Preparation of Biocomposite Microfibers Ready for Processing into Biologically Active Textile Fabrics for Bioremediation

Patrick Kaiser, Steffen Reich, Andreas Greiner, and Ruth Freitag*

Biocomposites, i.e., materials consisting of metabolically active microorganisms embedded in a synthetic extracellular matrix, may find applications as highly specific catalysts in bioproduction and bioremediation. 3D constructs based on fibrous biocomposites, so-called “artificial biofilms,” are of particular interest in this context. The inability to produce biocomposite fibers of sufficient mechanical strength for processing into bioactive fabrics has so far hindered progress in the area. Herein a method is proposed for the direct wet spinning of microfibers suitable for weaving and knitting. Metabolically active bacteria (either Shewanella oneidensis or Nitrobacter winogradskyi (N. winogradskyi)) are embedded in these fibers, using poly(vinyl alcohol) as matrix. The produced microfibers have a partially crystalline structure and are stable in water without further treatment, such as coating. In a first application, their potential for nitrite removal (N. winogradskyi) is demonstrated, a typical challenge in potable water treatment.

1. Introduction

Biofilms are omnipresent in nature and tend to form on any accessible surface.[1] Natural biofilms typically consist of syntrophic microbial consortia embedded in a mucus layer, the so-called extracellular matrix. Chemically speaking, the extracellular matrix is a hydrogel formed by biopolymers such as proteins, lipids, and various polysaccharides, in particular polyanionic and neutral uronic acids.[2] Large amounts of water are retained in this matrix via the formation of hydrogen bonds, thereby preventing the drying out of the bacteria. In addition, the extracellular matrix adsorbs and retains nutrients and protects the organisms against toxins, UV light, and temperature fluctuations. Antibiotics, for example, are bound by components of the extracellular matrix and can no longer reach and harm the organisms.[3]

Since biofilms retain, stabilize, and protect metabolically active organisms, they are attractive for technical applications, e.g., in bioproduction or for environmental remediation. Whenever a natural biofilm consortium is available for the task, such approaches are known to work well. However, many interesting biotransformations require only a specific species or metabolic function. The group of Li has identified a number of microorganisms that can form single species biofilms, such as Corynebacterium glutamicum (C. glutamicum) and Bacillus megaterium (B. megaterium).[4] C. glutamicum has been used in industrial biotechnology since 1970 for the production of biogenic amino acids and vitamins.[5] B. megaterium is used for the production of high-quality recombinant proteins such as penicillin G-acylases or β-galactosidases.[6] Most other microorganisms, however, including Escherichia coli, do not form single species biofilms but need help from other adherent microorganisms to become part of such a structure.[7] Moreover, none of the single species biofilms described so far are comparable to the natural multispecies biofilms in terms of mechanical, chemical, and biological stability. It is therefore unlikely that an extension of the use of biofilms for bioprocess intensification is possible on the basis of natural biofilms.

We have recently introduced the concept of the “artificial biofilm” as a fundamentally new approach to integrating single species biofilms into technical processes.[8,9] Artificial biofilms are defined as biocomposites where specific microorganisms are embedded in a synthetic hydrogel matrix of tunable properties. In a first embodiment, nonwoven gauzes were prepared by electrospinning bacterial suspensions in poly(vinyl alcohol) (PVA) as biomimetic extracellular matrix. PVA is a very biocompatible material, but the produced biocomposites had to be stabilized against dissolution in water by the application of a thin coating of poly(p-xylylene) (PPX).[10–13] Electrospun gauzes containing Shewanella oneidensis (S. oneidensis) subsequently outperformed the corresponding natural S. oneidensis biofilms in biofuel cells. However, other potential applications would benefit or even require the integration of the artificial biofilm in the form of a woven or knitted fabric. Unfortunately, fibers produced by
standard wet spinning were not of sufficiently mechanical sta- 

tility for processing into fabrics, while for all artificial biofilms 

produced so far the lengthy (several hours) PPX coating pro-

dure put an additional stress on the embedded organisms. 

