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Review article

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Beneficial applications of biofilms

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Abstract

Many microorganisms live in the form of a biofilm. Although they are feared in the medical sector, biofilms that are composed of non-pathogenic organisms can be highly beneficial in many applications, including the production of bulk and fine chemicals. Biofilm systems are natural retentostats in which the biocatalysts can adapt and optimize their metabolism to different conditions over time. The adherent nature of biofilms allows them to be used in continuous systems in which the hydraulic retention time is much shorter than the doubling time of the biocatalysts. Moreover, the resilience of organisms growing in biofilms, together with the potential of uncoupling growth from catalytic activity, offers a wide range of opportunities. The ability to work with continuous systems using a potentially self-advancing whole-cell biocatalyst is attracting interest from a range of disciplines, from applied microbiology to materials science and from bioengineering to process engineering. The field of beneficial biofilms is rapidly evolving, with an increasing number of applications being explored, and the surge in demand for sustainable and biobased solutions and processes is accelerating advances in the field. This Review provides an overview of the research topics, challenges, applications and future directions in beneficial and applied biofilm research.

Sections

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Introduction

In recent years, interest in developing biofilm systems to produce value-added compounds has grown, and the term 'productive biofilms' is used in the research community for such systems[1]. These biofilms can be composed of pure cultures or can be highly diverse communities. Biofilm systems, in which organisms interact closely, allow the formation of niches characterized by sharp gradients that alter the range of thermodynamic and processing opportunities over distances of just a few micrometres[2]. Productive biofilm systems have been covered in several reviews exploring various applications, reactor designs and imaging methods[3–5]. In this Review, we expand our focus to 'beneficial biofilms', encompassing a broader research spectrum that includes the study, transfer and practical applications of biofilm systems. Such benefits include the use of biofilms for bioremediation, in agriculture[6], as programmable materials or as surface-covering entities[7,8].

The increasing interest in beneficial biofilm research in recent years has been driven by the fundamental properties of biofilms: surface aggregation, adherence, continuous operation, matrix development and heterogeneous metabolism. With the exception of granules, biofilms are cellular aggregates that are mostly bound to surfaces, which gives them the character of a retentostat (a bioreactor wherein growing cells reach a steady-state condition, in which culture liquid is removed from the bioreactor but a filter retains the biomass) with a stationary biocatalyst operating in a continuous mode. Thus, long-term operations are possible, with a reduction in the amount of labour invested in product–biocatalyst separation. The adherent behaviour of the cells is due to complex regulatory routines that lead to the production of an extracellular matrix with varying composition and properties. The matrix holds the cells together and forms a tight connection with the biofilm substratum[9,10]. Shape, composition and biofilm density are modulated by the interplay of outer conditions and the corresponding reactions and capabilities of the biological system. At the same time, these characteristics will influence diffusion coefficients and hence the mass transfer kinetics. Operating within continuous biofilm systems allows the biocatalysts to evolve and adapt to the process conditions, which can lead to a self-advancing system. However, biofilms also display heterogeneity in metabolic activity, and we are just starting to understand the various underlying reasons[2]. However, heterogeneity and the development of a biofilm matrix aid in the stability and resilience of biofilms against harsh conditions and toxic substances[11]. This heterogeneity is a key feature for many applications in which certain compound concentrations inhibit the growth or survival of planktonic (free-living) cells, yet cells within biofilms are still able to thrive[12].

The application of biofilm systems often necessitates specific reactor designs. The prototype for the operation of biotechnological processes is the stirred tank reactor. Nevertheless, this reactor design is only suitable for processes operating with planktonic biocatalysts or flocculent biomass. All reactor types established for biofilms bound to a surface thus far share the goal of achieving a very high surface area to volume ratio. The limit for this ratio is the point at which biofilms will bridge surfaces in such a way that a predictable fluidic regime can no longer be sustained and parts of the reactor are clogged. The surface on which the biofilms grow is called the substratum. In this Review, we differentiate between active and inactive substrata. Inactive substrata serve simply as growth supports for the organisms; besides certain material characteristics such as high roughness or an appropriate charge to enhance interactions with the usually negatively charged surface of the microorganisms, they do not further influence cell metabolism[13]. Active substratas have a dual purpose: they provide a surface on which the biofilms grow and also consist of, or deliver, ingredients for growth of the microorganism. One of the simplest active substrata for the application of biofilms is wood chips. They serve as a slowly degradable carbon and electron source for biofilms that cover their surface. This interaction between biofilms and wood chips is already applied on a large scale in field-denitrification plants[14,15]. Further examples include membranes that supply gaseous substrates to biofilms or the anode and cathode in a bioelectrochemical system, which act as an electron acceptor or donor, respectively[16]. Moreover, in some cases, biofilm catalysts might not need a growth substratum. An example is aerobic granular sludge, which is a mixed-species microbial biofilm that forms densely packed granules in the wastewater, aiding in the removal of carbon, nitrogen and sometimes phosphate[17]. Besides aerobic granular sludge, anaerobic granules are also used in wastewater treatment. Here, the biofilms are mainly used for nitrogen elimination. For instance, anammox bacteria have been applied in the form of anammox granules to aid in anaerobic conversion of ammonium as the electron donor with nitrite as the electron acceptor to dinitrogen[18,19]. The formation of granules allows us to apply these slow-growing organisms in wastewater treatment plants (the hydraulic retention time would lead to a dilution of the organism if it were planktonic). Similarly, granules formed by methane-oxidizing, nitrite-reducing bacteria and nitrate-reducing archaea have also been shown to be applicable in wastewater treatment. Recent work has suggested that both types of granule (anammox and nitrate/nitrite-dependent methane oxidizing) can be combined in one biocatalytic anaerobic granular system[20].

