Bacterial Materials: Applications of Natural and Modified Biofilms

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Over millennia, bacteria have developed clever strategies to build biopolymer-based communities in which they can survive even extremely challenging conditions. Such bacterial biofilms come with a broad range of fascinating material properties that—in settings such as medicine, food production, or other areas of industry—make it difficult to remove or inactivate them: they can stick to many surfaces, repel water and oils, and can even transport electrons. Inspired by the outstanding versatility and sturdiness of such bacterial biofilms, material scientists have set out to harness those properties and to create bacterial materials for different applications. However, as the range of technological applications employing biofilms keeps expanding, improved material properties or broader functionalities are desired. Here, such attempts where materials with improved properties were created by making use of either natural or modified bacterial biofilms are reviewed. The areas in which those bacterial materials may be used range from agriculture and (environmental) biotechnology over biomedical and electrical engineering to construction engineering.

1. Introduction

Bacterial biofilms are sticky and slimy substances that come with a variety of unique and sometimes annoying properties. In those biofilms, bacteria embed themselves into self-secreted, extracellular polymeric substances (EPSs).[1–6] Depending on the particular bacterial strain and the growth conditions during biofilm generation, the composition of this EPS can vary quite a bit.[5] Yet, in any case, these EPS crucially determine many biofilm properties and allow the resident bacteria to survive in challenging environments.[4,8]

Biofilms can colonize a broad range of different surfaces including those of natural materials such as stones, teeth, and plant roots[9–11] as well as man-made objects including pipes, hulls, and catheters.[12–14] Yet, bacterial biofilms not only adhere well to the surface of objects they colonize; the upper surface of many biofilms is sticky as well, and this property enables biofilms to adhere to each other and to neighboring materials.[15–19]

Another central property of bacterial biofilms is their slimy consistency. In most cases, bacterial biofilms can be described as viscoelastic solids, i.e., materials that combine liquid-like and solid-like characteristics but are dominated by the latter.[8,20–26] Depending on the bacterial species, the stiffness of biofilms grown in the lab ranges from a few hundred to several kPa.[15,20,27] However, when exposed to certain metal ions, which can be part of the natural environment the biofilms grow in, those stiffness values can be increased up to 1000-fold.[15,20,22] This finding already indicates the high adaptability of this biomaterial. Even more curious is the ability of biofilms to self-heal: even after exposure to large shear forces, they are able to quickly and fully recover their initial viscoelastic properties.[20,22] Together, those properties enable biofilms to permanently settle on solid surfaces—even in the presence of shear forces.[21,28,29]

Another key property some bacterial biofilms are able to develop is the ability to efficiently repel a broad range of fluids ranging from water to oils.[30,31] With such a high wetting resistance, biofilms can withstand erosion by flowing or dripping water, and they can protect themselves from toxic substances (such as antibiotics or metal ions) dissolved in liquids.[21,31,12] Moreover, even if bacterial biofilms can be successfully wetted, they still can restrict the diffusive entry of molecules into their core.[33–35] The macromolecular network established by the bacterial EPS is mainly responsible for this effect: molecules (or particles) that bind to the EPS are prevented from reaching the bacteria—and this can limit the efficiency of antibiotics or other antibacterial substances.[21,36]

Of course, the viability and proliferation of biofilm bacteria requires the metabolic conversion of nutrients, and certain biofilms have developed a specific internal architecture to allow for their perfusion.[25,37] As a side product of their metabolic activity, a subset of biofilm bacteria liberate electrons, which originate from the chemical decomposition of organic substances,[38] and there are even conductive biofilms that are able to generate an electric current.[39]

Altogether, these properties render bacterial biofilms sturdy and unique materials. In many cases, typically in industrial or medical
settings, biofilm growth has negative consequences for humans as the bacteria can contaminate food production processes or lead to infections.\cite{4,40–43} With the range of properties discussed above, it is typically quite difficult to remove biofilms from the surfaces they colonize or to chemically inactivate bacteria residing within a protective biofilm matrix. However, from a material scientist’s point of view, some of the unique properties of bacterial biofilms do not have to be a burden only—they also offer a variety of possibilities for applications in biotechnology, medicine, and even civil engineering (Figure 1). One option to make this happen is using genetic engineering tools, and there are many examples where this strategy was successfully implemented.\cite{24,44,45} As an alternative approach, the properties of biofilms can also be modified by manipulating the composition of this biomaterial, e.g., by adding (bio)polymers, nanoparticles (NPs), small molecules, or other bacteria. In the following section, we highlight selected examples of the latter. There, either natural biofilms or artificial combinations of bacteria with microscopic objects have demonstrated high potential to serve mankind by doing exactly what they are good at: being resilient, sticky, and liquid-repellent as well as chemically converting molecules into other products.

