AEROGELS FROM BACTERIAL CELLULOSE: A NEW DIMENSION IN PREPARING SHAPED CELLULOSE AEROGELS

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ABSTRACT

Cellulosic aerogels, being the "young" third generation of aerogels succeeding those based on silica and synthetic polymers, are intriguing materials featuring properties similar to their antecessors, with the additional advantages and characteristics of the re-growing biopolymer cellulose. The current paper communicates an straightforward approach to obtain highly porous, ultra-lightweight bacterial cellulose aerogels with densities similar to those of silica aerogels utilizing supercritical carbon dioxide as drying medium. The resulting aerogels showed good dimensional stability during processing. The novel materials were found to be quantitatively rewettable without significant shrinking, which render bacterial cellulose aerogels interesting as controlled release matrices. Supercritical antisolvent precipitation within the cellulose matrix was found to be an effective method for loading bioactive compounds onto bacterial cellulose aerogels without necessitating additional process steps. Reinforcement of bacterial cellulose aerogels is a promising approach to overcome the lack of mechanical stability.

INTRODUCTION

Aerogels are highly porous solids of extremely low density. They are obtained by drying gels under suitable, mild conditions, as is the case for supercritical dry. The unique properties of aerogels, namely extremely low densities, large, open pores, high specific surface areas, low thermal conductivity and low sound velocity, make these materials interesting for a broad field of applications reaching from biomedical to construction purposes [1].

Inorganic aerogels, and in particular silica aerogels, have been known already for a long time and were the topic of numerous scientific studies. Furthermore, there are a couple of large-scale applications, mainly in construction engineering where these materials are used for thermal or acoustic insulation or shock absorption [2]. These materials are already known and investigated beyond academic research. Another large group of aerogels comprise those based on organic polymers, and much research has been conducted in this field since the late 80ies of the previous century [3]. Organic aerogels are primarily considered as precursors for nanostructured carbon, but more and more research is done for chemically utilizing the functional groups of the organic backbones [4].

Also biopolymers, in particular polysaccharides, increasingly attract interest as educts for the preparation of aerogels comprising both the intriguing properties of aerogels (very low density, inter-connected open pores, high pore volume, e.g.), and the special properties of the particular renewable raw material. Much research has been dedicated to the development, characterization, and testing of cellulose-based aerogels as cellulose is the most abundant natural polymer [5]. While cellulosic aerogels were initially almost exclusively based on cellulose derivatives [6,7], other techniques starting from native, underivatized plant cellulose have been meanwhile developed, too [8,9].

However, preserving cellulose chemical integrity as well as maintaining a high dimensional stability of shaped cellulosic aerogels throughout the different preparation steps is a challenging task. All attempts to prepare dimensional stable, shaped cellulosic aerogels with densities as low as those of silica aerogels (<10 mg cm^{-3}) failed till now.

In the present paper we will communicate a facile procedure that allows for preparation of ultra-lightweight aerogels from non-derivatized bacterial cellulose at zero shrinking throughout the process, reaching densities of less than 10 mg cm^{-3}. Based on its rewetting characteristics, the novel materials have been tested as slow release matrices.

Supercritical antisolvent precipitation from Ethanol is a suitable method of loading bacterial cellulose aerogels with bioactive compounds. Selected results for the loading/release behavior will be reported for bioactive model compounds. As the extremely low density of the bacterial cellulose aerogels would exclude applications requiring higher mechanical strength, several attempts to reinforce these materials have been made.

MATERIALS AND METHODS

Cultivation of Gluconacetobacter xylinum was performed in aquarium-type glass tanks filled with steam-sterilized culture medium consisting 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} $Na_2HPO_4 \cdot 12\ H_2O$. The culture medium was inoculated with a suspension of Gluconacetobacter xylinum. After 30 days under static cultivation at 30°C most of the glucose was consumed and the thickness of the bacteria layer amounted to about 3-4 cm. The harvested cellulose was cut into block-shaped pieces which were briefly boiled with water, treated three times with 0.1 M aqueous NaOH at 90°C for 20 min each, and finally neutralized by rinsing with deionized water for 24 h.