Here we propose a different approach to the wet spinning of 

fibrous biocomposites. The PVA microfibers produced through 
this procedure are more stable in water than the previously 

prepared ones and therefore do not require coating, while being 

of sufficient strength for weaving and knitting. This is expected to 

considerably extend the application range of the artificial biofilms, 

since now applications in the form of filters or adsorbers become 
possible. For a proof-of-concept application, Nitrobacter winograd-

skyi (N. winogradskyi) bacteria were embedded into the bioactive 

fabric. Under aerobic conditions this organism is chemolithoau-

trophic and uses nitrite (NO$_3^-$) as main oxygen acceptor.[14] The 
nitrate (NO$_3^-$) produced in this process becomes subsequently 

available, e.g., as nitrogen source for plants.[15] This ability makes 

N. winogradskyi species important agents in the natural nitrogen 

cycle, while opening various options for technical applications, 
e.g., in sewage and water treatment (denitrification step).

2. Experimental Section

2.1. Materials

Standard chemicals, culture media components, supplements, 
plastic materials, and culture flasks were from established 
suppliers and used as received. High-quality water was pre-
pared by a Millipore unit. Phosphate buffered saline (PBS) 
was prepared in house as follows: 8.0 g NaCl, 0.2 g KCl, 
1.42 g Na$_2$HPO$_4$, and 0.24 g KH$_2$PO$_4$ per liter of Milli-
pore water, pH adjusted to 7.3 with 2 mM NaOH, sterilized by 
autoclaving (121 °C, 20 min). PVA Clariant Mowiol 28-99 
(M$_w$ 145 000 g mol$^{-1}$, 99.4 % hydrolysis) was from Sigma-
Aldrich. To obtain a 10 w% PVA solution was prepared through 
autoclaving (121 °C, 20 min). Afterward the solution was stirred at 100 rpm 
during cooling to prevent phase separation. N. winogradskyi (#10237) 
was obtained from DSMZ (Braunschweig, Germany). S. oneidensis 
MR-1 was from ATCC (Manassas, Virginia). Nitrobrother basal 
medium (NB medium) was prepared in house as follows: 2 g 
2-((2-Hydroxyethyl)-1-piperazinyl)-ethansulfonsäure (HEPES), 
100 mL mineral mix (0.198 g NaCl, 0.01 g MgSO$_4$ · 7 H$_2$O, 
0.0526 g KH$_2$PO$_4$, and 0.0035 g CaCO$_3$ per liter of Milli-
pore water), pH adjusted to 7.0, if necessary, and sterilized by 
autoclaving. Afterward the solution was stirred at 100 rpm 
during cooling to prevent phase separation. N. winogradskyi 
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e.g., in sewage and water treatment (denitrification step).

2.2. Analytical Methods

Nitrate concentrations were determined using a photometric 
test kit (1.14776.0001, Merck, Darmstadt, Germany) following 
the supplier’s instructions. For viability testing, biocomposites 
were stained (“Live/Dead” stain) with the BacLight Bacterial 
Viability Kit from Life Technologies (Eugene, OR) according 
to the manufacturer’s instructions using Syto9 (green, living 
bacteria) and propidium iodide (red, dead bacteria) as dyes. 
Stained biocomposites were subsequently analyzed by confocal 
microscopy (LSM510, Zeiss, Oberkochen, Germany). A phase 
contrast microscope Olympus BX51 equipped with a 600x oil 
immersion objective UPPlanFI, Olympus and camera CC-12, 
from Olympus Soft Imaging Systems (Tokyo, Japan), was 
used for light microscopy. Software package 3.2 (Build 883) 
from Olympus Soft Imaging Systems was used for treating 
and storing the pictures. Microfibers were in addition characterized 
by scanning electron microscopy (LEO 1530, also Zeiss). 
Samples were attached to the holder with water-based conductive 
carbon glue and sputter coated with P (coat thickness: 1.3 nm, 
sputter coater: 208 HR from Cressington, Watford, England). A 
polarized microscope (DMRX, Polarisator ICT/P, Leica, Wetzlar, 
Germany) was used to demonstrate the crystallinity of the fibers. 