Some biofilm-based processes have already been developed to allow industrial application, especially in the water-treatment sector, where sand and trickling filters have been used for many years in water purification[21]. In wastewater treatment, rotating biological contactors use biofilms on rotating disks that constantly alternate between wastewater and air, facilitating carbon elimination and ensuring an energy-efficient oxygen supply. Aerated membrane biofilm reactors have similarly been implemented on an industrial scale in wastewater treatment. In this setting, the biofilms grow on a membrane surface that supplies the organisms with oxygen. Also used in water treatment, biofilms of nitrifying and anammox bacteria flourish on plastic growth substrata. These biofilms grow on suspended biofilm carriers that were designed to provide as much surface area as possible for biofilm growth while protecting the biofilm from shear-force-based dissolution[11,22].

Moreover, industrial–scale biomethanation and vinegar production processes have been developed using biofilms of methanogens or acetate-producing microorganisms, respectively, growing on inactive substrata in a trickle bed reactor[23,24]. Yet, aside from these examples, biofilm-based processes have not been developed sufficiently to reach competitive space-time yields in white biotechnology (the production of compounds relevant to the chemical industry utilizing microbial cells as catalysts) or green biotechnology (biotechnological techniques using photosynthetic organisms and agriculture for sustainable biomass production, improved growth and disease resistance in agriculture).

This Review aims to broaden the understanding of productive biofilms to a diverse audience. We provide an overview of the chemistry and molecular biology of biofilms in Box 1 and then explore the benefits and challenges associated with beneficial biofilms, including an overview of the reactor technologies that are available so far to harness the potential of biofilm systems. Thereafter, we highlight key innovations

Box 1

Biology and chemistry of biofilm systems

It has been estimated that 40–80% of microbial cells on the Earth grow as biofilms[143]. The topic of whether microbial biofilm formation is based on a dedicated developmental programme leading to multicellularity has been discussed extensively[144]. However, after an initial selection of a regulatory routine for surface growth or granule formation, the further stages of biofilm maturation could certainly be due to an adaptation of single cells to existing or developing gradients, and these routines do not necessarily seem to be dependent on biofilm lifestyle and multicellularity itself. Also under discussion are the frequently described individual stages of biofilm formation. A model was recently proposed describing the process of biofilm development and disassembly[145]. In doing so, the authors abandoned the assumption that all biofilms develop from individual cells on surfaces and that after some time, these cells adopt mushroom-like structures. Instead, they advance the model to incorporate environmental multispecies systems and posit that biofilms do not need a solid substratum, that multicellular cell clumps can also initiate biofilm formation and that the architecture of mature biofilms can vary to a large extent[146].

Biofilm formation can be triggered by a variety of environmental cues, but two have been studied and understood in more detail. The process that these triggers have in common in most bacterial model organisms is that they directly or indirectly lead to an increase in the intracellular concentration of the messenger molecule cyclic diguanylate (c-di-GMP). An increase in intracellular c-di-GMP concentration usually triggers biofilm formation, whereas low concentrations suppress biofilm formation[147]. Key enzymes for formation and hydrolysis are c-di-GMP cyclases and phosphodiesterases. Important triggers for the regulation of these enzymes are the recognition of surface adhesion or the cellular recognition of population density by quorum sensing. Interactions of cells with surfaces lead to increased torque during flagella rotation, a physical parameter that seems to cause alterations in the composition of bacterial flagella. Free stator components in the model organism *Pseudomonas aeruginosa* were shown to interact with the membrane-bound c-di-GMP cyclase SadC, and these interactions are associated with activation of this enzyme[148]. Moreover, the activity of the cyclase seems to be further dependent on type IV pili, which are also thought to be involved in surface sensing and the primary stages of biofilm formation[149]. Interestingly, the immobility of the cells, which is a typical characteristic of cells in biofilms, can then be further strengthened by specific dissolution of the motor–stator complexes mediated by the c-di-GMP-dependent activity of the protein FlgZ[150,151]. Not only flagella and pili but also several other surface structures, including curli fibres, can mediate initial surface adhesion[152]. Besides the direct surface recognition, quorum sensing can also be a trigger for biofilm formation. In this context, individual cells produce subthreshold concentrations of a messenger molecule. When the cell density and thus the concentration of the molecule exceeds a certain value, a concerted overall reaction of the microbial community occurs. One of these reactions can be the formation of biofilms. Interestingly, in mature biofilms operating in a continuous system, the concentration of quorum-sensing molecules will decline with the decrease in distance between a cell and the biofilm surface at the bulk phase. Thus, over micrometre distances, quorum-sensing-mediated regulation can lead to drastically different regulatory programmes operating in biofilms[153].

Only a subset of individual cells that attach to a surface will form microcolonies. These microcolonies might merge into superstructures or potentially compete for the substratum[154]. The maturing biofilm is characterized by the production of extracellular polymeric matrix components that can have several functions for the organism but are foremost a 'glue' that keeps the biofilm in place. We have a general knowledge of the monomers that build the matrix but no clear understanding about the polymeric structure and the structure–function relationship[10]. Nevertheless, the packing of cells in biofilms is not uniform and growth kinetics, substrates, shear forces, and/or process or environmental conditions in general can have a tremendous effect on biofilm shape and porosity in addition to cell–cell distance[155]. Thus, packing will have an impact on diffusive mass transfer limitation and several other factors modulating biofilm physiology.

Finally, biofilm dissolution ends the biofilm development cycle. The latter can be caused by various cues, including the availability of nutrients, electron acceptors and variations in quorum-sensing signalling. In general, cells dispersing from biofilms are characterized by lower cytoplasmic c-di-GMP concentrations[156]. Recently, a biophysical model was developed that can be used to predict quorum-sensing-based biofilm formation and biofilm stability. The model is based on dimensionless parameters that integrate information concerning cell concentration and motility, nutrient diffusion, consumption, chemotactic sensing and autoinducer production. This model's simplicity will allow wide applicability and extension to more factors controlling biofilm formation[157].

and applications in white and green biotechnology as well as agriculture and wastewater resource recovery. We then explore the application of biofilms as materials and coatings, and discuss future trends in biofilm engineering and the applications of beneficial biofilms.