2. Applications of Natural and Modified Biofilms in Different Fields

2.1. Biofilms for Agricultural, Environmental, and Industrial Biotechnology

Several biotechnological applications highly benefit from mankind’s ability to make use of bacteria and their products. For instance, a large range of bacterial biocatalysts (enzymes or whole cells) have been developed to produce valuable molecules such as fine chemicals, pharmaceuticals, and ingredients of cosmetics.\cite{46} In addition, bacteria themselves can be interesting products themselves, e.g., as food ingredients\cite{47–49} or as additives to increase the sustainability of agriculture. The latter is achieved by the microorganisms acting as biocontrol agents,\cite{50} plant-growth promoters,\cite{51} or biofertilizers.\cite{52} Of course, also bioremediation approaches heavily depend on microbial activity\cite{53}—without them, wastewater treatment would not be efficient at all. In the following section, we highlight a few examples from those areas, where bacterial biofilms with dedicated properties were developed.

To obtain biofilms with tailored functionalities, synthetic biology tools can be employed, where genetic modifications on the bacterial genome are employed to change the biofilm properties.\cite{24,45} Typically, such a strategy is based on the bacteria secreting additional (or altered) biofilm matrix components, enzymes, or other functional molecules. Alternatively, different bacterial strains can be combined with each other or with synthetic components (molecules, polymers, or nanoparticles) during biofilm cultivation (Figure 2). In nature, biofilms comprise multispecies microbial consortia, which follow a symbiotic life style to better adapt to the environment. Inspired by this natural collaboration, cocultivation of bacteria producing cellulose (e.g., acetic acid bacteria or acetobacteria) with other, catalytic microorganisms can result in functional, living materials with increased production efficiency. Here, the cellulose-environment generated by one bacterial strain can act as an encapsulation agent for the other strain and thus provides protection to the latter.\cite{54,55} In another example where several additional functionalities were installed into a biofilm, the cellulose matrix secreted by the biofilm bacteria was modified by enzymes produced by yeast cells. There, engineered yeast cells were artificially integrated into the biofilm matrix by cocultivation, and this resulted in biofilms with altered mechanical properties: the biofilms were converted into viscoelastic fluids.\cite{56}

A different strategy aims at immobilizing enzymes in biofilms to obtain an enhanced bioprocess performance such as increased activity, robustness toward alterations in pH and temperature, and reusability. For instance, Romero et al.\cite{57} demonstrated how biofilm matrix components contribute to the immobilization of an extracellular bacterial enzyme. They showed that secreted lipase molecules are fully trapped in the biofilm matrix—there was no (undesired) loss of enzyme from the biofilm pellicle into the aqueous phase it was grown on. In the protected microenvironment of the biofilm matrix, the specific activity of this immobilized enzyme was increased, and the immobilized enzymes maintained 42% of their activity even after three catalytic cycles. Botyanszki et al.\cite{58} achieved an immobilization of amylase onto the curli fibers of Escherichia coli biofilms; to make this possible, they used genetic tools to achieve site-specific binding to the curli fibers, and this entailed improved enzymatic activity: As a consequence of this immobilization, the pH range within which the enzyme has good activity, was increased and the biocatalyst maintained a high activity even in the presence of solvents. Such improvements and those building on them\cite{59} may provide a big advantage in industrial applications, where organic solvents are necessary—
thus broadening the range of possible applications. Similarly, Dong et al.\[60\] made use of a chemical immobilization strategy based on carbodiimide coupling to covalently link an enzyme to the EPS of a *Bacillus subtilis* biofilm matrix without reducing enzymatic activity. Moreover, in the same study, magnetic nanoparticles were added to the enzyme-enriched biofilm. The authors suggested that this second modification may help retrieving the biofilm by magnetic forces, e.g., after it has been added to a complex environment, in which it is supposed to perform its catalytic activity.