Prior to supercritical drying, the resulting bacteria cellulose aquogels were subject to a solvent exchange step by gently shaking in the twenty-fold volume of absolute ethanol (Sigma-Aldrich). After 6 and 12 h, the gels were transferred to a second and third bath with the same amount of fresh solvent. After another 6 h, the resulting alcogels were $scCO_2$ dried.

Supercritical drying was performed on a supercritical fluid formulation apparatus SF1 (Separex, France). Alcogels were placed on a stack which was loaded into the preheated 500 ml autoclave (40°C). The autoclave was pressurized to 10 MPa. After reaching the final pressure, the outlet valve was opened and the autoclave was flushed with pure CO_2 for 1 hour at a flow rate of 2.5 kg h^{-1}. Ethanol was separated in a cyclone separator. After

the drying period, the autoclave was depressurized slowly and the dry aerogels were removed.

Mechanical tests were performed on an electromechanical universal testing machine (Quicktest QTS 10, Prüfpartner GmbH) equipped with a 500N load cell. For each type of cellulosic aerogel, two individual specimens were loaded under compression at a constant strain rate of approximately 8.10^{-4} s^{-1}. Compressive strains were calculated from the cross-head displacement measured and corrected for the load train compliance of the testing machine. To avoid possible effects of change in the environmental conditions, compression tests were performed in a controlled environment at 24.8°C and 27% relative humidity.

For ***loading of the aerogels*** with bioactive compounds, the bacterial cellulose alcogels were placed into ethanolic solutions of dexpanthenol or L-ascorbic acid instead of the third solvent exchange bath. The gels were kept in the bath for 24 hours at minimum, and the gels were dried according to the supercritical route described above.

RESULTS

Shaped cellulose aerogels obtained from underivatized plant cellulose via the Lyocell route - i.e. a) dissolution in *N*-methylmorpholine *N*-oxide monohydrate (NMMO-H_2O), b) moulding, c) regeneration (reprecipitation) of cellulose with water or ethanol and d) drying of the obtained alcogel with supercritical CO_2 - lack dimensional stability especially at low solid contents [8].

As shown in figure 1, shrinking of Lyocell gels having a solid content of about 3% is considerable especially during the $scCO_2$ drying step. The resulting remaining volumes drastically decrease if the solid content is further reduced.

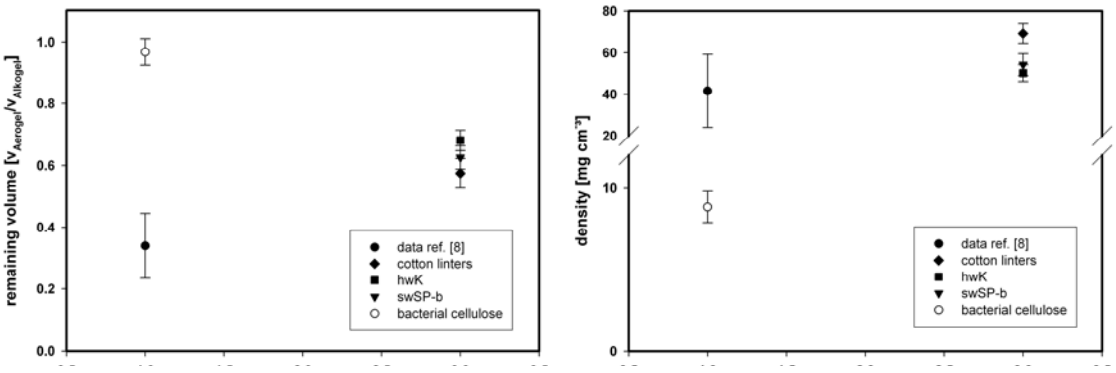

Figure 1: Remaining volumes (left) and densities of aerogels (right) obtained from commercial pulps and bacterial cellulose in dependence of the solid content for alcogel

Due to the high shrinking rates, densities below 40 mg cm^{-3} can hardly be obtained for shaped cellulose aerogels prepared according to Lyocell technique [10]. In contrast, bacterial cellulose alcogels, which are not subject to dissolution, shaping, and regeneration as the pulps used in the Lyocell route, were found to be largely dimensionally stable upon drying with supercritical carbon dioxide.

