shrinkage when applied to BC (Liebner et al. 2010a).

Drying with supercritical CO₂

For the laymen mostly prominent as an effective greenhouse gas, carbon dioxide has had mainly negative publicity. However, from a technological point of view CO₂ features very interesting properties; it is non-toxic, non-flammable, abundant and cheap. Considering the comparably low temperature and pressure needed to reach the critical point (31.1° C, 73.8 bar), the gas has gained increased attention as a working medium in a broad

variey of processes already in the late 1970s and early 1980s (McHugh and Krukoni 1994). Figure 16 gives an overview of some technical relevant CO₂ based processes.

The technique as used in the present work to convert BC lyogels into aerogels is actually an extraction of ethanol by means of scCO₂, however will be referred to as "drying", as the end product of interest is

not the extracted ethanol, but the "dry" aerogel. A schematic of the process is given in Figure 17 which shows a p-x,y diagram for the binary system CO₂/ethanol at 40° C. The diagram shows the dew and bubble lines enclosing a region where the two compounds are not miscible and the system separates into a liquid and a gaseous part. At the intersection of the due and bubble lines lies the critical point of the system (marked P_c) at a somewhat higher pressure than that of pure CO₂. Since the drying process is run at constant temperature, it is possible to demonstrate all its stages in a single diagram. Point A depicts the bacterial cellulose gel soaked with ethanol when it is placed in the autoclave. During pressurization of the autoclave above the critical point (A to B), the CO₂ concentration is also increasing. The actual drying step is preformed by flushing the autoclave with fresh CO₂ which slowly removes the ethanol from the system at constant

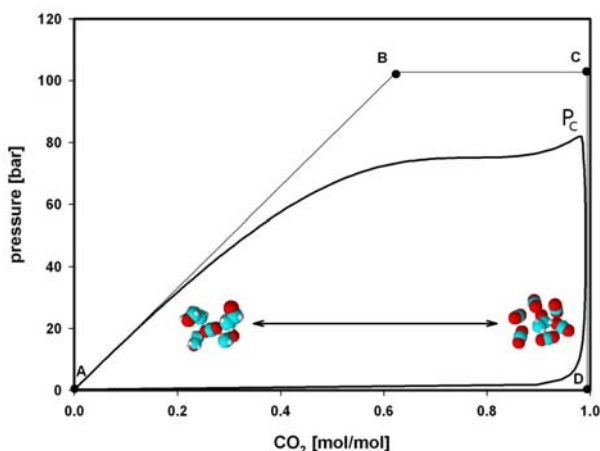


Figure 17: Phase equilibrium for CO₂/ethanol mixtures at 40° C and process steps of drying aerogels with supercritical CO₂. P_c indicates the critical point. A: ethanol swollen gel. B: CO₂/ethanol mixture in pressurized autoclave. C: ethanol replaced by supercritical CO₂ after flushing the autoclave. D: autoclave depressurized, gel filled with gaseous CO₂. (adapted from Haimer 2010)

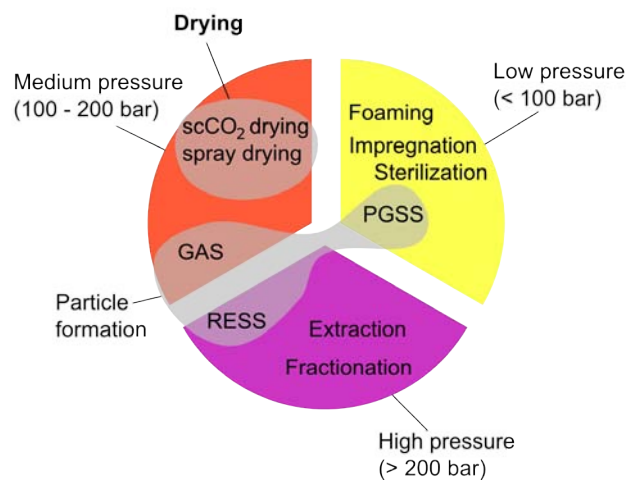


Figure 16: Non exhaustive listing of CO₂ based processes. GAS: Gas Anti Solvent, RESS: Rapid Expansion of Supercritical Solutions, PGSS: Particles from Gas Saturated Solutions

pressure until only CO₂ is present (shift from B to C). The last step (C to D) is the depressurization of pure CO₂ to atmospheric pressure. At this final stage, care has to be taken that depressurization happens at a rate that allows the heating system of the autoclave to compensate for the cooling caused by the Joule-thomson effect. If the CO₂ reaches a subcritical temperature, it will turn liquid and the phase boundary problem of conventional drying will affect the end product.

One remarkable point about the process is that even though pressurization into the supercritical region takes several minutes, no phase boundary due to evaporating solvent progresses into the gel. The principle of the formation of an CO_2 -expanded liquid phase under subcritical conditions and of the flushing step with scCO_2 is shown in Figure 18. In the beginning (Sketch I) when the gel is subject to ambient conditions, it is possible that some solvent evaporates, however this state only lasts over a very short period of time when the gels are taken out of the solvent bath and placed onto the rack in the autoclave. The effect can also be further minimized by putting the rack above a bath with the appropriate solvent that will saturate the atmosphere around it. During the comparably long period of pressurization, CO_2 partly dissolves in the solvent forming a CO_2 -expanded ethanolic phase (Sketch II). During this time the volume of the solvent in the gel increases forcing some of it out of the pores. Additionally, only very little ethanol can evaporate into the gaseous CO_2 phase since the cylinder pressure of approximately 60 bar is reached quickly. These effects ensure that the liquid/gas phase boundary never enters the gel and thus does not destroy the pore structure. During this period also some of the expanding solvent will penetrate into the frit below the gel, which does not greatly affect the gel if pure solvents are used. However, as soon as substances are precipitated into the gel by CO_2 as the antisolvent, some will precipitate between gel and frit making them stick to each other. This circumstance does not only render the removal of dried aerogels more difficult. When measurements on the aerogel's surface are carried out (e. g. FTIR), one has to keep in mind that the surface that faced the frit may easily have characteristics different from the other surfaces.

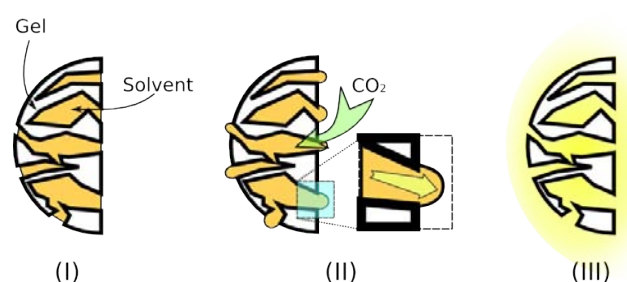


Figure 18: Schematic presentation of scCO_2 -drying: I: ambient pressure, gel is swollen with solvent. II: pressure is below critical pressure, gaseous CO_2 diffuses into solvent. III: pressure above critical pressure, mixture of CO_2 and solvent evenly distributed in autoclave.

Finally, after the critical pressure was reached, no phase boundaries are present between ethanol and CO_2 (Sketch III) and the drying step as described before can be performed.

Summarizing it can be stated that the conversion from a swollen alcogel to aerogel is best conducted by means of drying with supercritical CO_2 , as it is the most gentle of all procedures that preserves best the structure of the material.

Experimental part

Materials

Bacterial cellulose (BC) specimens were kindly provided by fzmb Bad Langensalza. The material was obtained from a static cultivation of *Gluconacetobacter xylinum* AX5 wild type strain on Hestrin-Schramm's medium for 30 days at 30° C. The BC layer was then cut in cuboids measuring 120 mm x 20 mm x 20 mm or 40 mm, boiled in 0.1 M aqueous NaOH at 90° C three times (each 20 min) and finally rinsed with deionized water for 24 h. The cleaned samples were received already engaged in ethanol, however prior to drying at least three further solvent exchanges with ethanol abs. were performed to eliminate all remaining water.

The chemicals used can be found in Table 1. All chemicals were used as received.

Table 1: Chemicals used, abbreviation in text, type and origin

Substance	Abbr.	Type and origin
Ethanol abs.	EtOH abs.	Ethanol absolute, p.a., Merck
Dimethylsulfoxide	DMSO	Rotipuran $\geq 99.8\%$, p.a., Roth
Acetone	-	Rotipuran $\geq 99.8\%$, p.a., Roth
Terephthalic acid	TA	98%, Sigma Aldrich
Cellulose acetate	CA	39.8% Acetyl cont. Mn ~ 30000 , Sigma Aldrich
Tetraethyl orthosilicate	TEOS	98%, Sigma Aldrich
Soluble glass	SG	Sodium silicate solution, Sigma Aldrich
Dilactide	DL	3,6-Dimethyl-1,4-dioxane-2,5-dione, Sigma Aldrich
Methoxyhydroquinone	2-MHQ	2-Methoxyhydroquinone, 98%, Sigma Aldrich
Carbon dioxide	CO ₂	Biogon C, Linde

Methods

Drying with supercritical CO₂ (scCO₂)

Two apparati were available for scCO₂ drying, the main difference being that one was equipped with a windowed autoclave that allows the observation of ongoing processes inside it. This apparatus will be referred to as “small”, while the other one will be referred to as “big”, as it featured the bigger autoclave. The apparatus used was chosen according to the needs of the drying process.

The small apparatus was equipped with a custom made 43.5 cm³ cylindrical autoclave with two opposed sapphire windows. In order to control the temperature in the autoclave, heated water was pumped through an aluminum encasing. A schematic can be seen in Figure 19. Other parts are described in Table 2.

The drying itself was performed by placing wet gels in the autoclave and then pressurizing it first to cylinder pressure and then to the desired operating pressure.

After this pressure had been reached, an outlet valve was opened to a small extent so that the autoclave pressure remained constant. This way fresh CO₂ slowly replaced the CO₂-expanded ethanolic phase. Depressurization after the drying itself was performed through the same outlet valve. Neither solvent, nor CO₂ recovery were possible with this setup, as no separators were available.

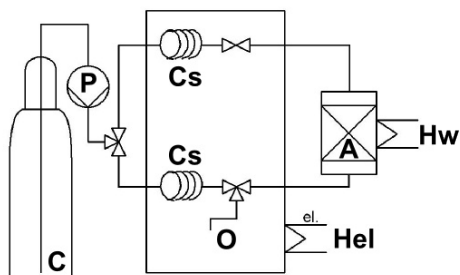


Figure 19: Small apparatus for scCO₂. C: CO₂ tank, P: Pump, A: autoclave, O: outlet valve, Cs: spirals of capillary tubes for CO₂ preheating, Hel: electrical heating, Hw: water heating for autoclave, rectangle around Cs and O: insulated heating chamber.