The advantages of applying biofilms

Biofilms offer a wide range of opportunities for biotechnological application and in materials science. Products can be separated from biomass with ease and processes can operate with hydraulic retention times that do not depend on the growth rate of the biocatalyst. An example of the latter is the previously mentioned introduction of the anammox process in wastewater treatment. Here, a biofilm of nitrifying and anammox bacteria on suspended biofilm substrata is key[25]. Both groups of organisms are chemolithoautotrophic and growth rates are too low to sustain a continuous wastewater treatment process using planktonic cells. In fact, shortening the hydraulic retention time below to less than the doubling rate is a clear selection parameter for biofilm formation. The interaction of nitrifying bacteria with anammox bacteria is also a

good example of another advantage of biofilm processes. Organisms with drastically different growth requirements can operate together in these systems because the localized biological activity will lead to the formation of sharp gradients[2]. As an example, aerobic respiration by nitrifying bacteria leads to anoxic conditions within the biofilm core and an accumulation of nitrite. Anammox bacteria require anoxic conditions because they use nitrite as an electron acceptor for a respiratory process in which ammonium is the electron donor. Another advantage is the rather high biocatalyst concentration. Densities of 200–300 grams dry weight of cells per litre were reported for biofilm processes. This exceeds what is typically reached in processes that rely on planktonic cells and can only be reached by high-cell-density fermentations that have been reported to operate with cell dry weights of 100–200 g per litre[26,27]. Moreover, some biocatalysts cannot be cultivated to competitive cell densities in planktonic reactor systems compared with biofilm systems[28–30]. The high biocatalyst concentration in biofilm processes has a positive influence on achievable space–time yields. The operation of biofilm-based plug–flow processes can lead to adaptations of the organisms to the stable substrates and product gradients, which may come with the advantage of self-optimization of the biocatalyst. In other words, selective evolution of the biocatalysts can advance the organisms towards a specific function if the process conditions are stable. Such stable process conditions can easily be achieved in biofilm systems. By contrast, the traditional operation of a batch system requires the whole biocatalyst community to continuously adapt to the decreasing substrate and increasing product concentration.

Along these lines, microorganisms thriving in biofilms are characterized by higher stability or resilience, mostly thanks to the extracellular matrix, which confers a diffusion barrier, and to the heterogeneity of growth rates within the biofilm community[31]. Cells with lower growth rates are less susceptible, for instance, to antibiotics that target cell-wall biogenesis because the susceptibility correlates with microbial growth rate[32]. The resilience is also an important factor in applied biofilm technologies because the biofilm-organized cells tolerate higher substrate concentrations or organic solvents. For instance, it was revealed that biofilm systems can be operated with higher concentrations of organic acids compared with planktonic cells in bioelectrochemical systems, and that biofilms can tolerate higher concentrations of styrene, which is used as a substrate to produce styrene oxide[12,33]. This biofilm resilience can be modulated by process conditions. The concerted action of compression of the biofilms and increased flow velocities leads to a decrease in the diffusive mass transfer limitation, which can cause an increase in the susceptibility to toxic substances[12]. This general characteristic of increased resilience of cells in biofilms is also important for the application of biofilms in the field of bioremediation. In this context, biofilms are used to degrade xenobiotic substances, change the oxidation state of toxic metals to lower their bioavailability or are applied as nonspecific absorbers for pollutants[34]. These processes typically use inexpensive materials (for instance, activated carbon, sand or rocks) as inactive biofilm substrata[11].

The abovementioned adaptive responses to gradients during continuous operation are enabled by a diverse set of mechanisms, including simple regulatory routines or genomic alterations caused by insertion sequences, transposons, prophages and/or the mutation rate of DNA polymerases[35]. These elements and the genome repair machinery tune genetic stability and allow most members of a community to adapt optimally to an environmental condition using the available genetic information. However, some members of the community will not be isogenetic with the majority or will at least follow another regulatory programme. Because of its diversity, this fraction ensures stability and the flexibility to react to changes in the environment[36]. The power of mutation and selective forces as drivers of genomic instability is illustrated by the *Escherichia coli* long-term evolution experiment (LTEE), which was conducted by Richard Lenski's group[37]. In 1988, the group started with 12 *E. coli* populations that have been ever since always transferred within the same medium. After 50,000 generations, genome sequencing revealed more than 14,000 point mutations and more than 2,000 insertions and deletions. What is the connection between microbial genetic stability and productive biofilms? Considering the development of axenic biofilm processes with genetically modified organisms, the benefit of operating biofilm systems with high biomass concentrations over very long production campaigns in continuous mode could be questioned if the genetic stability of the production organisms would lead to process unpredictability. Thus, work with productive biofilm systems must follow one of two directions: (1) either the biocatalyst must derive a fitness benefit from being productive; or (2) the biocatalyst must be synthetically stabilized. Very simple examples of the first direction are acetogenic or methanogenic biofilms grown on membrane supports. The organisms gain energy from producing either methane or acetate from hydrogen and CO_2 (or CO) that can be added through the membrane. Thus, natural selection will favour productive organisms that not only have fitness benefits but are also beneficial with respect to their process productivity. Conversely, the genetic modifications necessary for many production processes are likely to lead to a decrease in the organism's fitness. If an organism is forced to use a certain percentage of its substrate for production processes, it will have a lower biomass-formation rate compared with the wild type. Thus, selection will probably lead to unwanted events in strain evolution. Nevertheless, several groups have focused on establishing higher genetic robustness in microbial strains[38]. This robustness can, for instance, be gained by reducing the genome and concomitantly deleting mobile DNA elements or cryptic virulence genes[39–41]. This robustness can be further enhanced by disabling some stress-induced DNA-repair mechanisms that are conducted by error-prone DNA polymerases[42].