The tensile strength of the microfibers was tested using a 
material tester Zwickiline Z0.5 from Zwick/Roell (Ulm, Ger-
many). Measurement specimens were stored overnight in the 
tractor of the instrument, to adapt them to the measurement 
conditions (humidity and temperature). A KAF-TC from Zwick/ 
Roell (nominal load: 200 N, serial number: 1002557) was used 
as force receiver. The software package TestXpert II (Version3.0, 
Zwick/Roell) was used to process the data. The clamped fiber 
length was 20 mm. A preload of 0.008 N mm$^{-1}$ was applied. 
Then the fiber was stretched at a speed of 100 mm min$^{-1}$ until 
rupture. For the determination of Young’s modulus via tensile 
strength tests, the microfibers were cut into pieces of 20 mm 
and individually glued with nail polish on carriers made of 
polypropylene (PP) film (Quick n’Go Astor, Mainz, Germany.) 
After a drying time of 1 d, the carrier was clamped into the test 
instrument (ElectroForce 3200, Bose, Framingham, Massachu-
setts) and then cut in such a way that stress was subsequently 
only applied to the fiber. Thereafter, the fiber was pulled with 
0.01 mm s$^{-1}$ and the force recorded (max. 2.45 N). Young’s 
modulus was calculated from the data according to Equation (1)

\[ E (\text{MPa}) = \frac{\text{tensile stress (N mm}^{-2}\text{)}}{\text{extensional strain}} \]  

(1)

The linear mass density of the fibers was calculated in tex 
(mass in grams of 1000 m fiber).

The fiber tension was calculated according to Equation (2)

\[ \text{fiber tension (cN tex}^{-1}\text{)} = \frac{\text{Yield Strength (MPa)} \times \text{cross–section area (mm}^{2}\text{)} \times 100}{\text{linear mass density (tex)}} \]  

(2)

A thermal analyzer (821 DSC, Mettler Toledo, Columbus, OH) 
was utilized for the thermal analysis of the microfibers. Differen-
tial scanning calorimetry (DSC) scans were recorded in nitrogen 
atmosphere and heating rates of 10 K min$^{-1}$ from 0 to 250 °C.
2.3. Microbiology

For the production of *S. oneidensis* MR-1, 30 mL of LB medium was inoculated with *S. oneidensis* from kryo stock (*OD_{600} = 1.0*) and cultivated overnight in 100 mL shake flasks at 30 °C and 150 rpm in a Forma Orbital shaker (Thermo Fisher Scientific, Marietta, OH). The *OD_{600}* of the culture was determined photometrically (Biophotometer, Eppendorf, Hamburg, Germany), with an *OD_{600}* of 1 equaling $8.5 \times 10^8$ bacteria mL$^{-1}$, as previously determined. For the production of *N. winogradskyi*, 300 mL NB medium was inoculated with *N. winogradskyi* from kryo stock (*OD_{600} = 0.2*) and cultivated for 2 weeks in three 500 mL shake flasks (100 mL culture each) at 27 °C and 100 rpm in the Forma Orbital shaker. The *OD_{600}* of the culture was determined photometrically, with an *OD_{600}* of 1 corresponding to $14 \times 10^6$ bacteria mL$^{-1}$, as previously determined.

2.4. Biocomposite Preparation

For biocomposite preparation, sufficient quantities of bacteria were harvested from an exponentially growing culture by centrifugation (10 min, 1200 g), washed twice with sterile PBS and resuspended (*S. oneidensis* MR-1) at a final concentration of $2 \times 10^9$ bacteria mL$^{-1}$ in 6 mL of a 10 wt% PVA solution in sterile PBS or (*N. winogradskyi*) at a bacterial concentration of $28 \times 10^8$ bacteria mL$^{-1}$ in 3 mL of the sterile 10 wt% PVA solution. The mixture was stirred with a magnetic stirrer (RH basic, IKA, Staufen, Germany) at 150 rpm for 1 h to obtain a homogenous suspension. All solutions coming into contact with the bacteria were sterilized by autoclaving (121 °C, 21 min) and cooled down to ambient temperature prior to use. For automated fiber production, the homemade wet spinning apparatus shown in Figure 1 was used.