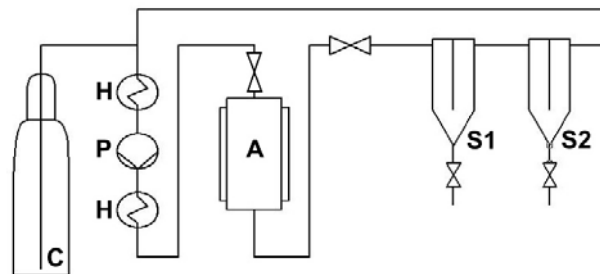


Figure 20: Schematic of the supercritical fluid formulation apparatus SF1. C: CO₂ tank, H: heat exchangers, P: pump, A: autoclave, S1 and S2: separators.

The big apparatus was a supercritical fluid formulation apparatus SF1 (Separex, France, see Figure 20 for schematic). Wet gels were placed on a rack with steel or glass frits inside the preheated 0.5 l

Table 2: Parts used for the small apparatus

Part	Type and origin
CO ₂ pump	HPLC mini pump by TSP, USA
CO ₂ thermostat	Julabo LC4
Water pump	Haake B12 pump with heating
Water thermostat	Haake N6

autoclave which was then first slowly pressurized to cylinder pressure and then to the operating pressure at a flow rate of 2.5 kg h⁻¹ CO₂. This setup allowed flushing of the autoclave through two cyclone separators (40° C and 60° C, both at cylinder pressure) which made it possible to recycle CO₂ in the process as well as to recover the solvent.

As mostly ethanol-engaged gels were dried, the drying conditions were usually 100 bar and 40° C. The drying (fresh CO₂ flushing) time depends on the dimensions of the gel and is known from previous experience to be approximately 1 h for samples with 1 cm edge length. Samples used in the present work did not deviate from this edge length, thus it was possible to use the 1 h drying time for all of the samples. However, for gels that were dried from DMSO or acetone, the operating pressure was usually 150 bar (due to a higher critical point of the corresponding mixtures with CO₂). Also, gels engaged in DMSO were let to equilibrate at operating pressure for 10 min before flushing and the flushing itself was performed for 2 h because of the worse miscibility of DMSO with CO₂.

Mechanical testing

Compressive tests were performed on a Zwick/Roell Z010 universal testing machine. The testing speed was 2.4 mm min^{-1} , displacement was measured with the cantilever, force with a 50 N load cell. For the determination of the Poisson's ratio a MINITRON camera and videoextensometer software were used. Prior to testing, specimens were kept under defined conditions (20° C , 65 % r. h.) for at least 24 h and only taken out of closed vessels shortly before testing.

The evaluation of the obtained stress strain curves was performed directly in the test software testXpert. In order to perform evaluations that were not possible with testXpert, the obtained stress strain curves were exported to text files and processed with custom scripts written in the Python programming language.

The E modulus was determined as the slope of a regression line through the linear elastic region of each stress-strain curve, boundaries were adjusted individually for each curve. Yield strength

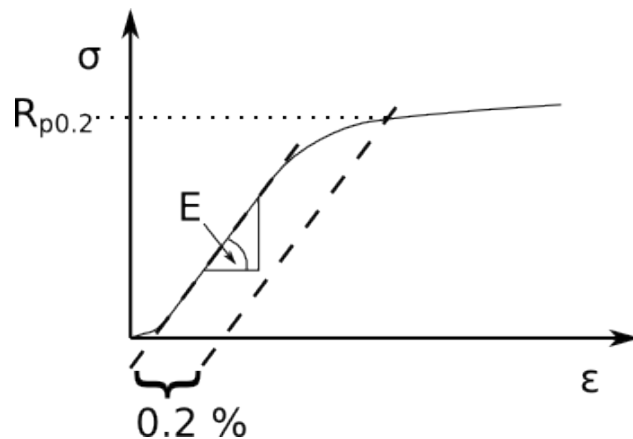


Figure 21: Schematic for the determination of the E modulus under compression and yield strength.

($R_{p0.2}$) was defined as the stress at 0.2 % plastic deformation (Figure 21), as this is a value used commonly in scientific and engineering practice.

Microscopy

Scanning electron microscopy (SEM) was performed on a Philips XL 30 ESEM. Up to six samples were mounted with electrically conductive double sided sticking pads on an aluminum plate and then sputtered with gold. Spot size, electron acceleration and magnification were chosen depending on what combination delivered best results.

Polarized light microscopy was performed on a Leica DM4000M microscope, images were recorded with a digital camera (Leica Microsystems Wetzlar GmbH, Germany).

Further measurements

Porosity and specific surface area were measured by N_2 sorption experiments at 77 K on a Micrometrics ASAP 2020 analyzer.

Thermoporosimetry results were obtained from DSC curves measured on a Mettler-Toledo DSC 30 with o-xylene.

ATR-FTIR was performed using a Vertex 70 (Bruker) with a DLaTGS detector. The ATR - sampling device was the PIKE MIRacle with a ZnSe crystal. Spectra were collected in the wavenumber range between 4000 cm^{-1} and 600 cm^{-1} , at a spectral resolution of 4 cm^{-1} and a zerofilling of 2 was applied.

Modification of BC gels

Soluble glass (SG) modification was carried out in aqueous solutions of SG (1 % wt. and 5% wt.). The solutions were prepared and water engaged BC specimens were placed over night in them. After 3 solvent exchanges to ethanol samples were dried with scCO_2 . Three different temperature levels - room temperature, 50° C and 75° C - during loading were investigated. 50° C and 75° C were achieved by placing the specimens engaged in the respective solutions in closed Schott flasks in a Memmert UFE 400 drying oven.

Tetraethyl orthosilicate (TEOS) modifications were carried out in 250 ml glass beakers under constant stirring for 24h. 5 solutions were prepared according to Table 3 and BC gels previously engaged in ethanol were added to them. The fifth solution contained 3-chloropropyltrimethoxy

Table 3: Modification solutions for TEOS modifications.

Sample	TEOS (ml)	CPTMS (ml)	HCl (ml)	Ethanol (ml)
1	40	0	80 (pH 1)	80
2	40	0	80 (pH 4)	80
3	66.7	0	66.7 (pH 1)	66.7
4	57.1	0	114.3 (pH 1)	28.6
5	20	20	80 (pH 1)	80

silane (CPTMS) as an additional precursor. After the reaction time, the gels were taken out and washed with EtOH abs. Three further solvent exchanges with EtOH abs. were performed prior to drying with scCO_2 .

Dilactide (DL) and the appropriate amount of catalyst (Zinc Octoate, 4.43 mg ml^{-1} in ethanol) were readily soluble in scCO_2 . Thus the compounds could be precipitated into the gel by dissolving them in the supercritical fluid and then depressurizing with an aerogel engaged in it. 1h was given the system to equilibrate in the supercritical state, i. e. to dissolve DL and diffuse into the pores of the aerogel. Loading was performed in the small apparatus at concentrations of 3.5, 7 and 10 mg ml^{-1} DL per autoclave volume (the volume of the solid fraction of dried aerogels was neglected). To induce the polymerization of DL, the samples were either heated in the drying oven at 160° C or in the autoclave of the big apparatus at 160° C and pressurized to 300 bar with CO_2 . For stirring the stirrer corresponding to the SF1 apparatus was used. Disregard the heating

type and loading concentration, all loaded aerogels had the same density after the heating step only slightly above the density of untreated aerogels.

2-MHQ modifications had to be carried out in ethanol because the amounts soluble in scCO_2 were too small to produce a series with different loading concentrations. Several loading baths in falcon tubes, each containing 20 mg ml^{-1} 2-MHQ were used. To precipitate the compound prior to scCO_2 drying, it was let react with different oxygen and ammonia sources, as described in Table 4. Pure oxygen was introduced into the solution by carefully bubbling the gas into the falcon tubes out of a pressure cylinder for 15 min. Except for the ammonium nitrate version, an excess of ammonia (3 ml 5% aqueous NH_3 solution per falcon tube) was added to all solutions.

Table 4: Types, duration and temperature of BC modification with MHQ in ethanol

Reactants	Modification duration	Temp.
NH_4NO_3	1 month	RT
$\text{H}_2\text{O}_2, \text{NH}_3$	3 days	50°C
O_2, NH_3	3 days	50°C
O_2, NH_3	3 hours	50°C

The two last modifications involved the precipitation of terephthalic acid (TA) and cellulose acetate (CA). The modification procedure itself was very similar for both compounds and the first steps are sketched in Figure 22. For TA, DMSO was used as a solvent, for CA - acetone. The precipitation took place directly during scCO_2 drying with CO_2 as the antisolvent. Finally, the TA modified aerogels were heated to 160°C at 300

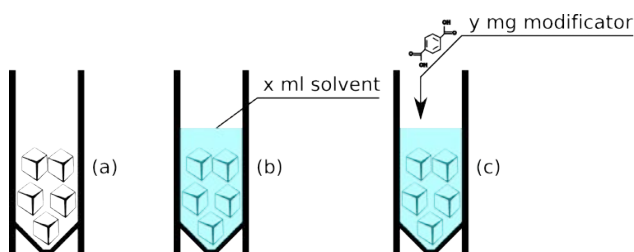


Figure 22: Procedure for modification of BC gels. (a): Gels already soaked in an appropriate solvent are placed in a falcon tube. (b): Falcon Tube is filled with solvent to a defined mark of $x \text{ ml}$. (c): $y \text{ mg}$ of the modification agent are added to the solvent; the concentration of the loading bath is thus given by $y/x \text{ mg/ml}$.

bar CO_2 pressure in the big apparatus immediately after drying in order to induce polycondensation of the compound. While neither TA, nor CA are soluble in pure scCO_2 , the solvents used (DMSO and acetone, respectively) acted as entrainers and lead to clogging of filters in some cases.