Of course, the benefit of including nanoparticles into biofilms can go beyond enabling material recovery. The antimicrobial properties of certain nanoparticles are well established, and there are many biotechnological applications where nanoparticles are combined with biofilms to harness this property.\[61\] In agriculture, nanoparticles have already been used quite often as biocontrol agents against plant pathogens.\[62,63\] Recently, Mahawar et al.\[64\] combined silver nanoparticles with cyanobacteria and observed improved plant growth as well as better resistance toward pathogens than when those two agents were applied individually. Timmusk et al.\[65\] formulated different bacterial inoculations with titanium dioxide (TiO\(_2\)) nanoparticles to improve the resilience of plants toward drought, salt, and pathogen stresses. Here, the addition of nanoparticles to the bacterial inoculates considerably amplified biofilm formation on the rhizosphere of the plant; consistently, NP-containing formulations performed better than bacterial fertilizers alone. Similarly, in a study conducted by Vishwakarma et al.\[66\] combining rhizobacteria with silicon gave rise to better protection of plants against toxic effects than a rhizobacterial biofilm could provide itself.

Similar to equipping biofilms with enzymes, some nanoparticles can also convey catalytic activity to bacterial materials. For instance, Wang et al.\[67\] immobilized several nanomaterials in engineered *E. coli* biofilms to enable the reduction of polynitrophenol, the photocatalytic degradation of organic dyes, or photoinduced hydrogen production. In addition to establishing catalytic processes, nanoparticles can also boost the existing catalytic activity of a biofilm by enhancing existing extracellular electron transfer mechanisms. For instance, bioremediation of hexavalent chromium by *Shewanella oneidensis* biofilms formed in carbon nanotube (CNT)-enriched alginate beads was improved compared to biofilms formed without CNTs.\[68\] Adding quinone-based electron mediators to the CNT-enriched
Biofilm materials further enhanced this effect, and the resulting bacterial material turned out to be useful for the bioremediation of uranium.\textsuperscript{[69]}

For many biotechnological applications, e.g., for protein or metabolite biosynthesis, planktonic bacteria are highly suitable,\textsuperscript{[70]} however, when other bacterial properties are required as part of a functional material, biofilms are typically preferred over planktonic cells.\textsuperscript{[71]} To a large extent, this is due to the superior material properties of biofilms. Nevertheless, there are efforts to further improve these material properties, e.g., by incorporating functional entities (molecules, ions, minerals, or polymers) into the biofilm matrix. As one of the main components of natural biofilm matrices, extracellular DNA (eDNA) has been shown to play a crucial role in biofilm formation.\textsuperscript{[72]} Bacterial aggregation,\textsuperscript{[73]} adherence,\textsuperscript{[74,75]} and eDNA affect the mechanical strength and integrity of biofilms.\textsuperscript{[76–78]} Additionally, extracellular DNA modulate extracellular electron transfer throughout the biofilm matrix.\textsuperscript{[79]} and provide enhanced resistance against antibiotics.\textsuperscript{[80]} In fact, Chaves et al.\textsuperscript{[81]} suggested that tuning the viscoelastic properties and surface topography of biofilms by controlling the amount of eDNA within the biofilm may offer opportunities in biotechnological applications—yet this still needs to be explored.

From a physicochemical point of view, interactions between eDNA and biofilm matrix components (or antibiotics) can be rationalized by electrostatic forces acting between the strongly anionic DNA molecules and cationic groups from biofilm constituents.\textsuperscript{[82,83]} Another strategy to alter the interactions between certain biofilm matrix components makes use of ionic crosslinks, which can be generated by incorporating cationic metal ions into the biofilm material.\textsuperscript{[20,21,84]} Kretschmer and Lieleg\textsuperscript{[22]} showed that the size and valency of the ions in combination with the molecular configuration of anionic residues on the biofilm matrix polymers dictates if and how strongly the stiffness of the biofilm is increased by this approach. The ability to boost the biofilm stiffness may open the door for novel applications: for instance, by adding Fe\textsuperscript{3+} ions to the biofilm matrix, Zhang et al.\textsuperscript{[85]} could increase the internal mechanical strength of biofilms, which were genetically engineered to become highly sticky. With such a “living glue,” surface damage could be successfully repaired. A similar self-healing activity accompanied by a strong anticorrosion protection was observed by Liu et al.\textsuperscript{[86]} when they enriched a culture of cellulose-overproducing bacteria with Ca\textsuperscript{2+}. Here, calcite (CaCO\textsubscript{3}) formation within the cellulose-rich biofilm provided improved stability of biofilm coatings. The authors suggested that such anticorrosive biocoatings could be useful tools to increase the life time of metallic objects in marine environments. A (reversibly) increased biofilm stiffness as achieved by the addition of metal ions can also result in enhanced erosion resistance\textsuperscript{[21,29]}—and such a property can be a desirable feature for biotechnological applications. A similar result was obtained by Hayta and Lieleg;\textsuperscript{[26]} yet, there, bacteria were allowed to establish a biofilm matrix in the presence of purified biopolymers. As a consequence, not the shear stiffness of the biofilm but its surface topography and thus its mode of interaction with water was modulated such that the stability of the biofilm toward erosion was enhanced.