The bacterial suspension was filled into a 10 mL syringe (0.8 × 120 mm cannula) connected by an adapter to a compressed air hose. Microfibers were produced by steadily pressing (0.6 bar) the suspension through the cannula into the coagulation bath (acetone) for hardening. Afterward, the microfibers were dried with compressed air in the drying unit and wound up at velocities between 50 and 170 U min$^{-1}$. Microfibers containing no bacteria were produced as controls.

In addition, the set up shown in Figure 2 was used for crafting shorter microfibers by hand.

In this case, the cannula of the 10 mL syringe containing the PVA solution was dipping directly into acetone bath. The fibers were produced by pulling a polymer drop from the tip of the cannula through the acetone bath and collecting the resulting fibers on a 6 × 2 cm metal frame. If indicated, the fibers were stored overnight at 4 °C and PPX coated the next day by chemical vapor deposition (of [2.2] paracyclophane (PPX) using a lab coater from SCS (Indianapolis, IN, USA). PPX was deposited at 20 °C and 60–70 mbar. Evaporation of the precursor took place at 150 °C, pyrolysis at 650 °C. Coating took ≈8 h. Fibers without bacteria were again prepared as controls.

2.5. Processing of the Fibers into Textiles

Microfibers produced with the automated apparatus were woven into 2.5 × 4.0 cm textiles using a handheld frame (Schmidt Spiele, Berlin, Germany). For warp threads a nylon fiber was used. A circular knitting machine (Krensler KT1, 10 needles per inch) was used to produce tubular fabrics. For this, the PVA microfibers were first twined with nylon fibers and then knitted by hand into the tubular fabrics (height: 5 mm, average diameter 70 mm) using a nylon auxiliary yarn.

2.6. Cultivation of Biocomposites

Biactive textiles containing *N. winogradskyi* and the negative controls thereof were cultivated in 100 mL NB medium in 500 mL shake flasks at 27 °C and 100 rpm in the Orbital shaker, as described above for the planktonic cultures. Samples of the culture medium were taken every other day for determination of the nitrite concentration and the *OD_{600}*. *S. oneidensis* biocomposites and negative controls thereof were cultured in 50 mL LB medium in 250 mL shake flasks at 30 °C and 100 rpm for 7 d. The *OD_{600}* in the culture medium was determined on a regular basis. Samples of the microfibers were taken before and after cultivation and stained with the Live/Dead assay. For the outgrowth experiments intact fibers were put onto solid growth agar plates (1.5 wt% of agar–agar (Carl Roth, Karlsruhe, Germany) was added to the culture medium and cast in sterile Petri dishes) and incubated for 72 h under the respective growth conditions.
3. Results and Discussion

3.1. Biocomposites

The development of the wet spinning process for mechanically stable biocomposite fibers was based on a set of microfibers containing *S. oneidensis* bacteria embedded in PVA as synthetic extracellular matrix. *S. oneidensis* was chosen for these experiments, as this bacterium has a comparatively high specific growth rate (0.42 h⁻¹) and thereby facilitates biomass production. Fibers were prepared by automatic and manual wet spinning of PVA suspensions containing $2 \times 10^9$ bacteria mL⁻¹ together with negative controls ("PVA fibers") containing no bacteria but otherwise prepared under identical conditions. Whereas the maximum length of the manually spun fibers was limited to 2–3 m, fibers with a length of over 130 m could be prepared using the automated wet spinning apparatus with a 5 mL syringe (4 mL fluid).