This is surprising insofar as their solid contents are only about 1vol%, and can be only explained by the comparatively high crystallinity, high degree of polymerization (5,000 < DP < 15,000), and thus the high molecular weight of bacterial cellulose.

Especially the high crystallinity seems to play a crucial role for the increased dimensional stability of bacterial cellulose aerogels. The cellobiose repeating unit of the cellulose macromolecules in combination of the string intra- and intermolecular hydrogen bonds is known to feature the ability to form highly ordered areas, i.e. crystallites, which are supposed to be randomly distributed in the remaining amorphous matrix as described by the fringed-micelle model (cf. figure 2, left).

These highly ordered areas which further aggregate to larger fractal structures significantly increase not only resistance to thermal, chemical or light degradation, but also mechanical stability. The higher crystallinity along with the higher DP values of bacterial cellulose as compared to processed plant celluloses (cellulosic pulps) thus prevents the fragile capillary structure of bacterial cellulose aerogels from collapsing during the $scCO_2$ drying step. Commercial pulps on the other hand suffer much more even from weak forces arising upon (re-)formation of phase boundaries prior to reaching and after leaving the supercritical state during drying.

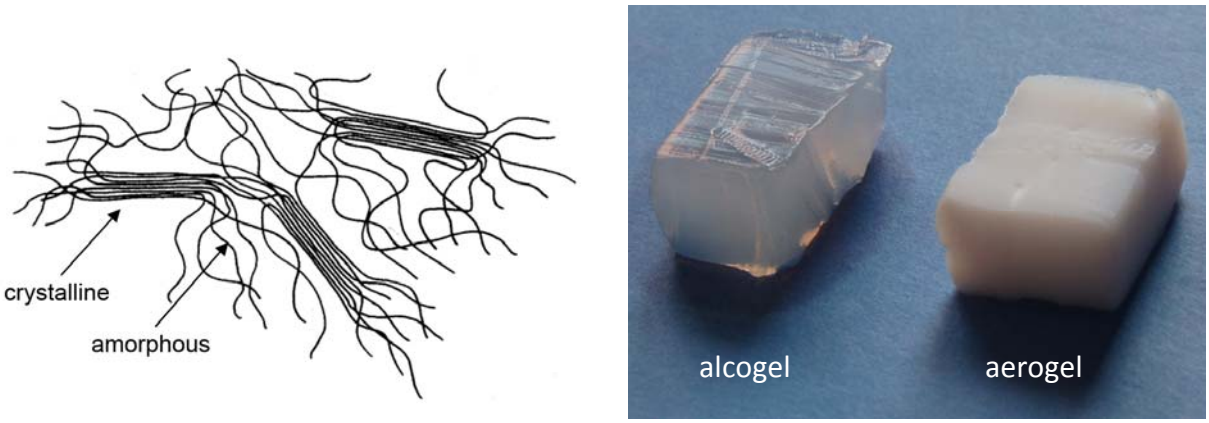

Figure 2: Fringed-micelle model of polymer cristallinity.cellulose (left). Bacterial cellulose alcogel prior to and after $scCO_2$ drying (right).

The special behavior of bacterial cellulose gels throughout solvent exchange and $scCO_2$ drying allowed for the preparation of largely dimensionally stable, ultra-lightweight aerogels with densities of down to 8 mg cm^{-3}. Such low densities were only reported for the most lightweight silica aerogels [12].