Mechanical properties under uniaxial compression and structure of bacterial cellulose aerogels

Results

Mechanical properties under uniaxial compression

Under uniaxial compression bacterial cellulose aerogels behave very much as expected for a cellular solid. A sample stress-strain curve is given in Illustration 23. The region within the first 3% of strain (Figure 23, (a)) is considered to be an adjustment region where mainly irregularities in specimen geometry are evened out. This region is relatively broad. Due to the fragility of the bulk material on the one hand and high strength of BC fibers on the other, it turned out to be very difficult to prepare specimens with

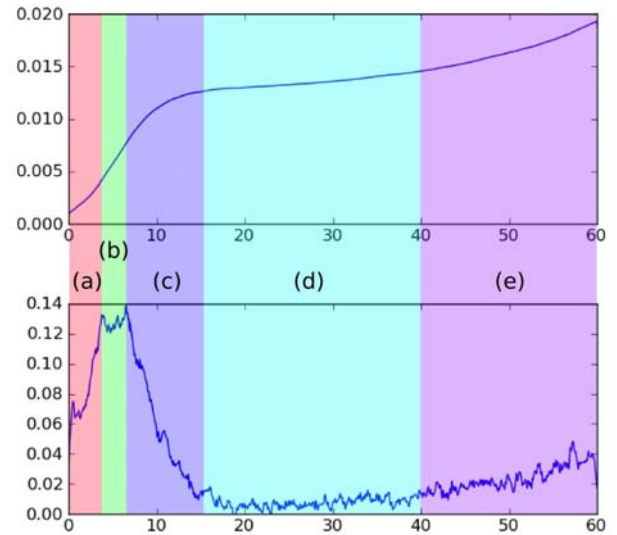


Figure 23: Sample stress-strain curve of a BC aerogel and its first derivative (Savitzky-Golay smoothed for representation purposes). (a): adjustment region, (b): linear elastic deformation, (c): non linear elastic and plastic deformation (d): plateau region, (e): densification.

exact geometry. The other regions of the stress-strain curve correspond very well to the work on cellular solids conducted by Gibson and Ashby (1982). At around 5% strain the stress-strain relation is linear with a more or less constant slope. This region is used to calculate the E modulus under compression (Figure 23, (b)). After that, the curve's slope drops (Figure 23, (c)) which is believed to happen due to non-linear elastic deformation (cell wall buckling) and beginning plastic deformation (cell collapse). Subsequently the stress-strain curve shows a so called plateau region (Figure 23, (d)) which is of considerable size (from 15% to 40% strain). Eventually the material densifies (Figure 23, (e)) leading to an exponential increase of stress over strain. The overall smoothness of the curve and the absence of a peak after the linear elastic region indicate that the material deforms in a ductile way on the microscale in contrast to brittle foams and silica aerogels.

Compression tests along the three spatial directions perpendicular to each other (orientation indicated in Figure 24, upper left) revealed that the material was transversely isotropic. Though, unlike a unidirectionally fiber reinforced composite, it

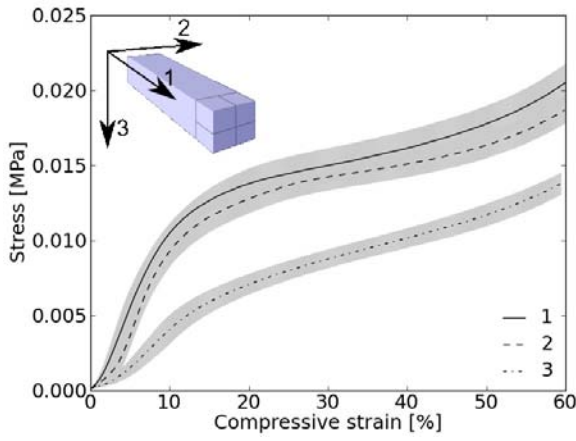


Figure 24: Stress-strain curves under compression for three different testing directions, charge fzBC 27. Direction 3 is assumed to be the direction of growth. Lines are mean values of stress, grey background represents standard deviation, sample size $n=5$.

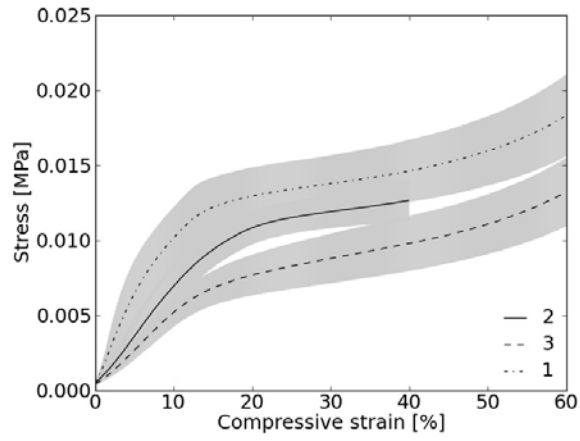


Figure 25: Stress-strain curves under compression for three different testing directions, charge fzBC 30. Direction 3 is known to be direction of growth. Lines are mean values of stress, grey background represents standard deviation, sample size $n=5$.

Table 5: Mechanical properties of charges fzBC 27 and fzBC 30 under uniaxial compression. Standard deviation given in brackets, sample size $n=5$.

	Experiment 1		Experiment 2		
Direction	E [MPa]	$R_{p0.2}$ [kPa]	E [MPa]	$R_{p0.2}$ [kPa]	ν [-]
1	0.149 (0.023)	7.05 (0.55)	0.189 (0.056)	6.88 (1.36)	0
2	0.140 (0.036)	7.84 (1.06)	0.078 (0.016)	6.86 (1.57)	0
3	0.057 (0.007)	4.65 (0.48)	0.059 (0.013)	4.88 (1.25)	0

features two directions with higher stiffness and strength and lower values for the third direction being the growth direction of the bacterial cellulose perpendicular to the interface between of culture medium and air. This can be seen qualitatively in Figure 24. The E moduli for the two stiffer directions 1 and 2 were determined to be 0.149 MPa and 0.140 MPa, respectively. The third direction featured an E modulus which was some 60 % less (0.057 MPa). Strength behaved similarly being 7.05 kPa and 7.84 kPa for the two stronger directions 1 and 2, respectively, and 4.65 kPa for the weaker direction 3 (see Table 5 for details on mechanical properties).

A second test aimed at measuring the Poisson's ratio, however on a different BC charge with known growth direction. The Poisson's ratio was determined to be 0, disregard loading or viewing direction. Interestingly, the anisotropy from the previous test was not fully reproducible. While two directions – one perpendicular to the growth direction and the growth direction itself – yielded similar results to the previous test with measured E moduli being 0.189 MPa and 0.059 MPa, respectively, the third one clearly deviated with an E modulus of 0.078 MPa (see Table 5 for detailed values). Figure 25 demonstrates this fact, as well as the observed higher scattering of results. The results presented here are clearly below the values reported by Liebner et al. (2009), for cotton linters based

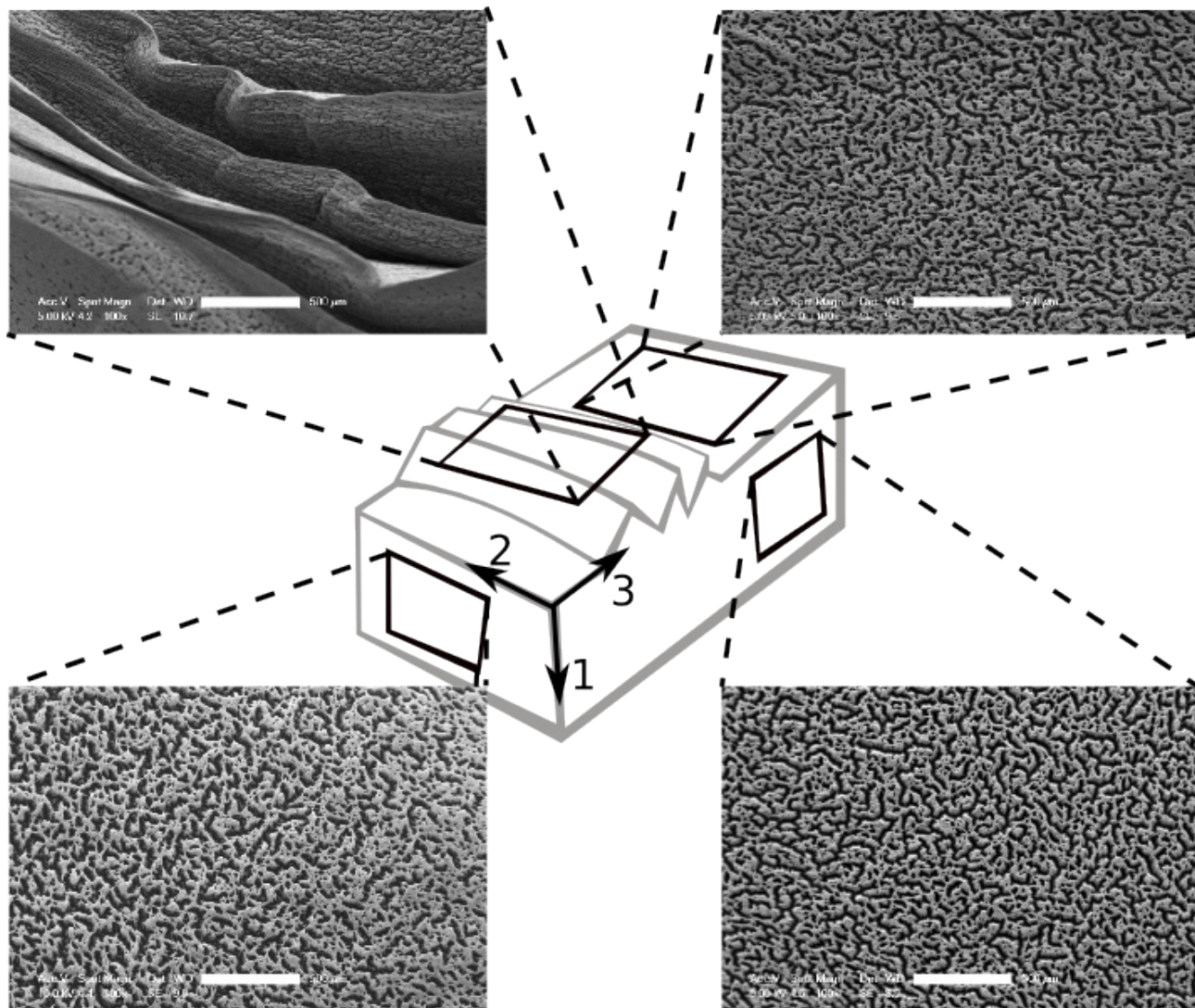


Figure 26: SEM images of three different planes of a bacterial cellulose aerogel. All images were taken from the same sample which is sketched in the center. Growth direction is direction 3. Bottom left image taken at 10 kV acceleration voltage, others at 5kV. Magnification: 100x, length of white bar corresponds 500 μm .

cellulose II aerogels (E moduli of 9.9 MPa and 42.9 MPa for 3% and 6% cellulose content, respectively). But considering the far higher density of cellulose II aerogels (up to 70 mg ml^{-1}), the comparison of the materials is not straightforward.