Embedding bacteria with polymers is a strategy not only used by naturally occurring biofilms—the same can be achieved artificially. In fact, the effects of embedding bacteria into purified biopolymers present in the matrix of certain biofilms (such as alginate and cellulose) has already been extensively studied—both, with the goal to investigate the bacterial behavior in different polymeric matrices\textsuperscript{[87,88]} and to create more robust bacterial catalysts for biotechnological applications.\textsuperscript{[89–91]} Recently, novel encapsulation techniques have been introduced to keep up with the latest developments in biotechnology. For instance, in a study conducted by Jaroch et al.,\textsuperscript{[92]} in situ encapsulation of mature biofilms was achieved, and the biofilms were grown on a hollow fiber membrane. As a result, the encapsulated biofilm could better resist the shear forces it was exposed to in a bioreactor. Importantly, this covering layer was permeable to air and nutrients, which guaranteed good cell viability. A different approach was followed by Panchal et al.,\textsuperscript{[93]} who filled liquid marbles generated from halloysite nanotubes with a bacterial culture. Here, the bacterial EPS produced inside the liquid marbles enhanced the mechanical strength of the spheres and stabilized their shape and volume by preventing evaporation. With these improvements, the encapsulated bacteria could be stored at room temperature for more than a week. Interestingly, a similar approach could be applied to nonbiofilm forming bacteria when the spheres were artificially enriched with polymers. A new, bioinspired method based on the self-assembly process of chitosan macromolecules was introduced by Park et al.\textsuperscript{[94]} Here, tyrosinase-producing bacteria modified the chitosan biopolymers such that they bound to the bacteria and formed a network around them. Artificial biofilms produced this way showed better cell loading capacity and cellular viability than those obtained via conventional encapsulation strategies. Also here, an application has already been identified: The authors showed that these synthetic biofilms can be employed in the bioremediation of crude oil: within 28 days, they could remove \approx90\% of oil from contaminated water.

Overall, these examples clearly highlight that, with further improvements in terms of production time, stability and recoverability, artificial biofilms have the potential to contribute to many other areas of biotechnology in the future.

2.2. Biofilms for Medical Applications

One of the natural habitats of bacterial biofilms is the gastrointestinal tract (GIT).\textsuperscript{[95]} In fact, in humans, there are approximately ten times more procaryotic cells than eucaryotic ones. Commensal bacteria are not only crucial for regulating our metabolism and immune system, they can also protect us against pathogens. Hence, avoiding (and, if necessary, curing) GIT dysbiosis is increasingly considered as a therapeutic approach to deal with GIT disorders. To maintain or regain a balanced microflora in the GIT, diet regulation, antibiotic treatment, and consumption of prebiotics or probiotics may be needed.\textsuperscript{[96]} The latter are living microorganisms which, when administered in adequate amounts, confer a health benefit to the host. More specifically, consumption of probiotics aims at regulating the gut microbiota by manipulating interspecies interactions.\textsuperscript{[97]}

During the industrial production process that is required to turn bacteria into food products suitable for oral consumption,
the probiotics are exposed to harsh conditions such as heat or cold; after production is completed, the prolonged storage, e.g., in fridges or cooling cabinets (4 °C), is not ideal for the bacteria either. In addition, until they reach the desired area (i.e., the intestines), probiotics pass through the extreme environment of the stomach—yet they need to be viable in large numbers when arriving in the gut where they are supposed to take effect. Thus, those beneficial bacteria require protection. Microencapsulation of probiotic bacteria is a well-established method to produce functional probiotic food products,[98] and several biopolymers or smaller molecules (such as milk proteins) have been employed to achieve this.[97,99] However, also the natural shell produced by the bacteria, i.e., the EPS, can provide the required protection: biofilm-embedded bacteria exhibit better resistance against extreme conditions and trigger a better immune response in the host.[100,101]