In general, variations of the remaining volumes and densities are lower compared to aerogels from solutions of the same cellulose pulp contents (cf. fig. 1). The given error bars are mainly due to uncertainities of measurement due to handling of the sensitive materials.
Due to the extremely low density of bacterial cellulose aerogels, electrostatic charge is sufficient to make the aerogel stick to a surface, which makes the material difficult to handle.

Electron micrographs revealed a hierarchical order of open-pores. In addition to a well-developed macropore network with pore diameters of up to about 100 µm, bacterial cellulose aerogels offer in addition a mesoporous substructure (Figure 3).

The high dimensional stability of the bacterial cellulose aerogels upon solvent exchange and $scCO_2$ drying is a pre-requisite for complete rehydration at dimensional stability ("quantitatively rewetting") of the aerogels. During rewetting of such highly porous and fragile materials, surface tension of the used solvent usually plays a crucial role for the dimensional stability of the gel.

As forces alongside a capillary gradient adjacent to the solvent menisci can lead to capillary contraction and resulting closure of the pores resulting in inaccessible volume fractions, rewetting of such sensitive materials is often difficult and mostly largely incomplete.

However, in the case of bacterial cellulose aerogels even water does not significantly affect the pore structure which is most likely due to the above-mentioned high DP values and the comparatively high portion of crystalline domains. Both, the maintained full accessibility of the pore volume and the negligible shrinking upon rewetting render shaped bacterial cellulose aerogels highly attractive materials for controlled release systems [13]. This is exemplarily demonstrated herein for loading a shaped bacterial cellulose aerogel with D-panthenol and its release kinetics.

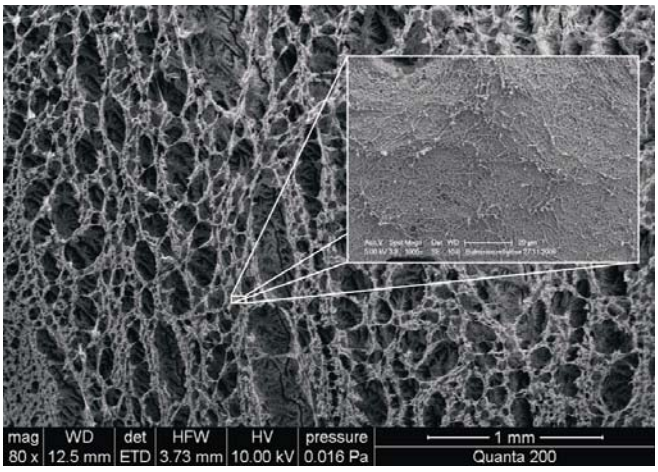

Figure 3: SEM-images of the macro- and microporous domains of a bacterial cellulose aerogel.

Carbon dioxide can act as an antisolvent, inducing precipitation of substances of low solubility in CO_2. The main advantage of this technique is that supercritical drying and loading of the gel with the active compound can be performed at the same time, in a single step, for appropriate solute/solvent systems. Figure 4 (left) shows the amount of loaded D-panthenol in relation to the D-panthenol concentration within the loading bath.

As only precipitation and thus the interaction between D-panthenol, ethanol, and carbon dioxide, influence the loading system, interactions between D-panthenol and the cellulosic matrix can be disregarded, resulting in a linear relationship for the loading isotherm.