Structural investigations

After tearing the samples apart prior to observation in the SEM, on some samples discontinuities were already visible to the unaided eye. However, the discontinuities were not homogeneously distributed throughout the sample. As can be seen in Figure 26, in a rectangular sample with an edge length of approximately 1 cm, the lamellar structures are only present in a region, while the rest of the sample has very similar appearance disregard the viewing plane. A close-up of a discontinuity (Figure 27) reveals that the material becomes increasingly dense towards it and there seems to be an overall orientation of cellulose fibrils along the discontinuity, which can not be seen in the more homogeneous regions (Figure 28). If more discontinuities are present, as in

Figure 29, their spacial distribution is regular with gaps of approximately 800 μm .

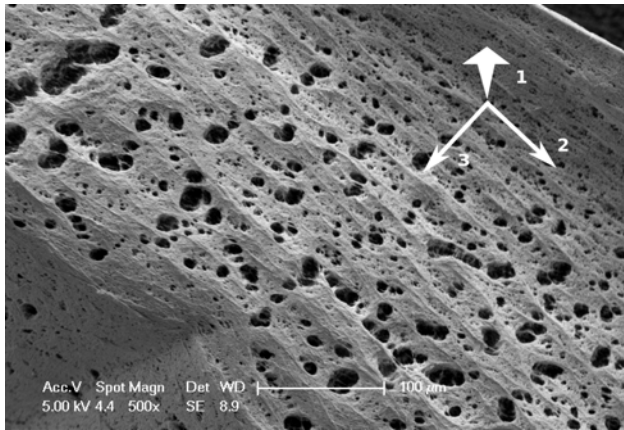


Figure 27: Close-up of a discontinuity on fzBC 27. Viewing direction along direction 1 (arrow pointing out of image plane).

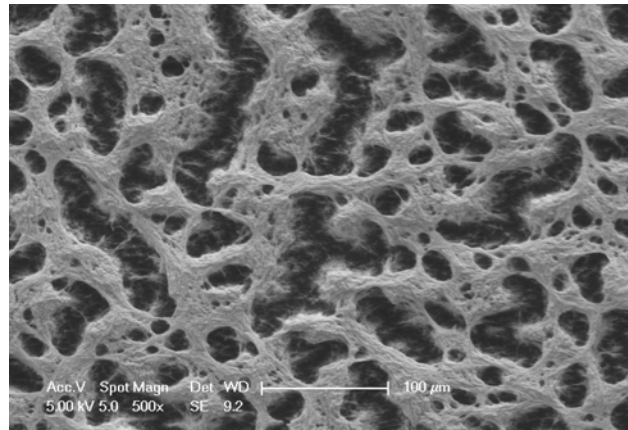


Figure 28: Close-up of BC structure in a region where no discontinuities are present.

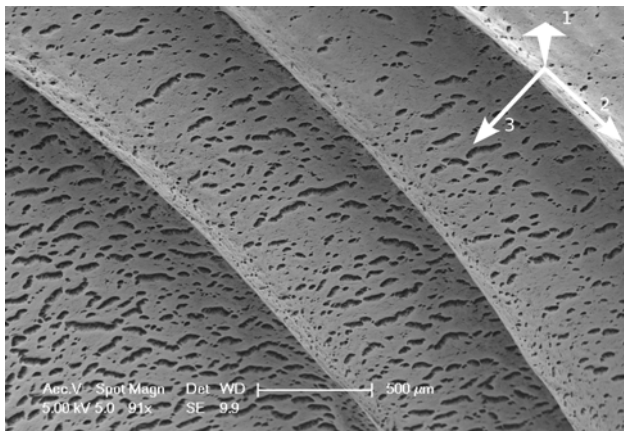


Figure 29: SEM Picture of fzBC30. Arrows at top right indicate directions as used in mechanical testing. Direction 1 points out of the image plane. Direction 3 is the growth direction.

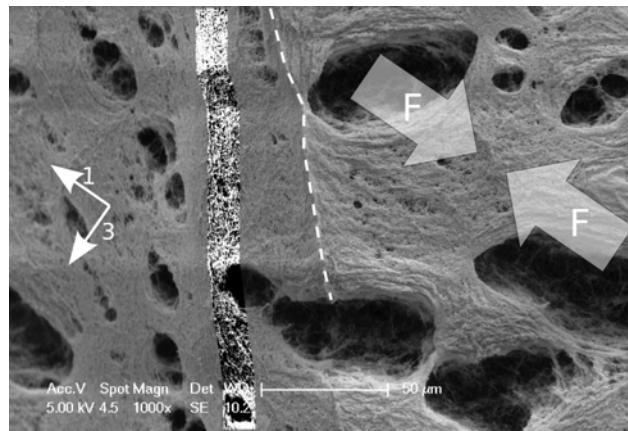


Figure 30: SEM image of fzBC27 after compression along direction 1. Bold Arrows marked F indicate loading direction. Dashed line indicates buckled density discontinuity. For better visibility of buckling, part of the image was altered in brightness and contrast.

The effect of the discontinuities on the material's reaction to an applied load can be seen in Figure 30. Although samples were compressed up to 60% strain, only very little deformation is visible on the microscale. The visible deformations look not so much like collapsed pores but rather like layers in the material that shifted to one side. It is noteworthy that these shifting planes are almost perpendicular to the lamellar discontinuity, even though the loading direction had an angle of approximately 45° with respect to the discontinuity. Also, this phenomenon seems not to appear throughout the whole sample (compare the parts to the left and right of the discontinuity in Figure 30).

Discussion

Structure

A stratified structure of BC was already proposed by Hestrin and Schramm (1954) and was visualized in more recent works (e. g. Bodin et al. 2007, Iguchi et al. 2000). The

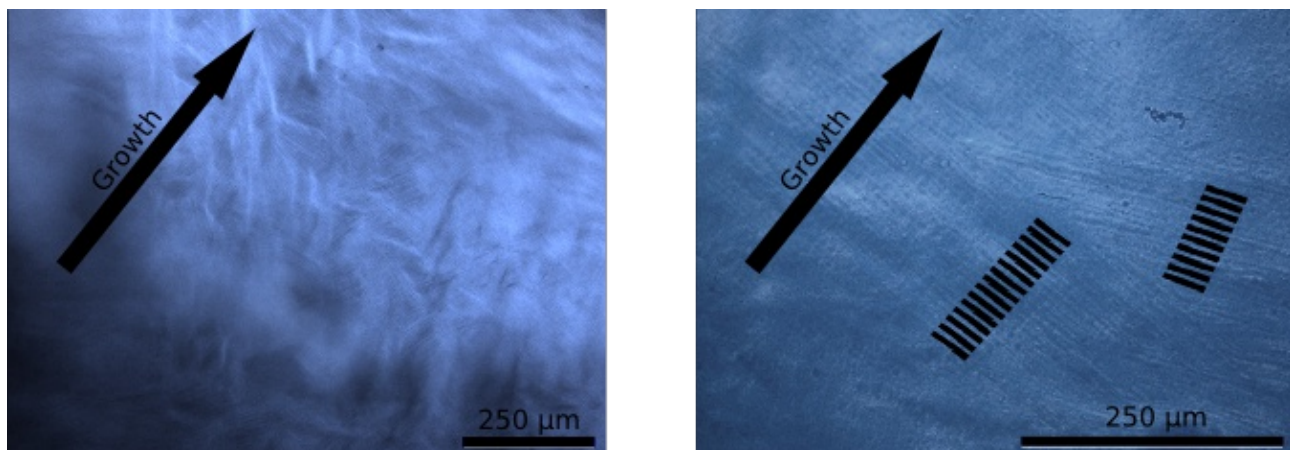


Figure 31: Water swollen frozen BC samples under cross polarized light. Black arrow indicated growth direction. Left: randomly oriented lamellar structures. Right: regions of uniformly oriented lamellar structures. Striped rectangles indicate direction.

structural influence on mechanical properties can be seen in the work by Nakayama et al. (2004) who reported a very low elastic modulus under compression (0.007 MPa) when loading BC hydrogels along their growth direction and a significantly higher value of 2.9 MPa perpendicular to it, however measured under a tensile load. The fact that the E modulus for hydrogels is about ten times smaller than that of aerogels in a similar loading situation suggests that the removal of solvent either results in hornification or allows the formation of additional hydrogen bonds between cellulose fibrils. Both phenomena can lead to higher stiffness.

While the BC aerogels used in the present work show the expected anisotropy with respect to mechanical properties (see Figure 24, Figure 25 and Table 5), their structure looks different from what would be expected. No regular layering was observed in the samples, which the author believes to be due to the fact that samples used in the present study were not freeze-dried. Figure 31 shows an image from polarized light microscopy of water swollen frozen samples which revealed lamellar structures with a spacing of approximately 10 μm - the same range as seen in the work by Bodin et al. (2007). Obviously, during freezing the expanding water pushes the cellulose network apart along its weak direction. This supports scCO_2 drying as the most gentle means of obtaining aerogels from BC.

In scCO_2 dried BC aerogels discontinuities in terms of density and fiber orientation were discovered that were not omnipresent (Figure 26). The interval of these discontinuities was shown to be approximately 800 μm which is some decimal powers bigger than the distance between layers as seen in other publications. The origin of these irregularities remains unclear. If the origin is biological, they must be triggered by something that

happens periodically over a period of the growth process. Assuming a BC layer thickness of 4 cm after 30 days and linear growth, such events would have to happen almost twice a day. However, this is only a very rough estimate, as the growth of BC is known to be non-linear (Iguchi et al. 2000) and neither the exact final BC layer thickness, nor the horizontal position where the sample was taken from the freshly grown BC layer are known to the author.

Another remarkable attribute of the scCO₂ dried BC are holes in the range of tenths to a few hundreds μm (Figures 26, 27, 28, 29 and 30). These holes represent the portion of the total porosity of BC with the largest pore diameter, visible by means of SEM. Evidence of such a BC morphology in literature is not known to the author. The cellulose producing bacteria are far too small to be the cause of the blank spaces. It could be that the voids are artifacts from the drying process. However, the full rewettability of BC dried by means of scCO₂ indicates that the process is very gentle and at least does not alter the morphological structure in an irreversible manner such as by collapsing pores. Investigations prior to drying by means of cross polarized light microscopy did not indicate the presence of such large pores. But considering that water engaged BC is almost transparent (due to the similar refractive indices of cellulose and water), it should not be excluded that the structures were present but not visible with the given setup.