Considerations for biofilm reactor design

In this section, we explore general aspects that need to be considered for the design and construction of bioreactors. These include, for example, the hydrodynamic conditions in the system and the directly coupled mass transfer. In addition, some developments will be described that enable the cultivation of biofilms in a medium mist, which reduces the amount of medium required to a minimum.

Hydrodynamic conditions and mass transfer

Hydrodynamic conditions inside biofilm reactors are of great importance for overall biofilm development. In addition to morphology, properties such as thickness and elasticity of the biofilm are influenced by flow. Furthermore, adhesion can change depending on hydrodynamic forces.

Regarding the mass transport to and especially within the biofilm, the mixing behaviour and boundary-layer thickness inside the reactor have a substantial impact on biocatalyst performance. Today, coupled computational fluid dynamic–discrete element methods[43] can be used to make predictions in biofilm reactor design to avoid flow obstacles, vortex formation and dead zones. In addition, good reproducibility of the flow conditions on top of the biofilm surface is

necessary. Furthermore, such models should facilitate the scaling-up of the reactors. In this situation, geometric (dimensions), kinematic (flow conditions) and dynamic (forces) similarities are important criteria when scaling up biofilm reactors[44]. The transport conditions (diffusion constants) in the biofilm itself are usually investigated using microprobes or fluorescence recovery after photobleaching methods[45] with the use of confocal laser scanning microscopes. Still, this method in addition to other optical methods can only be used for thin biofilms. Based on the fluorescence recovery after photobleaching method, a variety of models have been developed over the last five decades, starting with work[46] to calculate diffusion behaviour in biofilms[47]. Critical comparisons of the different methods usually show that, depending on the selected boundary conditions, very strongly diverging results are obtained, which sometimes lead to deviations of more than 30%[48]. Therefore, one study[48] ultimately concluded that "...it might be sufficient to use two relative diffusion coefficients in biofilm models: a high value of 0.5–0.8 for small solutes, such as oxygen, and a low value of 0.1–0.4 for medium-sized solutes, such as glucose and acetate". Some reactors have been developed for the cultivation of biofilms that meet the special requirements of these systems (Fig. 1). These requirements include, in particular, that the mass transfer (gas phase and dissolved substrates) between the bulk phase and the biofilm occurs only via the surface of the film. Designs that might address this are fixed-bed and fluidized-bed reactors, which are also frequently used in already-established industrial biofilm processes. A distinction can be made between submerged and surfaced reactor systems in addition to intermediate forms, such as rotating disk reactors. Despite the advantages (such as higher biomass concentrations and continuous operation), biofilm reactors have so far seen little use in industrial applications, because process control is still only poorly established. Therefore, their applications are currently mainly limited to the field of wastewater and drinking-water treatment. For a detailed review on biofilm reactors and their applications please refer to ref. 4.

Surface-to-volume ratio

In contrast to systems with suspended cells, not just the reactor volume is important but, in particular, the ratio of reactor volume to cultivation surface. This is not only important for the mass transport as explained above. Another characteristic of productive biofilms is, for example, the provision of non-dispersible substrates such as light and electrons. In these cases, a large surface-to-volume ratio is also required. Light and electrons cannot be uniformly distributed in the reactor using a stirrer or other mixing methods, unlike other substrates. Their supply is only possible via a surface (electrode) or irradiated reactor volume. When cultivations are carried out in suspension, this criterion can only be met by a particularly high surface-to-volume ratio. This parameter cannot be achieved with conventional designs. As biofilm reactors usually require a surface for growth, the light and/or electron supply requirements of the microorganisms can be considered in the design of the reactors. Of course, transparent growth bodies are suitable for phototrophic organisms and conductive systems in the field of electroactive biofilms. For example, a novel reactor system was developed for the cultivation of terrestrial phototrophic cyanobacteria. This system enables an optimal supply of light and nutrients to the organisms. Although cultivation of terrestrial cyanobacteria as a suspended culture in submerged systems is also possible, productivity is limited[49], and the physiological and production-specific properties of phototrophic biofilms cannot be exploited. Some of these terrestrial cyanobacteria (and other microorganisms) do not grow at all in an aqueous solution but grow optimally in a nutrient mist that prevents the biofilm from drying out. Thus, special reactors have been developed[49] to enable cultivation of terrestrial cyanobacteria as phototrophic biofilms (Fig. 2). These systems both let in light and provide an exceptionally high surface area[49]. To make this possible, the substratum is surrounded only by a nutrient mist and is fully illuminated so that the culture volume is only the volume of the biofilm (Fig. 2b,c). Such reactors have led to higher productivity and more efficient harvesting processes compared with suspended cultivation systems[50]. In immersed cultivation, the microorganisms are supplied with a mist-like nutrient aerosol. The aerosol provides the necessary moisture and nutrients for the biofilms and is generated from the medium by suitable vaporization techniques, such as ultrasonic vaporization. This process enables a resource-efficient operation as only the required amount of aerosol is generated and released into the reactor in a demand-controlled manner. Compared with submerged systems, the water requirement is minimized. In addition, the phototrophic biofilms can be selectively dried or supplied with less aerosol before harvesting to save energy for downstream processes. Furthermore, it has been demonstrated that an increased productivity of terrestrial cyanobacteria can be achieved by closely mimicking the natural terrestrial habitat in emersed photobioreactors[51].

The mist reactor is not the only system that meets the criterion of a large surface-to-volume ratio. Supplementary Table 1 details various other reactor types that meet this criterion. As observed in the table, this ratio can range from as low as 3 for tubular biofilm reactors to up to 500 for hollow-fibre systems. The entries in Supplementary Table 1 aim to illustrate the achievable surface-to-volume ratios for different reactors and their applications. For industrial use, the feasibility of the system scale-up is also important. In the case of a large area-to-volume ratio, a 'numbering-up' approach may be necessary (increasing the number of smaller reactors instead of scaling up the reactors), as individual facilities cannot always be expanded.