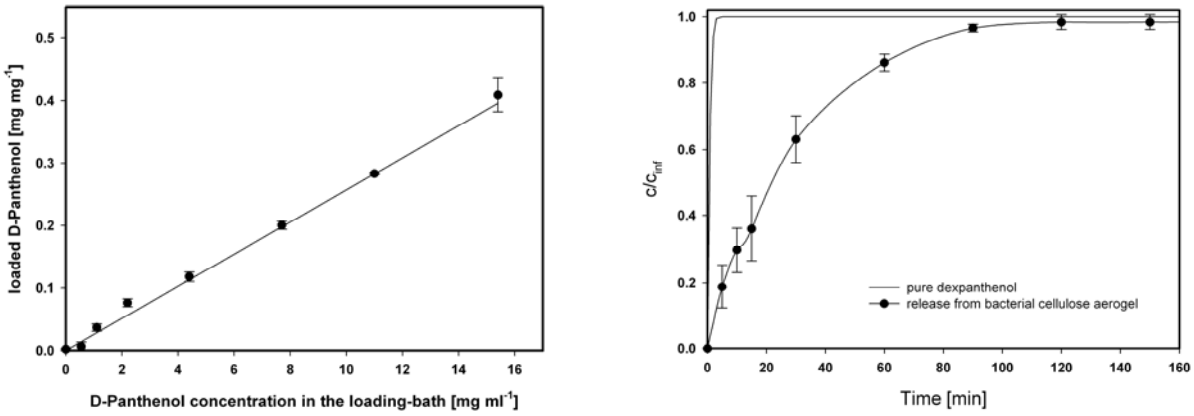

Figure 4: loading isotherm for the loading of D-panthenol (dexpanthenol) onto bacterial cellulose matrices (left), and comparison of the dissolution of pure D-panthenol and D-panthenol loaded onto a bacterial cellulose aerogel (right)

Figure 4 (right) compares the dissolution kinetics of the pure compound with its release kinetics from the loaded bacterial cellulose aerogel. It is evident from the data that the release of D-panthenol from the loaded bacterial cellulose aerogels is significantly delayed.

The fragile nature of bacterial cellulose aerogels can clearly be seen when comparing the mechanical properties of these materials to the properties of cellulose aerogels obtained from commercial pulps via the Lyocell route (see table 1).

Table 1 : Selected mechanical parameters of aerogels obtained from different cellulose polymers

Sample	solid content	E (N mm^{-2})	σ_y (N mm^{-2})	ε_y (%)
swSP-a	3%	9.1	0.16	4.0
swSP-a	6%	61.5	0.94	2.7
Cotton Linters	3%	9.9	0.18	4.0
Cotton Linters	6%	42.9	0.56	2.7
Bacterial cellulose	~ 1%	0.38	0.01	5.71

Young's modulus *E* and compressive yield stress σ_y of the bacterial cellulose aerogels indicate that there exists almost no elastic deformation domain. Reinforcement of shaped bacterial cellulose aerogels by interpenetrating networks based on inorganic or organic polymers is one approach to overcome this drawback. This is a topic of our current investigations.

CONCLUSIONS

Shaped ultra-lightweight aerogels can be obtained from purified bacterial cellulose (*G. xylinum*) according to a facile two-step procedure that comprises solvent exchange (water to ethanol) and subsequent $scCO_2$ drying (40°C, 10 MPa).

The novel aerogels were superior in terms of dimensional stability throughout the preparation procedure (very little shrinkage) when compared to analogous aerogels from dissolved

plant celluloses, either obtained from shaped Lyocell dopes or according to other dissolution / regeneration procedures. The extremely low density of down to 8 mg cm^{-3}, the resulting high pore volume along with the hierarchical structure of macro- and mesopores render the bacterial cellulose aerogels very attractive materials for a multitude of applications.

The utilization as controlled release matrices is considered as one particularly promising application for shaped bacterial cellulose aerogels. It is based on a quantitative rewettability of the aerogels and their dimensional stability upon loading with a particular bioactive compound, during subsequent scCO2 drying, and upon releasing the bioactive compound into an aqueous medium. All requirements have been confirmed to be met for loading the bacterial cellulose aerogels with D-panthenol. Furthermore, the release profiles were found to be independent on the amount of loaded compound and highly dependent on the thickness of the aerogel layer.

Due to their low cellulose content, compactness and stiffness of bacterial cellulose aerogels are rather low and render them very sensitive materials. Different approaches aiming at the preparation of reinforced cellulose aerogels are therefore currently studied.

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