Mechanical properties

It is interesting to see that even at high compressive strains (60%), BC aerogels show very little deformation on the microscale. Larger holes in the material do not seem to be affected at all and only buckling seems to take place in some regions of the specimen (Figure 30). In general this behavior supports well the measured zero-Poisson's ratio. This is in good agreement with Sescousse et al. (2011) who also reported a zero Poisson's ratio for cellulosic aerogels from EMIMAc-solution similar to cork. Considering the very low solid volume fraction of an aerogel, it is indeed possible that under compression the struts of the material are rearranged in a way that no macroscopic change of lateral dimensions during linear elastic deformation can be observed. Thus a zero Poisson's ratio seems plausible.

Figure 30 shows a specimen with a discontinuity running diagonally through it that was observed in the SEM after being subjected to compression tests. Obviously, the influence

of the discontinuity is severe; even though the loading direction on the pictured specimen was at an angle of approximately 45° with respect to the orientation of the discontinuity, the direction of the breaks is almost perpendicular to the discontinuity and not to the loading direction. Additionally, deformation can only be seen on one side of the specimen. These results indicate that as soon as discontinuities deviate from their usual orientation perpendicular to growth direction, the material fails partly due to shearing and not cell collapse on the microscale. The deviation in terms of orientation with respect to loading direction seems thus to be the main source of scattering of results observed during mechanical testing.

In general, the findings confront the further processing of BC aerogels with considerable problems. The discontinuities are obviously not homogeneously distributed throughout the material, nor equally oriented. Another unknown is their size; the tearing pattern suggests that they are rather planar than line-shaped (Figure 26), but the area they occupy can not be estimated, as only surfaces can be observed by SEM and it was not possible to produce a number of sufficiently thin slices to get an impression of the extension.

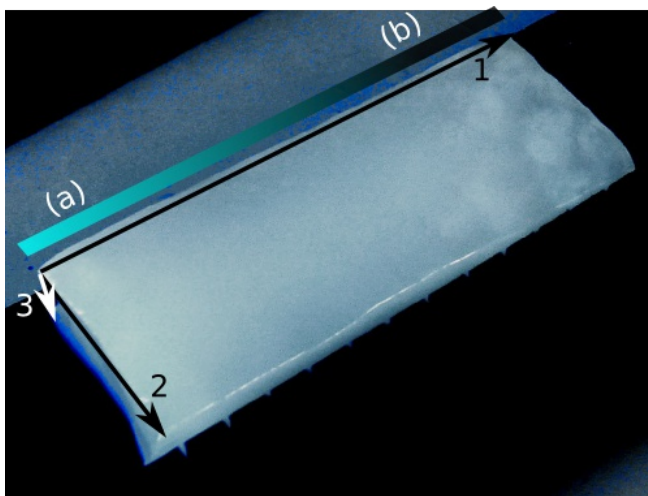


Figure 32: Gels, charge fzBC30. Left: swollen gel, $\sim 120 \times 40 \times 20$ mm, direction 3 indicates direction of growth. (a): homogeneously appearing section, (b): cloudy spots visible. Right: dried aerogels, $\sim 40 \times 10 \times 10$ mm. Homogeneously white appearance.

It is currently not possible to assess the presence and orientation of discontinuities without destroying the specimen. Dried specimens appear uniformly opaque white (Figure 32, right). In the more transparent swollen gels, some less transparent regions can be seen (Figure 32, left (b)), however due to their rather rounded appearance they do not resemble the structures seen in SEM images.

Polarized light microscopy of water swollen frozen specimens could deliver some indicators on the orientation of structures in BC, however results show that only some

rather small regions with uniform orientation are present (Figure 31, right) and other regions feature a completely random orientation (Figure 31, left). Even though the expansion of the freezing water introduces irreversible damage to the material, it may still serve as a comparably simple tool to identify the growth direction of BC by investigating a small sample of a BC piece in question.

Modification of bacterial cellulose aerogels

Introduction

It can be seen from mechanical testing of unmodified BC aerogels that the material is very fragile. Handling of the material with bare hands is almost impossible as it will stick to skin and removal will inevitably introduce mechanical damage. Even the careful use of tweezers usually leaves marks on the specimens. Apart from counteracting this fragility, the ability to adjust the properties of aerogels is desirable in order to be able to tailor them to a certain application. A very widespread material where this is possible, is foamed polyurethane. Patel et al. (2008) tested polyurethane foams with densities ranging from 90 mg cm^{-3} to 320 mg cm^{-3} and Young's moduli from 0.08 MPa to 151.4 MPa, respectively. The high specific Young's modulus ($\sim 15 \text{ MPa cm}^3 \text{ g}^{-1}$, along the stiffer directions) of bacterial cellulose aerogels compared to that of polyurethane (~ 0.9 to $10.3 \text{ MPa cm}^3 \text{ g}^{-1}$ as can be calculated from data provided by Patel et al. (2008) for the lowest densities tested) makes it a promising starting point for the development of an ultra-lightweight material.

There are also other reasons that make BC gels interesting for modification; their open porous structure allows diffusion through the material and the very low cellulose content (1% vol.) leaves sufficient space to use such materials for tissue engineering, slow release applications, filter systems or catalyst supports. Furthermore, particularly the highly crystalline bacterial cellulose can be engaged in a broad variety of solvents without being defibrillated, allowing an even broader variety of substances to be introduced into the BC gels.

scCO₂ is an elegant means that makes it possible to load BC aerogels with compounds via different pathways. Compounds that are soluble in scCO₂ can be dissolved in the supercritical fluid and homogeneously distributed in a porous matrix. Subsequent depressurization into the subcritical region will lead to the precepitation of the

compound. Other substances, that can not be dissolved in scCO_2 but in solvents that can be extracted with scCO_2 , can be precipitated directly during the drying procedure. In this case, scCO_2 will act as an antisolvent.

Most literature dealing with influences of certain process parameters on bacterial cellulose production communicate the results of investigations aiming at improved BC yields. According to Krystynowicz et al. (2002), BC in an agitated culture features a smaller index of crystallinity than BC from static cultivation. Liebner et al. (2011b) give an overview of possible modifications of BC during fermentation through the addition of various substances to the culture medium that will be integrated into the BC network. However, in the present work a different route, namely the modification after fermentation is considered. The post-fermentation modification does not only open new possibilities to tailor the properties of BC but is also likely to be more efficient than in-situ modifications when smaller amounts than a whole batch are to be modified.

At first modifications were conducted that aimed at investigating the suitability of various modification agents to be integrated into the established experimental setup (solvent exchange, scCO_2 drying). Afterwards, two substances, namely terephthalic acid (TA) and cellulose acetate (CA) were subjected to more extensive testing.

Two groups of modification agents were considered for the preliminary tests – one was silica, the other carbon based. The idea behind the use of the silicon-based compounds tetraethyl orthosilicate (TEOS) and soluble glass (SG) was to create a stiff inorganic network within the cellulosic network. The carbon based group consisted of dilactide (DL) and 2-methoxyhydroquinone (2-MHQ). The DL was supposed to be thermally processed after loading to polymerize to polylactic acid (PLA) and MHQ to be ammonoxidized to 2,5-diamino-[1,4]benzoquinones that can form semi-ladder oligomers.

Deformation of cellular solids

Thorough investigations of the behavior of cellular solids under mechanical loading were conducted by Gibson and Ashby (1982). They could show that the main deformation mechanism for very lightweight cellular materials is bending of the struts they consist of. This analysis led to the development of a model nowadays referred to as the Gibson & Ashby (GA) unit cell, which predicts the mechanical properties of a cellular material to scale according to a power law (Formula 1).

$$P_{cs}/P_{bulk} = C^*(\rho_{cs}/\rho_{bulk})^n$$

Formula 1: Scaling behavior of mechanical properties as a function of density.

P_{cs}/P_{bulk} are the mechanical properties of the cellular solid and the bulk material, respectively, and ρ_{cs}/ρ_{bulk} is the relative density of the material with respect to the density of the bulk material. C and n are constants, corresponding to the intercept and slope of regression lines in a loglog plot, respectively.

The value of n reflects the material's deformation behavior. An array of struts aligned along the loading direction would yield $n=1$, i. e. the relation would be linear. An analytical solution of the deformation of the GA unit cell predicts $n=2$, assuming that bending of struts is the main deformation mechanism.

In the present work, the power law was not fitted into relative values. Since substances were precipitated into the BC network, the final material was actually a composite. Estimates of the bulk densities do not seem necessary since the not fully crystalline cellulose alone will rather have a density distribution than one particular value that fits the bulk material in all places. The introduction of other substances further complicates the situation. Estimates of mechanical properties lead to similar problems. In addition, considering the time and technical equipment that was needed to estimate the mechanical properties of neat cellulose and the scattering of results (see chapter on bacterial cellulose - properties), this investigation would go clearly beyond the scope of this work.

Porosities

A remarkable feature of BC aerogels is their hierarchical pore size distribution. Results indicate that it is very broad for this material and there is currently no means of measuring the full distribution. Depending on the measurement technique, different pore sizes are determined. It can be seen from BET measurements that a portion of mesopores (2 nm - 50 nm) is present, thermoporosimetry indicates a big amount of macropores (~ 100 nm). However, from SEM imaging it can clearly be seen that the material also contains even bigger voids (up to several 100 μm). Unfortunately, Mercury porosimetry can not be applied because the fragile cellulose framework is crushed during the measurement, which has also been reported for aerogels from plant cellulose (Sescousse et al. 2011), cellulose acetate (Fischer et al. 2006) and polyurethane (Pirard et al. 2003).

Thus it is very hard to make exact statements about the pore related properties. However, results from various porosimetry techniques can allow to obtain information about some textural changes happening due to modifications.

Results

Pretests and TA modification were carried out on charge fzBC27, CA modification on charge fzBC30. Load was applied to the specimens along direction 1 (i. e. perpendicular to the growth direction - see chapter on unmodified BC).

Pretests

The results of the TEOS modification are summarized in Table 6. The modification seems to have had no effect neither on apparent density, nor on mechanical properties, except in two cases – TEOS 2 and 4. However, TEOS 2 was contaminated with aluminum during loading and TEOS 4 samples were covered with a brittle encrusting after scCO₂ drying (note the high standard deviation in Table 6). Porosity measurements by means of thermoporosimetry also support the assumption that the effect of the TEOS modification was negligible since no clear trends can be deduced from the pore sizes. Litschauer et al. (2011) performed similar modifications on cotton linters based cellulose II aerogels and also reported encrustings, however detailed studies on the interactions between TEOS and cellulose are in preparation. From the present results it can be concluded that the mechanical properties are either unaffected or affected in an uncontrollable manner by the performed TEOS modifications.