The benefits of transitioning from a submerged system to a biofilm system in terms of productivity is shown in Supplementary Table 2. The table shows the improvement in productivity when cultivating as a biofilm. Although the list is not suitable for comparing the productivity of the different systems, it clearly shows that for all biofilm reactor systems considered, productivity could be increased compared with purely submerged systems.

Catalytic biofilm systems in white biotechnology

Thanks to the intrinsic features of biofilm-grown organisms discussed above, laboratory-scale experiments have revealed that these systems are well suited for use as biocatalysts in industrial applications, particularly white biotechnology (Supplementary Tables 1 and 2). Over the past decade, the application of biofilms to produce bulk and fine chemicals has increased owing to the previously discussed characteristics and the following economic advantages[52]. As mentioned above, the substrata in or on which the biofilms grow can be divided into inactive substrata, which solely provide an attachment surface, and active substrata, such as membranes or electrodes, which also provide the biofilm-forming organisms with growth substrates (such as carbon or electrons)[4]. Furthermore, cells can adhere to each other, forming flocs or aggregates that are often observed in wastewater treatment.

Biofilms on inactive substrata

Biofilm-based production systems on inactive substrata may offer advantages over their planktonic counterparts, especially for anaerobic processes, gas fermentation, or whole-cell biocatalysis (production

of industrially relevant compounds using microbial cells like *E. coli* or *Saccharomyces* sp.) under harsh reaction conditions. In anaerobic fermentation, an increase in volumetric productivity has been reported, correlating to a higher biomass density compared with planktonic approaches[31]. In a study comparing the performance of planktonic to biofilm-grown *Lactobacillus delbrueckii* in terms of lactic acid production, cell density in the biofilm system increased by a factor of 19, corresponding to a 6- to 8-fold increase in volumetric productivity[31] (Fig. 3a). The process was performed in a simple tubular biofilm reactor system where the biofilm attached directly onto the glass material of the reactor. Tubular reactor systems are common in biofilm research because they are easy to operate and monitor on a laboratory scale. The surface-area-to-volume ratio may vary substantially, depending on the tube diameter. As previously mentioned, this parameter is crucial for biofilm reactors. Lactic acid production was measured in a system with a surface-area-to-volume ratio of 3.1 metres squared per metres cubed. When tube diameters are reduced to the microscale, the surface-area-to-volume ratio is increases tremendously and can reach values of 2,000 metres squared per metres cubed[53]. Microtubular biofilm reactors made from various materials have been used for the biotransformation of organic compounds via monooxygenase-catalysed reactions (Fig. 3b). The biotransformation substrates, such as styrene[54,55] or cyclohexane[56], are highly toxic and lethal to microorganisms at higher concentrations. Biofilm systems offer the advantage of long-term adaptation to harsh reaction conditions, and a continuous process lasting for several weeks to months could be achieved.