In the case of the automatically spun fibers the residence time of the fibers in the acetone coagulation bath was adjusted via the speed of the tracking (wind up) unit. This in turn affected the average diameter of the fibers (Table 1). In the case of the manually prepared fibers, the residence time in the acetone bath was less well defined due to the manual handling, but corresponded to only ≈5 s. In this case no variation of the residence time was possible. For all fibers no difference was observed in the average diameters between biocomposites and control fibers prepared under otherwise identical conditions. A second characteristic that was affected by the residence time in the acetone bath was the crystallinity of the PVA, determined by DSC (Table 1). In this case data were only determined for the PVA fibers, as the presence of bacteria would have made the correct determination of the crystallinity of the PVA matrix impossible in the case of the biocomposites. Crystallinity was verified by polarized microscopy for all PVA fibers.

According to these data, the crystallinity of the PVA fibers increased with increasing residence time in the acetone bath for up to 25 s. For longer exposure times, no further increase of the crystallinity in the microfibers is observed. With 42% the crystallinity of the automatically draw fibers was higher by 10% compared to that of the hand-drawn fibers (33%). Moreover, whereas the manually prepared fibers dissolved quickly, i.e., in less than 1 h, in water the microfibers (and biocomposites) produced by automatic wet spinning showed pronounced swelling (increase of the diameter by up to 50%) but no further dissolution in aqueous solution (water or growth medium) over several days of incubation. The automatically produced fibers were therefore significantly more stable in water than the hand-drawn one. No difference was observed in this regard between the PVA fibers and the biocomposites prepared under otherwise identical conditions.

Hand-spun microfibers can be stabilized against water by adding a protecting shell of a hydrophobic material such as PPX.[8] In addition, stabilization of PVA fibers via crosslinking[16,17] or admixing of other polymers before processing[18] has been described. However, this represents further steps in producing the fibers, while the coating or crosslinking presents an additional mass transfer barrier.[8] The improved stability of the automatically spun fibers and biocomposites in water is thus to be considered an advantage.

3.2. Biological Activity of the Biocomposites

Figure 3 shows scanning electron microscope (SEM) pictures of PVA fibers and the corresponding biocomposites prepared by automated wet spinning with a residence time of 32 s in the acetone bath as example. In these pictures the surfaces of the empty PVA fibers appeared smoother than that of the corresponding biocomposites, where the presence of the bacteria added to the roughness of the surface.

The presence and vitality of the embedded bacteria in the artificial extracellular matrix was subsequently tested in a Live/Dead stain assay. A particular concern was the acetone used in the hardening bath, which is known to be toxic for bacteria. Due to the change in fiber production from manual to automatic drawing, the contact time with the acetone had almost tripled, from 5 to at least 13 s. When the produced biocomposite fibers (13 and 25 s residence time in the acetone bath) were stained for live and dead bacteria (Figure 4), a thin layer of dead bacteria was found in the confocal microscopy pictures close to the surface (red color, indicative of dead cells), whereas the majority of

<table>
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<tr>
<th>Table 1. Characteristics of the PVA fibers.</th>
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<tr>
<td>Residence</td>
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<tr>
<td>Diameter[5]</td>
</tr>
<tr>
<td>Crystallinity</td>
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Figure 3. SEM pictures of microfibers A) without and B) with embedded *S. oneidensis*. 

the bacteria at the core of the fibers were alive (areas stained in green) (Figure 4A,C). As to be expected, the area dominated by dead cells was larger and more defined in the case of the biocomposite with a 25 s residence time in the hardening bath. When the microfibers were subsequently cultured for 7 d in LB medium (shake flasks) and then reexamined, the picture was similar. If anything, the areas of dead and live bacteria had become more distinct (Figure 4B,D). The confocal microscope was operated at its maximum resolution to obtain these images. In particular, in the frames of Figure 4B, D individual (dead) bacteria can be discerned as red dots close to the surface of the biocomposites.