Results from the other modifications are summarized in Table 7. Temperature had no

Table 6: Mechanical properties and density of TEOS modified BC. Sample size: n, standard deviation given in brackets. PR represents the most frequent pore radius measured with thermoporosimetry.

	E [MPa]	Rp _{0.2} [kPa]	ρ [mg cm ⁻³]	PR [nm]
Reference (n=5)	0.149 (0.023)	7.05 (0.55)	7.8 (0.5)	108.8
TEOS 1 (n=9)	0.177 (0.069)	11.61 (0.95)	7.42 (0.44)	128.5
TEOS 2 (n=9)	0.207 (0.073)	13.88 (3.5)	8.81 (0.56)	114.1
TEOS 3 (n=7)	0.139 (0.057)	10.2 (1.67)	7.61 (0.19)	153.4
TEOS 4 (n=9)	0.203 (0.117)	12.58 (4.02)	18.21 (14.29)	74.2
TEOS 5 (n=9)	0.166 (0.046)	12.03 (1.89)	8.27 (0.28)	78.6

significant influence neither on apparent density, nor on mechanical properties of SG modified BC, thus the results were merged. The SG concentration in the modification bath clearly had an influence on the results with respect to density, stiffness and yield strength.

Both of the carbon-based compounds were confirmed to increase the density and

mechanical properties of the BC aerogels. The DL modification was remarkable due to substantial impact on E modulus despite the very low amount of polylactic acid (PLA) deposited inside the BC aerogel. Higher amounts of polylactic acid are therefore expected to have a strongly reinforcing potential for such porous matrices. However the synthesis of higher molecular PLA would require anhydrous atmosphere and somewhat higher reaction temperatures which both was technically infeasible with the available equipment.

Table 7: Mechanical properties and density of SG, DL and MHQ samples. Sample size: n , standard deviation given in brackets.

	E [MPa]	Rp _{0.2} [kPa]	ρ [mg cm ⁻³]
Reference (n=5)	0.149 (0.023)	7.05 (0.55)	7.8 (0.5)
SG 1 (n=6)	0.228 (0.097)	12.82 (2.53)	12.56 (1.57)
SG 5 (n=6)	0.727 (0.244)	38.46 (1.94)	37.75 (3.35)
DL (n=15)	0.213 (0.083)	11.43 (2.85)	8.00 (0.55)
2-MHQ (n=14)	0.314 (0.107)	16.14 (2.73)	9.31 (1.05)

In general, the two organic compounds DL and 2-MHQ performed better than the inorganic ones as they had a relatively low impact on density but relatively high impact on mechanical properties. For this reason, carbon based chemicals were used in further modification experiments.

Terephthalic acid (TA)

Terephthalic acid was used in an attempt to introduce aromatic groups by thermal condensation reaction with the primary and secondary hydroxyl groups of the cellulose chains. Covalent linking of terephthalic acid in scCO₂ at 160°C in the presence of phosphorous pentoxide was considered as

a means to increase the pyrolytic yield when converting cellulosic aerogels into carbon aerogels. The apparent density of the loaded BC aerogels could be well controlled up to concentrations of about 30 mg ml⁻¹ TA in the DMSO bath. Above this threshold, some samples shrunk during drying (Figure 33, Table 8 for detailed values). Heating the autoclave to 160° C immediately after drying for 2h did not lead to the condensation reactions of TA,

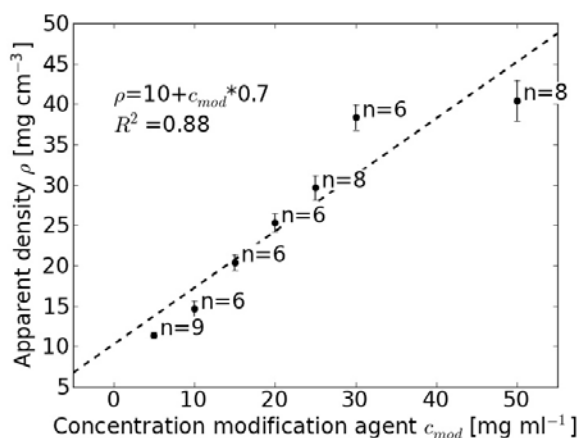


Figure 33: Apparent density of BC aerogels vs. concentration of TA in the loading bath. Full circles and whiskers indicate mean values and standard deviation, respectively. Sample size n is given in the graph for each measurement. Dashed line represents linear regression through data points; slope, intercept and coefficient of correlation are given in the graph.

even when P_2O_5 was added into the autoclave as a reagent to drain the water produced by condensation.

In terms of mechanical properties, no correlation could be established with density or loading bath concentration. The same applies for nitrogen sorption measurements – no dependence of BET results on TA loading can be seen. However, for all samples the specific surface area is low (19-73 $m^2 g^{-1}$), when compared to untreated BC aerogels (210 $m^2 g^{-1}$) (Table 9).

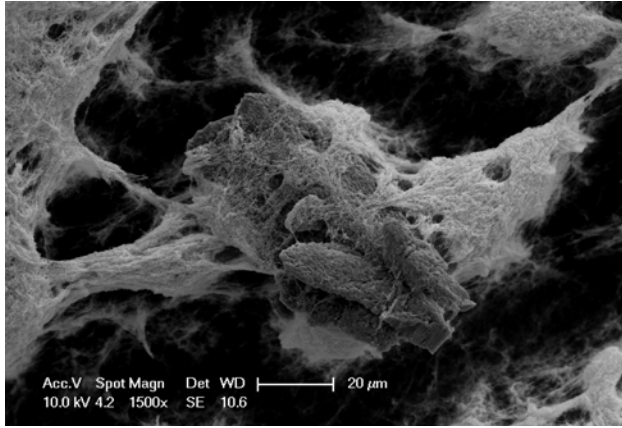


Figure 34: SEM image of a TA particle precipitated in a BC aerogel. Concentration of TA in loading bath: 30 $mg ml^{-1}$

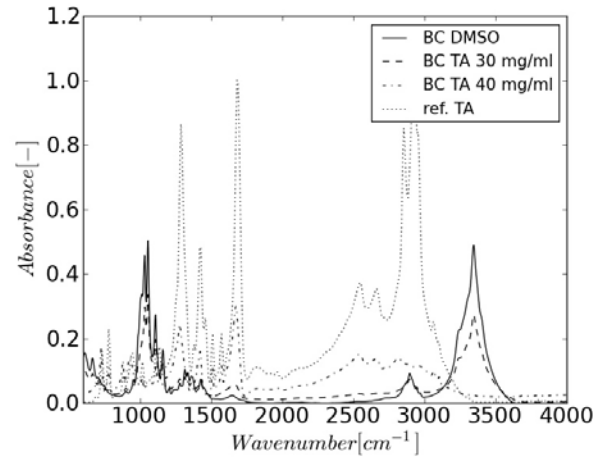


Figure 35: FTIR spectra two BC samples loaded with different amounts of TA compared with those of untreated BC and pure TA, respectively.

Table 8: Apparent densities, mechanical properties and results of BET measurements (specific area, sorbed volume, pore diameters from BET and BJH calculations and the BET constant) for BC aerogels modified with various concentrations of TA in the loading bath. For density and mechanical properties standard deviation and sample size are given in brackets before and after the slash, respectively.

c (TA) [$mg ml^{-1}$]	ρ [$mg cm^{-3}$]	E [MPa]	Rp0.2 [kPa]	Spec. area [$m^2 g^{-1}$]	Sorbed volume [$cm^3 g^{-1}$]	○ pores BET [nm]	○ pores BJH [nm]	c const [-]
5	11.37 (0.40/9)	0.360 (0.090/3)	11.33 (0.58/3)	-	-	-	-	-
10	14.65 (0.95/6)	0.217 (0.077/2)	12.00 (1.00/2)	73	0.15	8	30	84
15	20.40 (0.99/6)	0.301 (0.105/5)	10.80 (2.49/5)	37	0.09	10	8–40	108
20	25.31 (1.17/6)	0.480 (0.056/4)	16.50 (0.58/4)	47	0.08	7	25	60
25	29.64 (1.51/8)	0.305 (0.169/2)	15.50 (0.71/2)	61	0.10	7	25–30	79
30	38.36 (1.59/6)	0.413 (0.125/4)	19.75 (2.22/4)	19	0.05	10	25–30	64
50	40.44 (2.53/8)	0.415 (0.299/2)	13.50 (0.71/2)	-	-	-	-	-

SEM investigations revealed that TA precipitates as small particles in the gel rather than covering the cellulose fibrils in a homogeneous way (Figure 34).

The inhomogenous distribution of TA inside the cellulosic scaffold was confirmed by FTIR as the spectra of some sample segments strongly resembled untreated BC aerogels dried

from DMSO (Figure 35, solid and dashed lines). In contrast, spectra obtained from other samples did not contain any peaks typical for the untreated sample but rather peaks characteristic for pure TA (Figure 35, dotted and dash-dotted lines).

Cellulose acetate (CA)

A strong correlation could be found between the concentration of CA in the loading bath and the apparent density of BC aerogels ($R^2=0.998$, see Figure 36). The intercept of the regression line lies around 10 mg cm^{-3} , which is in good accordance with the apparent density of the unmodified BC aerogel (9.63 mg cm^{-3}).

Mechanical properties also scaled well with apparent density (Figure 37). The E modulus under compression increased by a factor of 15 from 0.235 MPa to 3.561 MPa over the tested density range. Yield strength behaved in a similar manner by increasing from 13 kPa to 123 kPa (see Table 9 for detailed values). It is noteworthy that density and mechanical properties of the unmodified BC aerogel which was swollen in pure acetone were slightly higher than those reported previously in the present work.

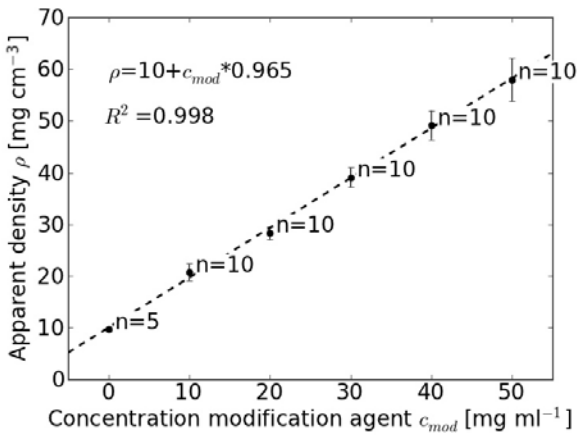


Figure 36: Apparent density of BC aerogels vs. concentration of CA in the loading bath. Full circles and whiskers indicate mean values and standard deviation, respectively. Sample size n is given in the graph for each measurement. Dashed line represents linear regression through data points; slope, intercept and coefficient of correlation are given in the graph.