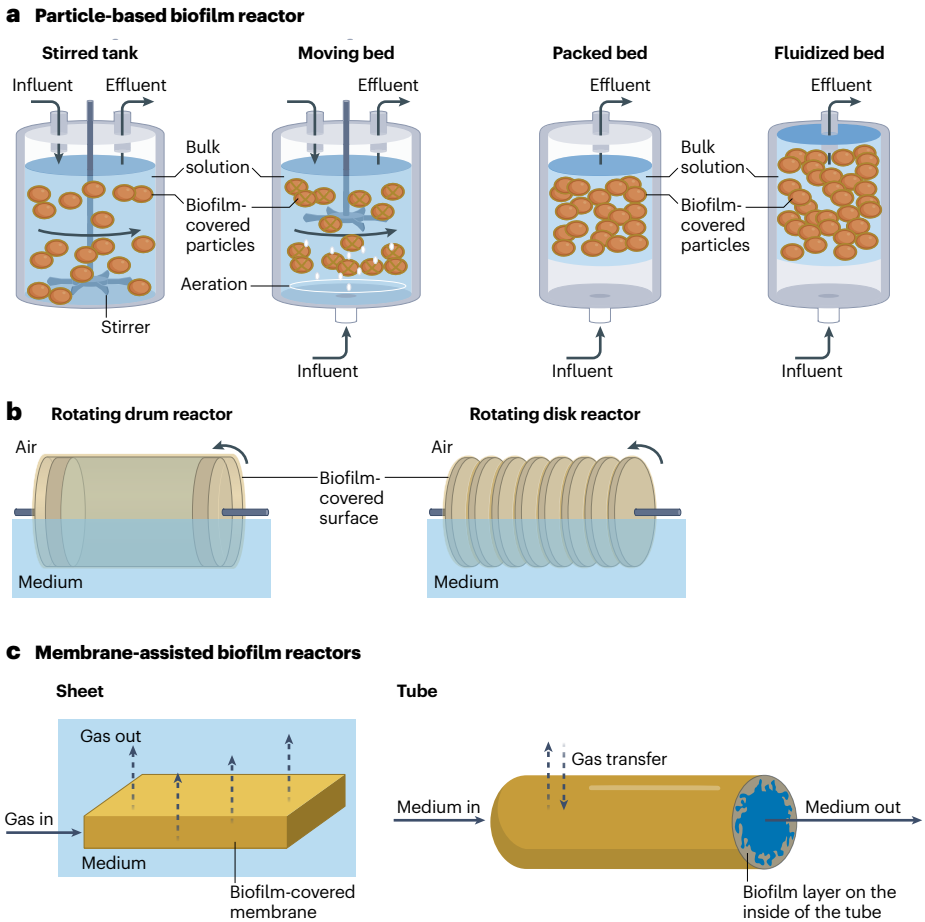

Fig. 1 | Prevalent biofilm reactor configurations. a, Particle-based systems are the most common biofilm reactors. Stirred tank reactors are usually operated with suspended cultures. However, when equipped with particles that can act as substrata for growing biofilms, they can also be operated as biofilm reactors, although operating conditions (for example, stirring speed) need to be adapted. The general principle of a moving bed biofilm reactor is very similar to that of a stirred tank. Owing to the hydrodynamic conditions applied, the particles form a floating bed in the reactor vessel. These systems are well established in wastewater treatment. In packed bed reactors, the reactor volume is filled with particles used as biofilm substratum. The substratum bed and the biofilm stay in a fixed position and do not move with the liquid. In fluidized bed reactors, the substratum particles are suspended by upward liquid velocity created by the feed. **b**, In a rotating drum biofilm reactor, a large cylinder is mounted on a central axis, which constantly turns, transferring the biofilm growing on the drum surface alternately from the liquid to the gas phase. The operation principle in a rotating disk biofilm reactor is basically the same, except that instead of a single drum, multiple disks are mounted on a central axis and serve as the attachment support for the biofilm so that the available surface area is increased compared with the drum. **c**, In membrane-assisted biofilm reactors, the membrane serves a dual purpose. It is utilized as a substratum for the biofilm and at the same time is essential for gas transfer in and out of the biofilm. Membrane-assisted biofilm reactors occur in various designs, such as sheets but also tubes, capillaries and hollow-fibre modules. Capillary systems provide an extraordinarily large surface-to-volume ratio. If transparent materials are used, these reactors can also be applied for the cultivation of phototrophic biofilms. For more information on biofilm reactors and their application please refer to ref. 4.

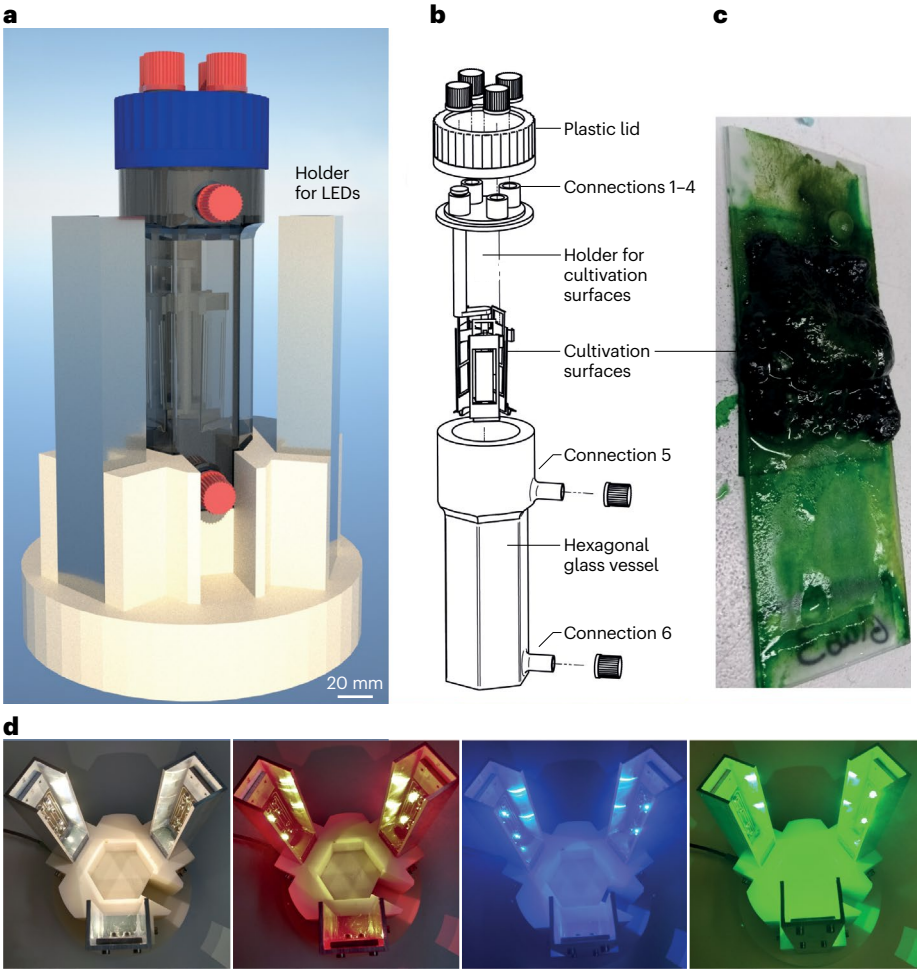

Fig. 2 | Aerosol reactor with illumination unit for the cultivation of phototrophic terrestrial biofilms. a, Computer-aided design model of the aerosol reactor integrated into the illumination unit. The holder inside the reactor allows the cultivation of biofilms on up to three variable surfaces. Moisture and media components are supplied via an aerosol that is fed into the reactor through one of the available ports (red caps). **b**, Detailed drawing of the aerosol reactor with its components. **c**, Biofilm of *Trichocoleus desertorum* on borosilicate glass after 14 days of cultivation in the aerosol reactor at 30 °C and illumination with red light. **d**, The illumination unit allows the setting of different light colours (white, red, green and blue) in addition to variation in light intensity. This process allows, for example, the pigment composition of cyanobacteria to be varied, because they are capable of chromatic adaptation. LED, light-emitting diode. Adapted with permission from J. Stiefelmaier.

Designing biofilm systems as cascades for multistep production processes may also be beneficial for solving diffusion and transport challenges. One study developed and reported an interesting approach in which they synthetically designed the biofilm matrix for the localized covalent display of enzymes on curli fibres, and using this approach, combined two extracellular reaction steps with one intracellular step in an *E. coli* system developed for D-phenyllactic acid production (Fig. 3c). This strategy resulted in production rates increasing by more than twofold compared with a basic whole-cell process[57].