Even though probiotics in biofilm form come with a range of advantages compared to their planktonic counterparts, they can still be further improved. For instance, Cheow and Hadianoto[102] encapsulated Lactobacillus rhamnosus bacteria into double-layered, chitosan-coated alginate or carrageenan polymeric beads and then further incubated these microcapsules to enable the formation of biofilms in their core. As expected, those shielded biofilms process showed superior freeze-drying resistance and thermostolerance. Moreover, bacterial release into the intestinal mucosa was higher for such encapsulated biofilms. In 2014, the same group of researchers improved their probiotic delivery system by adding locust bean gum to their chitosan-coated alginate formulation, which boosted the resilience of the probiotic.[103] Similarly, biofilm loaded calcium pectinate beads produced by Heumann et al.[104] lead to sturdier probiotics; from those biofilm-spheres, the bacteria were released to the colon as clusters which provided a better anti-inflammatory effect and protection against GIT disorders than other probiotic forms of this bacterial strain. A better release of biofilm bacteria was also achieved by Vega-Sagardia et al.[105] who enriched their formulation with vegetable oil to increase the residence time of the probiotic biofilm in the stomach so that a Helicobacter pylori infection could be efficiently dealt with. A different approach proposed by Praveschotinunt et al.[106] aimed at enriching a probiotic biofilm with a therapeutic peptide to promote epithelial restitution. By introducing these modified biofilms, the authors were able to achieve mucosal healing and immunomodulation in vivo.

In addition to enriching the biofilm matrix, also fine-tuning the growth conditions of biofilms can render probiotics more resilient. For instance, Kiew et al.[107] examined the effect of biofilm age and growth medium affect the stress-resistance of biofilms. Moreover, also adjusting the detailed production process of encapsulated biofilms[108] and cocultivation with a second bacterial strain, e.g., combining lactic acid bacteria with B. subtilis[109] can improve the resilience of the probiotic. In the latter example, the EPS produced by B. subtilis is mainly responsible for the obtained protection effect. Importantly, the presence of this second bacterial strain comes with another advantage: in addition to their ability to secrete exopolymers substanes, B. subtilis bacteria have recently been reported to be able to help maintaining the balance of the GIT microbiota.[110,111] Another promising usage of biofilms in the GIT was described by Duraj-Thatte et al.[112] Here, a robust, self-regenerative hydrogel containing living bacteria was developed that showed an increased retention time in the GIT in vivo. Expression of mucin binding proteins by genetic modifications resulted in specific and strong adhesion of the bacteria-loaded hydrogels to the GIT tissue. Furthermore, the viscoelastic properties of this bacterial hydrogel could be adjusted by varying the type of mucoadhesive protein and the DNA content of the gel, and the authors suggested that such a system has the potential to serve as a drug delivery system.

However, the benefits of bacterial biofilms for our health are not limited to regulating the gut flora. Bacterial pellicles generated by certain bacteria belonging to the genera Agrobacterium, Acetobacter, Pseudomonas, Rhizobium, Azotobacter, Alcaligenes, Achromobacter, and Sarcina comprise almost exclusively bacterial cellulose (BC), and those have applications in biomedicine—with the bacteria being inactivated and washed out.[113,114] Owing to their high biocompatibility, water uptake capacity, permeability to gases and liquids, and desirable mechanical properties such as high tensile strength and flexibility, those BC materials have turned out to be good candidates for drug delivery systems[115] and wound treatment.[116,117] In addition, the structural similarity of BC-biofilms and human collagenous extracellular matrix enables the use of the former as tissue scaffolds.[118] Also here, the chemical and physical properties of BC-based materials can be further improved by incorporating polymers, nanoparticles, minerals, or functional molecules.[113,119,120]

More recently, engineered living materials have been introduced. Here, genetic engineering tools are combined with material science approaches to create novel materials for medical applications. For instance, Wang et al.[121] employed light- inducible biomineralization of hydroxyapatite to repair site-specific damages: E. coli biofilms expressing adhesins act as a glue that connects polystyrene microspheres thus creating a biohybrid filler material that autonomously solidifies via mineralization processes. Possible future applications of this technique could be in the field of bone regeneration. A similar E. coli biofilm producing adhesive molecules (adhesin and DOPA) was used by An et al.[122] to fight blood-leakage. In the lab, this already works: using a microfluidic setup mimicking a (slightly) bleeding blood vessel, it was shown that this living glue can autonomously repair small damages, and this is triggered by exposing the bacteria to the molecule heme. Although those examples still need to be developed further to be applicable in vivo, they present innovative new concepts of how bacterial biofilms could serve as promising tools for medical problems.