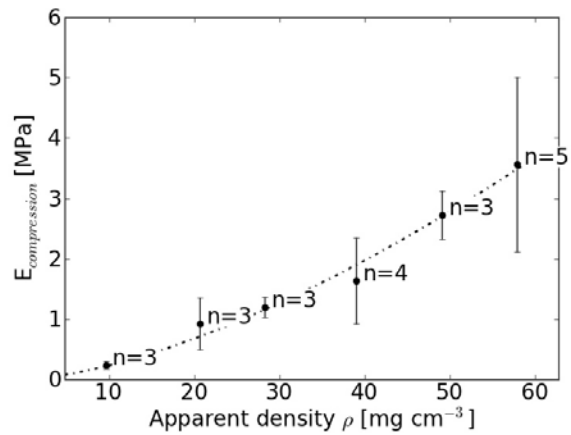


Figure 37: E modulus under compression vs. apparent density of CA modified BC aerogels. Full circles and whiskers indicate mean values and standard deviation, respectively. Sample size n is given in the graph for each measurement. Dashed line represents power law fit through data points. Error of fit: 0.12.

Table 9: Apparent densities and mechanical properties for BC aerogels modified with various concentrations of CA. For density and mechanical properties standard deviation and sample size are given in brackets before and after the slash, respectively

c (CA) [mg ml ⁻¹]	E [MPa]	Rp _{0.2} [kPa]	ρ [mg cm ⁻³]
0 (pure acetone)	9.63 (0.40/5)	0.235 (0.060/3)	0.013 (0.003/4)
10	20.69 (1.64/10)	0.921 (0.432/3)	0.024 (0.007/5)
20	28.31 (1.25/10)	1.193 (0.168/3)	0.051 (0.004/4)
30	39.05 (1.91/10)	1.634 (0.716/4)	0.070 (0.011/4)
40	49.12 (2.80/10)	2.722 (0.401/3)	0.101 (0.005/4)
50	57.94 (4.16/10)	3.561 (1.448/5)	0.123 (0.013/5)

Nitrogen sorption measurements were carried out with CA modified BC aerogels, but surprisingly no nitrogen sorption could be observed. Thermoporosimetry measurements (TPM) using o-xylene as the liquid phase showed that the maximum pore radius within the range of TPM-detectable pores increased with increasing

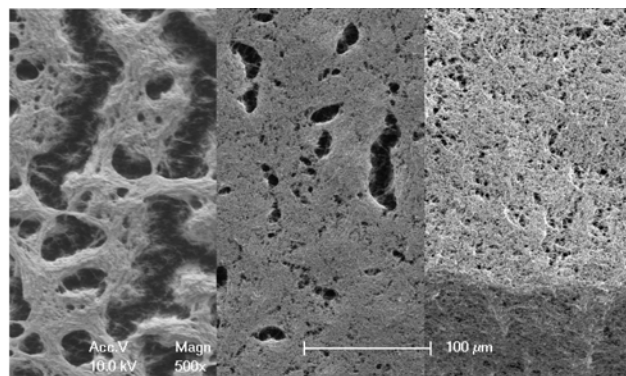


Figure 38: SEM images of unmodified and modified BC aerogel. Left to right: unmodified BC, 10 mg ml⁻¹ CA, 30 mg ml⁻¹ CA

CA loading (except for the highest loading of 50 mg ml⁻¹). Simultaneously, the number and size of the big voids present in scCO₂-dried unmodified BC aerogels decreased as shown by scanning electron microscopy (Figure 38). It is evident that precipitation of the fibrous CA inside the BC gel has a strong reinforcing effect as it prevents the gels from forming large voids upon supercritical drying. With increasing loading and homogeneous appearance the total pore volume decreased gradually (Table 10).

Table 10: Maximum pore radius (PR) and total pore volume (Vp) of CA modified BC determined by thermoporosimetry.

c (CA) [mg ml ⁻¹]	0	10	20	30	40	50
PR [nm]	108.76	63.47	66.95	70.19	89.04	57.26
Vp [cm ³ g ⁻¹]	41.17	36.45	18.45	22.99	17.69	14.88

2-Methoxyhydroquinone (2-MHQ)

Recently, it has been found that ammonoxidation – the reaction of a certain substrate in aqueous ammonia in the presence of molecular oxygen – of methoxyhydroquinone (cf. Figure 39, compound A) or 2,5-dihydroxy-[1,4]benzoquinone afford the metastable 2,5-diamino-[1,4]benzoquinone (B; Liebner et al. 2011c). A two-step reaction sequence starting with the generation of a phenoxy radical followed by replacement of the

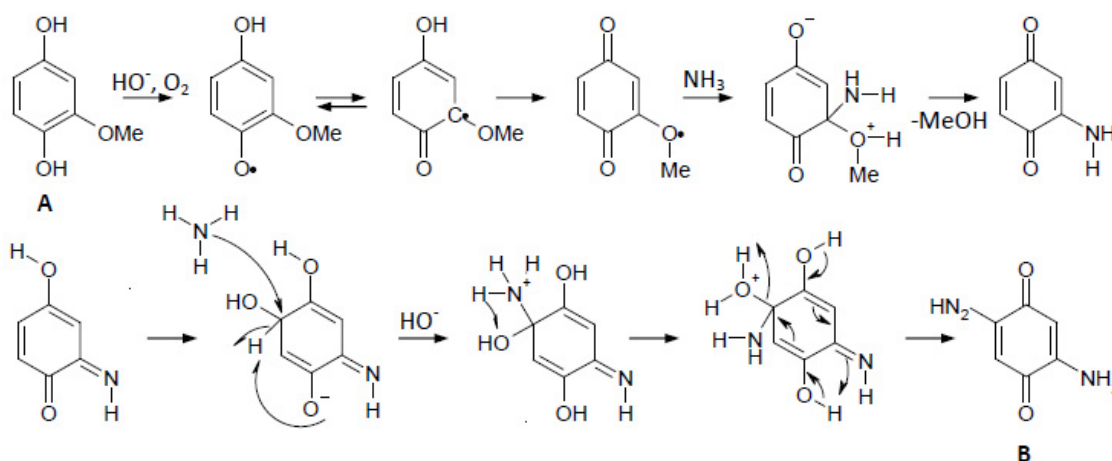


Figure 39: Proposed mechanism for the formation of 2,5-diamino-[1,4]benzoquinone (B) from methoxyhydroquinone (A) under ammonoxidative conditions (Liebner et al. 2010b).

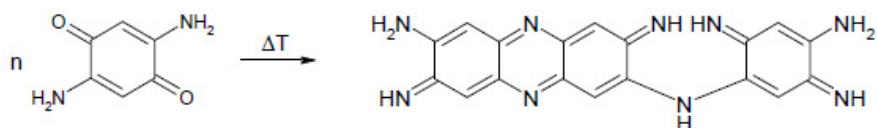


Figure 40: Formation of semi-ladder heterocyclic polymers from 2,5-diamino-[1,4]benzoquinone (Szita and Marvel 1969).

methoxyl group has been proposed (Liebner et al. 2010a).

2,5-Diamino-[1,4]benzoquinone was furthermore confirmed to easily undergo further condensation reactions under ammonoxidative conditions probably in a similar way as described by Szita and Marvel (1969, cf. Figure 40). Semi-ladder heterocyclic polymers of type C are high-temperature resistant materials. Interpenetrating networks consisting of such polymers are thus not only expected to have a stiffening effect but could be used for the preparation of high-temperature resistant aerogels via pyrolytic degradation of cellulose too.

2-MHQ lead to a strong color change of the samples (Figure 41) which was most pronounced for the specimen that was exposed to the modification bath for 3 days. Long exposure modification also lead to the highest density increase. However, the impact on mechanical properties was rather moderate and the measured values showed high scattering. Compared to the results from the pretests, the E modulus was even lower than that of samples with loaded directly in scCO₂ (Table 11). It is noteworthy that the long exposure sample showed a higher slope in the plateau region of the stress-strain curves than the other samples (not pictured).

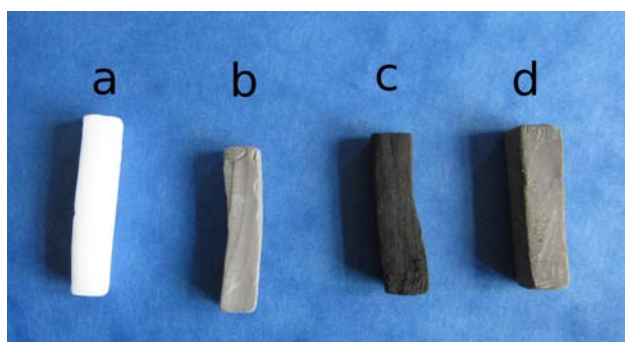


Figure 41: 2-MHQ modified gels after scCO₂ drying (a: unmodified, b: NH₄NO₃, c: O₂/NH₃ (3 days), d: H₂O₂/NH₃)

Table 11: Mechanical properties and densities of 2-MHQ modified BC aerogels. Standard deviation and sample size given in brackets before and after slash, respectively. The lower sample size for density evaluation is due the fact that samples were cut in smaller pieces prior to mechanical testing.

	E [MPa]	Rp _{0.2} [kPa]	ρ [mg cm ⁻³]
Reference (n=5)	0.149 (0.023)	7.05 (0.55)	7.8 (0.5)
NH ₄ NO ₃	0.156 (0.038/7)	12.9 (2.41/7)	11.4 (1.8/3)
H ₂ O ₂ , NH ₃	0.264 (0.063/5)	18.8 (3.22/5)	12.3 (1.0/3)
O ₂ , NH ₃ (3 days)	0.193 (0.037/5)	20.8 (4.68/5)	13.8 (0.8/3)
O ₂ , NH ₃ (3 hours)	0.281 (0.131/6)	17.9 (2.87/6)	8.8 (1.2/3)

Discussion

The results obtained from the pretests strongly suggest that organic compounds yield better results in terms of mechanical reinforcement at comparable density and amount of added reinforcing material. Inorganic materials such as soluble glass or polymerised silanols obtained via TEOS hydrolysis do obviously not agglomerate in a way contributing to increased mechanical stability of the BC aerogels.