A direct comparison with the hand-spun fibers was not possible here, as these fibers would have dissolved under assay conditions (aqueous environment). Previously, however, hand-spun fibers stabilized with a PPX coating had been investigated. Due to the coating (thickness 1 µm), these microfibers had a diameter of 199 ± 16 µm. In these fibers, dead bacteria were also found close to the surface directly after spinning, but after some days in culture, live bacteria had resettled these areas. In the case of the automatically drawn biocomposites, on the other hand, the layer close to the surface of the biocomposite fibers was apparently inaccessible or at least unattractive to the bacteria. It is tempting to assume that a difference in structure is responsible for that, perhaps caused by the higher mechanical load (stretching) applied during automated fiber preparation. A different alignment of the polymer chains caused by the stretching during preparation could then be permanently affixed by the hardening bath in the regions close to the fiber surface. Inaccessibility or reduced attractiveness to the bacteria could, e.g., be caused by a difference in water solubility (lower water content). Then the presence of these areas would also be responsible for the higher stability of the automatically drawn fibers/biocomposites in water.

Finally, the cultivation of the biocomposites in nutrition media served also a test for a possible release of the bacteria from the biocomposites into the surrounding solution. Escaped bacteria would have multiplied under these conditions and based on the known growth rate of *S. oneidensis* (0.42 h⁻¹) should have resulted in a measurable OD₆₀₀ within the day. From the fact that the nutrition medium was still clear even after 7 d of culture we concluded that in spite of the noticeable swelling of the automatically drawn biocomposites no release of the bacteria took place. This was subsequently corroborated by the cultivation of individual biocomposites on solid agar plates. Even a single bacterium released from the fibers under these conditions would have resulted in the formation of a colony within 24 h. This was not observed.

### 3.3. Mechanical Properties of the Microfibers

All fibers were subsequently subjected to standardized stress–strain curve measurements in order to evaluate the mechanical properties. Yield strength, ultimate strength, and strain were determined for microfibers with and without bacteria. In the case of the automatically spun fibers, the residence times in the acetone hardening bath were again adjusted to between 13 and 45 s. All data are compiled in Table 2.

In the case of the “empty” PVA fibers prepared as controls in the automatic spinning apparatus, the residence time in the hardening bath seems to have been of little consequence concerning the mechanical properties of the fibers. The values for yield strength, ultimate strength, and strain were similar for all fibers regardless of the time spent in the acetone bath. At the same time, the corresponding values for the PVA fibers created by hand were roughly four times lower. The corresponding
biocomposites showed a distinctly different behavior in that for these fibers there was a clear influence of the residence time in the acetone bath on the mechanical properties. While yield strength and ultimate strength of biocomposite microfibers with 13 and 25 s residence time were in the same range as for the corresponding empty PVA fibers, the strain was significantly lower. On the other hand, biocomposites, which had spent 32 and 45 s in the acetone bath showed similar strain as the corresponding empty PVA fibers, but increasingly lower yield strength and ultimate strength. Biocomposites drawn by hand were again comparable to the corresponding hand-drawn PVA fibers in terms of yield strength and ultimate strength, but showed higher strain.

Young’s modulus (the “elasticity coefficient”) was then derived from additional strain–stress measurements and describes the relationship between applied stress and resulting strain in the deformation of a solid body with linear-elastic behavior. The larger the Young's modulus, the more resistant the material becomes to elastic deformation. Figure 5 compiles the data for the PVA fibers and the biocomposites. As to be expected from the pronounced similarity in the mechanical properties, all PVA microfibers produced in the automated apparatus showed elasticity coefficient in a similar range, namely, ≈0.25 GPa. In the case of the PVA fibers drawn by hand, Young’s modulus was twice as high (0.51 GPa). Based on these results, the hand-drawn PVA fibers are significantly less elastic than the ones produced in the automated units, in spite of a significantly lower crystallinity. At present no explanation can be given.