### 2.3. Biofilms with Enhanced Electrochemical Activity

Already in 1911, Potter could demonstrate that the decomposition of organic substances by bacteria or fungi can generate an electrical current.[123] However, this finding did not receive much attention until it was realized how the microbes make use of electron mediators in such a biological, electrogenic system.[123,124] Exoelectrogenic bacteria can transfer electrons directly to each other or to the surface of electrodes, and they
achieve this by employing outer membrane cytochromes, excreted mediators (i.e., electron shuttles) or biological “nanowires” (i.e., conductive pili). Recent improvements in the electron transfer capability of microbial communities promoted the development of applications making use of them. Examples of such bioelectrochemical systems (BESs) include microbial fuel cells (MFCs), \textsuperscript{124,125} microbial electrolysis cells (MECs), \textsuperscript{126,127} biological photovoltaics (BPVs), \textsuperscript{128,129} microbial desalination cells (MDCs), \textsuperscript{130,131} microbial electrolysisynthesis (ME), \textsuperscript{126,132,133} and microbial electrochemical biosensors (MEBs). \textsuperscript{144}

In BESs, a diverse range of microorganisms (typically, those are bacteria; however, there are also examples where algae or fungi are used) can be employed—both as isolated strains and mixed cultures, \textsuperscript{135} and either in form of planktonic cells \textsuperscript{136} or as biofilms. \textsuperscript{137} One of the major factors hindering the practical application of BES is the low electron transfer efficiency at the electrode. To overcome this issue, biofilms can be a convenient solution as they come with the advantage that they can grow directly on the electrode surface; moreover, in protective biofilm matrix, the bacteria are well connected to each other, which facilitates the electron transfer process from one bacterium to another. However, when this biofilm matrix becomes too thick, it may become an obstacle that limits the diffusive transport of nutrients and electrons. \textsuperscript{138} Hence, there is still a need to maximize the transport properties within biofilms as well as the electron transfer efficiency at the biofilm–electrode interface.

To improve the electron transfer process to electrodes, researchers have pursued several approaches, and they can be divided into two groups: the first strategy is based on a manipulation of biofilm growth to increase both, biofilm formation and the electroactivity of the biofilm bacteria. The latter is typically achieved by creating more options to transfer electrons from donors to acceptors. One option to achieve this is to increase the number of extracellular electron carriers (cytochromes, flavin- or quinone-based mediators, or conductive pili) by means genetic engineering. \textsuperscript{144,149} Of course, maximizing the number of microbial cells producing these carriers, e.g., by nutrient optimization, has a similar effect. \textsuperscript{139,140} An alternative approach aims at manufacturing electrodes with enhanced conductivity of with larger surface areas. Here, lots of effort has been made to investigate various electrode materials and surface treatments. \textsuperscript{123,141}

The second strategy aims at improving extracellular electron transfer through the BESs by integrating artificial components into either the liquid part of the BES (containing planktonic bacteria) or into the biofilm matrix—and the latter is typically achieved by growing the biofilms in the presence of those artificial objects. The first steps taken in this area were based on the addition of soluble electron mediators to the bacterial culture during microbial growth. Flavin and quinone containing compounds such as riboflavin, Neutral Red, Brilliant Blue, Methyl Violetene, and humic acid have been approved as electron mediators for indirect electron transfer purposes. \textsuperscript{126,142–145} Wu et al. \textsuperscript{144} have studied the performance of a BES making use of a \textit{S. oneidensis} strain in combination with five different mediators. They observed that, after 4 days of incubation, the current generated by the mediator-enriched samples was 20–60 times higher than the one generated in the control sample. Further-
2.4. Bacterial Construction Materials

Creating more sustainable building materials is one of the major goals in the field of civil engineering,[166] especially alternatives for cementitious construction materials are needed to reduce the CO₂ emission originating from the production of cement.[167] Interestingly, bacteria and bacterial products can also help here.[168,169] Even though the chemical conditions inside cementitious materials (such as the high pH levels occurring during the hydration reaction and the lack of nutrients) are not ideal for promoting bacterial growth, innovative concepts have been introduced that improve the functionality of construction materials by using bacterial additives (Figure 3).

One reason why biobased admixtures derived from bacterial sources have attracted lots of interest is their ability to replace commonly used, partially noxious or even toxic additives; at the same time, those biological additives can often be produced such that their environmental impact is comparably low. Prominent examples for such bacterial products are biopolymers generated by bacterial fermentation: examples include welan gum,[170–172] or xanthan gum,[173–175] both of which are used as viscosity modifying agents in concrete.[176,177] Similar effects were obtained with other bacterial additives, such as extracellular polysaccharides,[178] bacterial cell walls,[179] whole prokaryotic cells,[180,183] or bacterial biofilms.[182] This is important as the viscosity determines the workability of the uncured construction material—and this parameter often needs to be adjusted to meet the requirements of different applications.