Though, an example underlining that the mere ability to bring a compound into an aerogel does not necessarily contribute to enhanced mechanical properties is 2-MHQ. Long exposure 2-MHQ modification showed a higher slope in the plateau region which indicates that ammonoxidation products are present as small particles lying in the pores. These particles do not alter the deformation characteristics of the fibers but instead reduce the crushable pore volume so that the densification region is initiated at lower compressive strains than usual.

TA modification

A similar behavior could be observed for the TA modification, but TA precipitated in comparably big particles (Figure 42, left) and altered the stress-strain behavior in an unpredictable manner, as only random scattering of mechanical properties was observed.

The findings from FTIR measurements are supported by SEM investigations that showed that TA precipitated as single crystals into the cellulosic network (Figure 34). Since TA did not fully cover the gel's surface but was only present in some domains, the FTIR sensor was randomly pointed at TA containing regions or neat cellulose (see Figure 35).

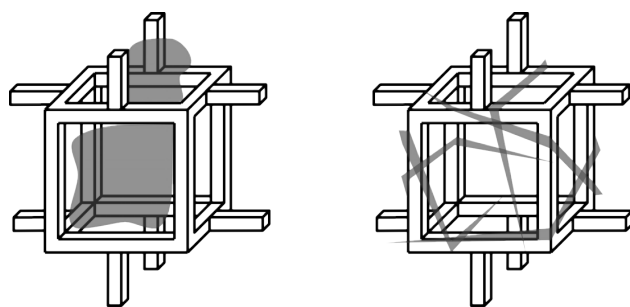


Figure 42: Schematic of different types of precipitation on a GA unit cell. Left: precipitated TA particle (gray) in BC aerogel. Right: precipitated CA fibers (gray) in BC aerogel.

pointed at TA containing regions or neat cellulose (see Figure 35).

Also, the enforced thermal treatment of the loaded gels after loading did not improve the mechanical properties of the modified BC aerogels. Obviously, most of the precipitated TA did not undergo noteworthy cross-linking which is supposed to be due to a presence of smaller amounts of water in scCO_2 which would also explain the unsatisfactory polymerisation of DL in the presence of (zinc octoate) at 160°C in scCO_2 at 300 bar. Unfortunately, the addition of P_2O_5 did not resolve the problem because of

its reaction with the supercritical CO₂ / DMSO mixture.

CA modification

CA effectively created a second, interpenetrating fibrous network that was capable of increasingly reducing the big voids present in scCO₂-dried gels from unmodified bacterial cellulose with increasing amounts of CA brought into the aerogel (Figure 38). It is noteworthy, that obviously no adsorptive interaction took place between BC and CA. This is indicated by the slope of the regression line in Figure 36 (loading bath concentration vs. apparent density), which is very close to 1. The lacking interaction can be explained by the hydrophobic nature of the almost per-acetylated CA (39.8% acetyl content) whereas bacterial cellulose is highly

hydrophilic due to the abundance of hydroxyl groups. The hydrophobicity of CA is also believed to be the reason for the failed nitrogen sorption measurements at 77K. Indeed, the CA modification seems to have rendered the aerogels hydrophobic so that they could be barely wetted with water even after being immersed in it for more than one week (Figure 43). Thermoporosimetry measurements on the other hand were possible due to the lipophilic nature of o-xylene and yielded results that indicated some weak trends such as the reduction of pore volume with increasing CA content. Unfortunately, TP covers only a pore range of up to about 5 µm and thus only the fact that the introduction of CA into the network altered the pore structure in terms of reducing the number and size of the big voids present in unmodified aerogels but increasing the average pore size of the fraction detectable by TP can be stated.

The fibrous precipitation of CA clearly contributed to an enhancement of mechanical properties. The power law (Formula 1, see page 36) could be fitted into the measured E modulus data with little error (Figure 37). Considering the scaling exponent n , the material clearly differs from a regular open porous cellular solid. From the current data, the reasons for the low exponent can only be guessed. First of all, the material's anisotropy has to be considered. When loading a sample perpendicular to its growth direction, the portion of fibers oriented along the loading direction will be bigger than in a material with fully randomly oriented fibers, thus reducing the exponent.



Figure 43: Hydrophobic effects prevent this BC aerogel modified with CA (30 mg ml⁻¹) from sinking even after 10 days of immersion in water.

Furthermore, CA modified BC aerogels are not a uniform material but actually a composite of two polymers. The different hydrophobicities of the compounds and the fact that no adsorption effects were observed during loading, suggest that the interface is comparably weak. This opens the possibility of shear deformation zones inside the struts which is also believed to have a considerable impact on the dimension of the exponent.

Comparison with similar materials

Putting the results of the present study in context with similar materials yields an interesting image. Figure 44 compares aerogels obtained from silica or cellulose II (regeneration from N-methylmorpholine-N-oxide monohydrate; NMMO) with those from cellulose Ib (BC) modified with CA. It can be seen that cellulosic aerogels generally perform better than their silica counterpart when considering specific stiffness (with respect to density). This means that theoretically cellulose allows to reduce the weight of aerogel-made parts even further without altering the stiffness.

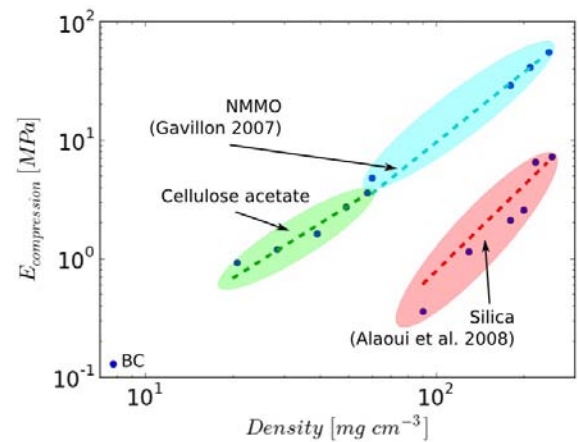


Figure 44: Log-log plot E modulus under uniaxial compression vs. density for various aerogels.

For NMMO based aerogels the low-end density limit is around 40 mg cm^{-3} , thus there is a comparably big gap between these aerogels and neat BC ($<10 \text{ mg cm}^{-3}$). This gap was successfully filled by introducing CA into BC aerogels extending the density range of cellulosic aerogels towards the ultra-low end.

Finally, it should also be stressed that the three materials pictured in Figure 42 are quite different from each other. Considering the values obtained from power law fits into the given data (Table 12), the deformation mechanisms are clearly different. While NMMO based aerogels correspond well to the GA unit cell ($n=1.96$), silica ($n=2.39$) and BC ($n=1.54$) aerogels have different scaling behavior. The higher exponents of silica aerogels

Table 12: Fitted values for the power law for different types of aerogels.

	CA BC aerogels	NMMO (Gavillon 2007)	Silica Alaoui et al. 2008
C	0.0066747	0.0011490	0.0000129
n	1.5417496	1.9583699	2.3897537
Error	0.1204300	2.9178216	5.5375969

are believed to be caused by dead ends in the framework, its structure and strut twisting as one of the deformation mechanisms (Scherer et al. 1995). The previously discussed scaling behavior of CA modified BC aerogels has to remain unexplained in the present study.

Conclusions and outlook

It was shown that scCO_2 can be used to prepare dimensionally stable ultra lightweight BC aerogels. Structural analyses showed that the scCO_2 dried material had a distinctly different appearance than for example freeze-dried BC pictured elsewhere in literature. A similar appearance to that in literature was obtained by freezing water swollen BC and observing it in cross polarized light. Considering that scCO_2 dried aerogels are 100% rewettable, while freeze dried material is not, it was concluded that freeze drying introduced irreversible damage to the cellulose framework because of the expansion of the solvent during freezing. This finding supported scCO_2 as a very gentle method to dry BC aerogels.

Furthermore structural investigations with SEM revealed lamellar discontinuities in terms of density and fiber orientation. These discontinuities had an interval of about 800 μm and in most cases they correlated with the growth direction of the BC pellicle, insofar as they lay in a plane perpendicular to the growth direction. The discontinuities were only observed in some regions, while others appeared homogeneous.

In terms of mechanical properties under uniaxial compressive loading it was shown that BC from static cultivation was anisotropic. The anisotropy suggested a layered structure which lead to significantly lower stiffness and strength along the growth direction than perpendicular to it. These results were in accordance with those from observation of frozen water swollen BC under cross polarized light. The formation of lamellar structures perpendicular to the growth direction was believed to be caused by slippage along the weakest direction due to the pressure caused by the freezing solvent.

Several modifications were applied in order to obtain reinforced BC aerogels. Pretests showed that in general, carbon-based organic modification agents performed better than silica-based ones because they had a lower impact on density while affecting the mechanical properties in a similarly positive way. BC aerogels were successfully reinforced by precipitating cellulose acetate (CA) into to the cellulosic network. Even though, no interactions between BC and CA could be observed, the modification allowed

to effectively scale the density as well as the mechanical properties by adjusting the CA concentration of the modification bath.

Structural investigations with SEM showed that CA precipitated as an evenly distributed fibrous network into BC giving it an increasingly homogeneous appearance. The disappearing of large voids with increasing amounts of CA, indicated that the voids were artifacts from the drying process with scCO_2 . However, considering the full rewettability of scCO_2 dried aerogels, this finding did not invalidate scCO_2 a particularly gentle drying technique.

Finally, the CA modification allowed to close a gap in terms of density between neat BC and the lowest density cellulosic aerogels obtained via the NMMO route. Comparing the results obtained in the present work with results on mechanical testing of silica based aerogels it was evident, that cellulosic aerogels performed better in terms of specific E modulus than their silica based inorganic counterpart.

However, several open questions remain when considering the results of the present work and more research is needed to elucidate them. First of all, the origin of discontinuities discovered during structural investigation with SEM has remained unclear. It was suggested that they occurred due to events during fermentation. The clarification of this question requires investigations from a biological point of view.

Secondly, the scaling behavior of mechanical properties over the apparent density opened another question. The scaling exponent of the fitted power law did not agree with predictions from an analytical solution for a Gibson and Ashby unit cell. Possible reasons for this behavior are the anisotropy of statically cultivated BC and the fact that CA modified BC is actually a composite material that features a poor interface between its constituents. However, a proper explanation of the observed effect would require deformation investigations on the micro- or even nanoscale.

As a last somewhat problematic point, porosity measurements have to be mentioned. BC aerogels were shown to have a very broad hierarchical pore size distribution ranging over several powers from the nanometer scale to the micrometer scale. Currently no technique is capable of monitoring the full pore size distribution. The combination of several techniques could provide a clearer image of the porosity and contribute to a better understanding of this intriguing material.

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