With values of 0.64 ± 0.01 GPa (13 s residence time) and 0.62 ± 0.01 GPa (25 s residence time) Young’s modulus was more than three times as high for the more rapidly drawn biocomposites as for the corresponding “empty” PVA fibers. For residence times of 32 and 45 s Young’s modulus of the biocomposites with 0.23 ± 0.04 and 0.17 ± 0.03 GPa, respectively, falls into the same range as that of the corresponding PVA fibers. The same is true for the hand-drawn biocomposites (Young’s modulus: 0.20 GPa). A possible reason for these observations can be found in the assumption that in particular live bacteria serve as quasi-crystalline regions or enhance the effect of the crystalline regions on the stiffness of the fibers. The more quickly drawn fibers have a lower contact time and in consequence a higher fraction of surviving bacteria in the fringe regions of the fibers, where presumably the crystalline areas are also located. In the case of the hand-drawn biocomposites the presence of live bacteria has thus a similar effect as a crystallinity of 30–40%.

Finally, the fiber tension was calculated from the results of the tensile tests (Figure 6). Especially for shorter residence times in the acetone bath, the fiber tension under otherwise identical conditions is higher for the biocomposites (3.1 ± 0.02 cN tex⁻¹) than for the corresponding PVA fibers (2.1 ± 0.01 cN tex⁻¹). Furthermore, they are tenfold higher than PVA fibers, which are blended with soy protein (0.15–0.34 cN tex⁻¹) and 20-fold higher than the wet-spun fibers of PVA (0.145 ± 0.016 cN tex⁻¹) of the group from Katoh,[20] In all cases, the fiber tension decreases with increasing residence time in the hardening bath, with the most pronounced jump between 13 and 25 s, i.e., the range where also the increase in crystallinity was most pronounced. It was not possible to determine the fiber tension of the hand spun fibers, since not enough material could be produced in each case for a determination of the fiber weight.

3.4. Fiber Processing

The automatic wet-spinning unit thus made the preparation of biocomposite fibers possible, which were sufficiently

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Table 2. Stress–strain values of the wet-spun microfibers (n = 3).

<table>
<thead>
<tr>
<th>Residence time [s]</th>
<th>PVA fiber</th>
<th>Biocomposite</th>
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<tbody>
<tr>
<td>13</td>
<td>14.1 ± 1.2</td>
<td>13 ± 0.8</td>
</tr>
<tr>
<td>25</td>
<td>10.0 ± 1.7</td>
<td>19.8 ± 2.0</td>
</tr>
<tr>
<td>32</td>
<td>12.4 ± 0.5</td>
<td>22.1 ± 0.8</td>
</tr>
<tr>
<td>45</td>
<td>13.3 ± 1.4</td>
<td>21.5 ± 0.3</td>
</tr>
<tr>
<td>5*</td>
<td>3.8*</td>
<td>4.5*</td>
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</table>

Table 2a. Yield strength and ultimate strength of the wet-spun microfibers (n = 3).

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<td>13</td>
<td>27.1 ± 0.4</td>
<td>467 ± 49</td>
</tr>
<tr>
<td>25</td>
<td>19.8 ± 2.0</td>
<td>424 ± 108</td>
</tr>
<tr>
<td>32</td>
<td>22.1 ± 0.8</td>
<td>488 ± 17</td>
</tr>
<tr>
<td>45</td>
<td>21.5 ± 0.3</td>
<td>477 ± 0.1</td>
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<tr>
<td>5*</td>
<td>4.5*</td>
<td>110*</td>
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Table 2b. Strain of the wet-spun microfibers (n = 3).