In the literature, the viscosity-increasing effect of bacterial additives was attributed to a combination of different mechanisms.[183] First, water molecules can be bound by the additives via hydrogen bonds, and this can increase the viscosity of the hybrid material. Second, long polysaccharide chains present in the bacterial additives can, in combination with water, create a gel-like structure, and this boosts the viscosity. Third, bacterial additives are often charged; different anionic motifs from a polymer chain can interact with several positively charged cement particles, leading to bridging flocculation, and also this effect can tune the viscosity of the material.

In addition to modifying the viscosity of cementitious materials, a second important effect brought about by bacterial additives aims at improving the properties of the cured material. For a variety of different bacterial additives, e.g., bacterial cell walls,[184] and bacterial solutions,[185,186] such an improvement of the mechanical competence of the final, cured material has been reported.[187–189] Moreover, bacterial additives can enhance the corrosion resistance of load-bearing steel elements in reinforced concrete[190–192]—and this increases the durability and

Figure 3. Benefits obtained by using bacterial additives in construction materials. Bacterial additives such as bacterial suspensions, spores, secreted macromolecules or cell fragments, as well as whole biofilms have been shown to improve the functionality of cementitious materials. For instance, a) self-healing of microcracks and b) water repellency was achieved. Moreover, c) by replacing toxic chemicals with bacterial ingredients and d) by enabling biocementation processes, more sustainable construction materials were developed.
thus lifetime of objects making use of this class of building materials, e.g., bridge pillars, walls in high rise buildings, and tunnel constructions.

The microscopic mechanisms responsible for these properties can be as follows: as the porosity and the strength of cementitious materials are related,\(^{[193]}\) strength improvement is often achieved by calcite precipitation, which reduces the porosity. In addition, precipitated calcite can act as a diffusion barrier and therefore protect steel elements in concrete from corrosion, and reduced rates of oxygen ingress can contribute to a higher corrosion resistance as well.\(^{[194]}\)

Corrosion of steel elements in concrete is driven by the ingress of chloride and sulfate ions into the bulk of the material—and this is made possible by invading water transporting the ions. The latter can occur via rain or water splashes, or it can originate from capillary water uptake when cementitious structures are erected in moist environments. Importantly, also in this context, bacterial additives have turned out to be extremely helpful: both, the external wetting resistance of mortar and the suppression of the capillary water uptake into the material can be enhanced using fresh\(^{[195,196]}\) or freeze-dried bacterial biofilm\(^{[197]}\) bacterial solutions\(^{[197]}\) or bacterial spores.\(^{[198]}\)

In those cases, it was suggested that a modification of the microstructure of the mortar material is responsible for the increased water resistance: increased roughness features on the inner and outer surface of the mortar as well as alterations in the density of the material were observed when bacterial additives were used. Yet, it remains to be shown which particular microarchitecture of bacterial hybrid mortar provides the overall optimal set of material properties—and how this ideal microstructure can be achieved.

Increasing the service life of cementitious structures is certainly a great step toward more sustainable building concepts. Yet, emerging trends from this field aim at developing cement-free building materials to completely erase the greenhouse gas emission caused by the cement production. Here, alternative binders, e.g., alkali activated slag, may offer a possible option.\(^{[199]}\) One limitation of this approach is the considerable material shrinkage triggered by alkali activation as well as insufficient containment of moisture in the material volume. Again, by using bacterial biofilm as an additive, those two issues could be successfully addressed.\(^{[200]}\)

Whereas, in the examples discussed above, a modification of the material properties was directly achieved by the addition of bacteria or bacterial products, a second strategy employed in the area of civil engineering aims at exploiting the unique ability of bacteria to take part in, control, or initiate biomineralization processes.\(^{[201]}\) Indeed, also this approach has led to many new developments toward the creation of more sustainable building materials,\(^{[202]}\) and most of them make use of microbial-induced calcite precipitation. One prominent example from this area is the concept of self-healing cementitious materials.\(^{[203–205]}\) In this approach, bacterial spores are added to the bulk of concrete. Due to their unique structure, bacterial spores can withstand harsh conditions without losing their viability.\(^{[206,207]}\) Instead, they remain dormant without any perceivable metabolic activity until they are reactivated by contact with moisture and oxygen. The latter is made possible when cracks have formed in the material through which water and air can enter. In other words, damage to the material serves as a “wake-up call” which then triggers autonomous repair: The metabolic activity of the reactivated bacteria induces calcite precipitation,\(^{[208]}\) and this, in turn, can seal microcracks (in the range of 0.46 mm).\(^{[209]}\) For such self-healing concrete, different types of bacteria have been identified,\(^{[209]}\) and they utilize different precipitation mechanisms\(^{[210]}\) to achieve this effect.