Syngas fermentation, which utilizes a mixture of CO_2, CO and H_2 with flexible molar ratios, is an alternative to classical Fischer–Tropsch synthesis, which is a thermochemical conversion technology that uses syngas generated by gasification[58]. With this method, various products, including alcohols, organic acids and hydrogen, can be generated at near-ambient temperature under anoxic conditions using acetogenic bacteria[59–62]. For syngas fermentation, the respective biofilms are either grown on solid supports inserted into a long column (trickle bed reactor) or suspended in the liquid phase of a so-called slurry reactor[63]. Furthermore, biofilms may be grown on materials operating in a rotating packed bed biofilm reactor or on hollow-fibre membranes (Fig. 3d), or monolith structures (monolith biofilm reactors), which will be discussed in the following subsection on active substrata. Gas–liquid mass transfer rates are critical for the performance of the respective system (recently reviewed in ref. 57). Unlike the abovementioned tubular biofilm reactor, scaling up these reactor types is feasible. Indeed, these reactor configurations can also be applied for biomethanation, and trickle bed reactors for this purpose are already in industrial use (Fig. 3e). This biofilm application is currently of interest because using natural biogas (typically comprising around 50% of carbon dioxide) and hydrogen produced from water electrolysis as the electron and energy sources can effectively double the methane yield of a biogas plant[64].

Biofilms on active substrata

The development of biofilms on substrata that support cellular metabolism has the benefit of encouraging a stable interaction between the cells and the substratum. Electroactive biofilms are a very active research area in this domain. The interactions between microorganisms and electrodes have been harnessed for various applications ranging from hydrogen and bioplastics production[65] to wastewater treatment[66] and the production of value-added chemicals[67] depending on the reactor design and the physiological conditions of the biofilm. Common to these applications is the dependence of the microorganisms on electron transfer from the biofilm to an electrode surface as seen in microbial fuel cells and electrolysis cells, or vice versa, as in microbial electrosynthesis cells. For an in-depth overview please refer to ref. 68. Thus, the processes are typically counter-diffusional, with either the electron

donor or acceptor coming from one side and other substrates introduced from the opposite side. Reports have documented maximum biofilm heights of 400 µm for both anodic (electron transfer to the electrode) and cathodic (electron transfer from the electrode) systems operating with single-species biofilms[69,70]. However, reports on microbial activities in anodic biofilms suggest that microbial activity is limited to 100-µm-thick biofilms because of pH gradients building up from the bulk phase towards the electrode[71] (Fig. 4a). Thus, in thicker biofilms,