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*Manually spun fiber.
long and mechanically stable to allow the processing into textiles. Fibers with Fiber tensions above 3 cN tex−1 are suitable for further processing with knitting machines. In view of a first exemplary application, biocomposite fibers (retention time = 13 s) for textile production were prepared with N. winogradskyi (28 × 10^6 bacteria mL−1) rather than S. oniedensis. The bacterium N. winogradskyi is slow growing (growth rate 0.014 h−1) compared to S. oniedensis. The preparation of sufficient amounts of biomass is therefore more challenging. However, the bacterium is used in environmental biotechnology, e.g., the removal of nitrite in wastewater. N. winogradskyi microfibers, used for knitting and weaving, had an average diameter of 199 ± 16 µm (n = 25) and were up to 12.5 m long, the mechanical properties were similar as previously determined for the S. oneidensis biocomposite fibers (data not shown). The exemplary machine processing into a knitted textile took place at the Fraunhofer Application Center for textile fiber ceramics in Münchberg, Germany. A circular knitting machine with a hand crank was used to ensure a controlled and, above all, low-force feeding of the microfiber into the machine. Figure 7 shows the resulting textile, namely, a tubular fabric with a total height of 12 cm (height of the bioactive stretch: 1 cm) and an average diameter of 8 cm. The white area of the tubular fabric was prepared using the biocomposite fiber, whereas the grey area was produced with a nylon yarn, which served as auxiliary yarn. This approach thus opens up new possibilities for processing biocomposite textiles in larger quantities.

In addition, a manual weaving frame was used for producing a woven textile. Due to the manual processing, only small tensile forces occurred during weaving, which prevented a breakage of the yarn. Using this frame, tissues with a stable structure could be produced in sizes of up to 150 cm^2. It was also possible to work under sterile conditions throughout, from fiber to textile production, since the apparatus was placed in a sterile safety cabinet. Figure 8 shows a hand-woven 2.5 × 4 cm textile made from a 7.5 m N. winogradskyi biocomposite fiber as filling thread and a nylon fiber as warp thread. A similar textile was prepared from an “empty” PVA fiber and used as negative control (no metabolic activity).

Both fabrics were then incubated for 48 h in a 50 mL nitrite containing nutrient medium (NB medium) in shaking flasks. Table 3 lists the nitrate concentrations measured during this time in the culture medium as well as the corresponding calculated consumption rates. While nitrite was rapidly consumed in the culture medium of the bioactive tissue, there was no detectable decrease in the culture medium of the control tissue (tissue without bacteria) detectable. Neither fabric showed any sign of dissolution during the cultivation.

Moreover, based on the decrease of the nitrite concentration in the culture medium, an estimation of the number of metabolically active bacteria in the biocomposite can take place. With a diameter of 300 µm (45 s residence time in the acetone bath) and a fiber length of 3 m, a total volume of 0.21 mL is calculated for the fabric. By means of the concentration of bacteria used in the PVA suspension of 28.7 × 10^6 bacteria mL−1, the entire fabric therefore theoretically contains a maximum of 6.1 × 10^6 bacteria. If the measured nitrite consumption rate is compared with the nitrite consumption rate of a planktonic culture performed in parallel, namely 62.4 mg d−1 10^−6 bacteria, it corresponds to the presence of 3.4 × 10^6 metabolically active bacteria in the tissue. Thus, if mass transport limitations were not considered, ≈60% of the embedded bacteria are still metabolically active. This is in the same range of dissolution during the cultivation.
as previously determined for the hand-spun PPX-coated single fibers.[6] In addition to the nitrite concentration, the OD$_{600}$ of the culture medium was regularly controlled during these experiments. Again, no increase in the OD$_{600}$ was observed, arguing that no bacteria were released from the textiles. This was subsequently verified by the cultivation of a small piece of textile on a suitable solid agar. Again, no colony formation was detected.

4. Conclusions

Using an automate wet-spinning apparatus, PVA fibers and biocomposites can be produced that are stable in aqueous solutions even without a protective coating. The maximum attainable length of the fibers is currently only limited by the size of the syringe holding the spinning solution. Concomitantly, the mechanical stability of the fibers is improved to a point where further processing (weaving, knitting) into fabrics becomes possible, if necessary with the help of auxiliary yarns. The bacteria in the biocomposites are alive and metabolically active. Using this approach, it becomes now possible to produce biologically active woven/knitted tissues and thereby significantly expand the application range of the “artificial biofilm” in industrial and environmental biotechnology.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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