Bacterial precipitation is also the basis for the patented concept of “biocementation.”\(^{[211,212]}\) Here, instead of sealing cracks in the cured construction material, bacteria are employed to produce calcium carbonate, and this mineral can solidify sand or other gravel particles without the need of a binding agent.\(^{[213]}\) The properties of such “bacterial soil” depend on several factors including the concentration of added bacteria and urea, and the grain size distribution.\(^{[214,215]}\) However, real-life applications of this idea have not been tested yet. Along the same lines—yet taking this idea one step further—the concept of “living building materials” is discussed by Heveran et al.\(^{[216]}\) As the name already suggests, microorganisms inside such a material remain viable and thus can react to alterations in environmental conditions such as temperature and humidity by switching on (or off) material growth.

For such a novel class of living buildings, it was even suggested that—once the end of the service life of the building is reached—the material can be largely recycled. Whether or not this is really possible, future research will have to show. Overall, the results we highlight above clearly demonstrate the great potential innovative bacterial materials hold for developing a novel, more sustainable class of construction materials with improved properties.

### 3. Outlook

Bacterial biofilms have the potential to be so much more than just a nuisance. The examples we highlight here stem from selected areas of bioprocess, biomedical, agricultural, environmental, electrical, and civil engineering and demonstrate how different material properties of biofilms can be used to generate objects with tailored functionalities. Together with fundamental insights into how those material properties can be further boosted or modified, a broad range of applications have already been identified that make use of bacterial materials. With the current improvements in additive manufacturing techniques,\(^{[217–219]}\) our ability to control the composition, architecture and shape of objects is improving day by day.

Indeed, there are already a few recent examples where such advanced manufacturing methods have been applied to create bacterial materials.\(^{[220–222]}\) For the purpose of wastewater treatment, an artificial biofilm was printed into a grid-like structure to obtain an object with a very high surface area. As a bacterial strain for this particular application, *Pseudomonas putida* was selected, which is capable of degrading phenol and converting it into biomass.\(^{[223]}\) As an example of a medical application, we would like to highlight 3D-printed *Acetobacter xylinum* bacteria, which—once embedded into a hydrogel matrix—produced cellulose.\(^{[224]}\) Once enough cellulose was secreted, the bacteria and the hydrogel were removed by washing leaving a cellulose
scaffold in a predefined shape as realized by the printing process. Such cellulose scaffolds were suggested to support the wound healing process—especially in areas having complex shapes such as the face. Also in electrical engineering, 3D-printing of bacterial structures has been attempted and living anodes for microbial fuel cells were produced. With this technique, bioelectrodes could be fabricated with a high level of control in terms of geometry and porosity. A different approach was realized by Moser et al., who used light signals to pattern E. coli on solid surfaces to induce the biofilm formation in a desired shape.

To arrange any printable material into a dedicated 3D shape, the viscoelastic properties of the “ink” need to be just right—and the same holds true when attempting to print biofilms. During printing, the biofilm needs to have the properties of a liquid; yet afterward, it has to stay in place and maintain its shape which requires elastic properties. Owing to their viscoelasticity and stickiness in combination with their self-healing abilities, “naturally grown” bacterial biofilms meet these requirements. However, when specific functions are desired, artificial biofilms are preferred and a viscoelastic matrix (typically a hydrogel comprising either alginate, hyaluronic acid, carrageenan, or fumed silica) is loaded with the bacteria of choice or the expression of bacterial EPS is manipulated. Here, the artificial biofilm matrix not only needs to provide the required mechanical stability but has also to ensure bacterial survival and metabolic activity. Duraj-Thatte et al. used a nanofiber gel produced by bacteria as a matrix for 3D-printing. Then, the original bacteria were removed from the gel by washing and replaced with different bacterial cells and selected additives.

In addition to 3D-printing, several other methods have been reported to control the structure of cellulose-based biofilms and to create complex shapes. For instance, biofilm spheres could be produced by adding PTFE nanoparticles by employing microfluidics methods using alginate–agarose as a shell structure or by making use of water-in-oil emulsions. With this range of methods, spherical biofilms with tunable sizes can be produced which might be useful for encapsulation purposes in food engineering and biomedical applications.

At this point, material science, microbiology, and manufacturing science meet and open up a plethora of new avenues that still need to be explored. Considering the huge variety of bacterial species and our growing ability to control the properties of bacterial biofilms, many new and exciting developments are possible in this area.

Keywords
bacterial additives in construction materials, bacterial biofilms, biofilms for medical applications, biofilms in agriculture, biofilms with electrochemical activity

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Conflict of Interest
The authors declare no conflict of interest.

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