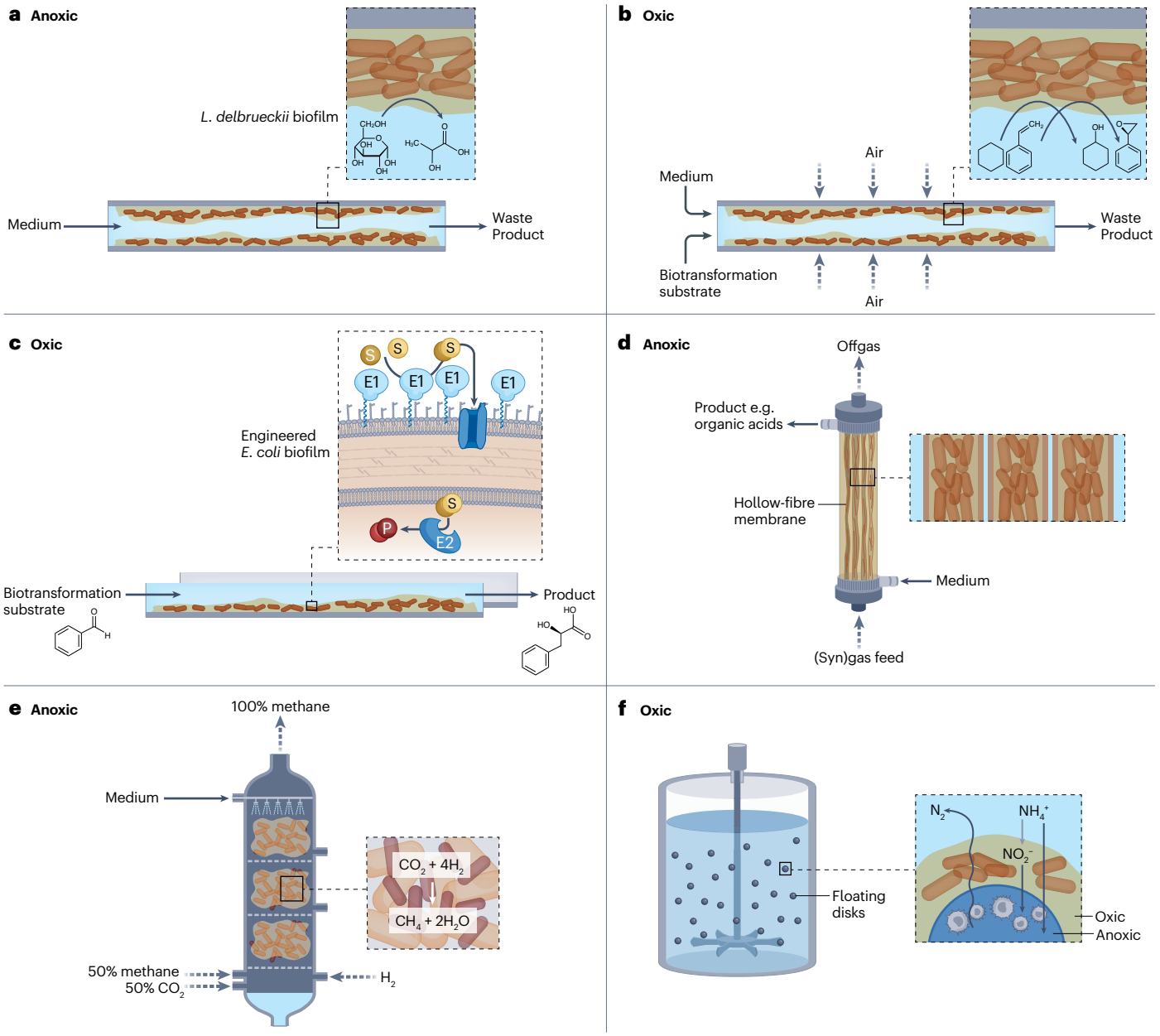

Fig. 3 | Different applications of productive biofilms on inactive substrata. **a**, Production of lactic acid directly from glucose by applying a biofilm of *Lactobacillus delbrueckii* growing attached to the inside of a tubular biofilm reactor (inner diameter 10 mm, length 400 mm). The system was run under anoxic conditions. **b**, Biotransformation of either cyclohexane[140] or styrene[141] to the corresponding products cyclohexanol or S-styrene oxide by applying a biofilm of *Pseudomonas taiwanensis* growing attached to the inside of a membrane-assisted biofilm reactor (inner diameter 2 mm, length 200 mm). In this multiphase system, biotransformation substrates are supplied as a pure organic phase, while air enters the system over the capillary membrane. **c**, Multistep biotransformation of benzaldehyde to D-phenyllactic acid using a biofilm of engineered *Escherichia coli*. Enzymes are assembled on the cell surface, in the membrane and in the cytoplasm. **d**, Anoxic syngas fermentation employing a biofilm consortium dominated by *Clostridium ljungdahlii*. The biofilm is grown in a membrane-assisted biofilm reactor on a hollow-fibre membrane, which supplies the syngas to the biofilm. **e**, Trickle bed reactor for methane production from biogas. **f**, Moving bed biofilm reactor with suspended biofilm carriers for removal of ammonia. E1 and E2, enzymes; P, product; S, substrates. Part **d** adapted with permission from ref. 61, Elsevier.

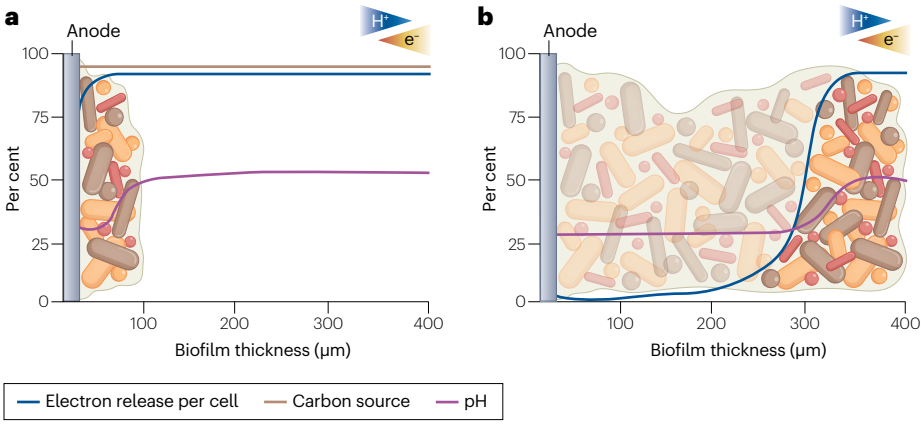

Fig. 4 | Dependency of biofilm height, pH gradient and activity of cells in anodic systems. **a**, Biofilms up to 100 μm in thickness seem to be limited mainly by the formation of a pH gradient with higher proton concentrations close to the anode. In most systems, the active parts of the biofilm seem to be in the middle or outer sections[142]. **b**, Biofilms that grow higher than the 100-μm threshold comprise an active outer layer of cells, whereas the inner biofilm layers seem to comprise inactive cells that function as conductive electron-transferring material.

only the upper layers of the biofilm will contribute to current production, whereas the other cells seem to be a conductive material that aids in electron transfer from the surface to the anode (Fig. 4b). Notably, anodic current densities between 5.7 and 9.3 A m^{-2} were measured for different species of the genus *Geobacter*, which are model organisms for bioelectrochemical studies[72]. This is astonishing if one considers that an average *Geobacter* spp. cell with an estimated volume of 0.91 μm^3 has been revealed to produce a current of about 82 fA (ref. 73). Considering that theoretically 1.1×10^{14} cells could reside in a 100-μm-thick biofilm, this would give theoretical current densities of 9 A m^{-2}. In other words, at least within medium-thick biofilms, the efficiency of *Geobacter* spp. biofilms seems to be very high. This is due to their conductivity, which arises from conductive structures that the organism integrates between cells[74,75]. We note that *Shewanella oneidensis*, another anodic model organism, is not capable of building comparable structures and indeed, a study has revealed that, for this organism, performance does not increase with increasing biofilm height[76].

In cathodic systems, it is often not direct electron transfer between the cathode and the biocatalysts but hydrogen produced in situ that is used as an electron shuttle between the cathode and the microorganisms[77,78]. Nevertheless, even if electron transfer is not direct, the theoretical efficiency gains that could be achieved with these cathodic systems are considerable[79]. The voltage that is needed in bioelectrosynthesis cells is much lower compared with the voltage of typical water electrolysis systems that produce hydrogen as an electron donor for biocatalysts[64]. Moreover, because the hydrogen produced in situ can be directly depleted during its diffusion through the biofilm, the problem of low hydrogen solubility is circumvented. Nevertheless, the question in this situation is how many microorganisms per cathode area can be actively supplied with electrons or hydrogen from the cathode[79,80]. The problem in this situation is that high currents can lead to the formation of hydrogen gas bubbles, which can cause the biofilm to detach from the electrode.

Overall, it seems clear that bioelectrochemical biofilm technologies will become more relevant if the abovementioned limitations can be successfully addressed. These technologies allow microenergy harvesting from waste streams, for instance, to power sensors or simple lighting installations. They can also be used to combine biotechnological processes with the co-production of molecular hydrogen and can be a sustainable way to increase the methane content of biogas[64,81]. A way to increase efficiency further is synthetic biofilm development, in which the interaction of the cells with the electrodes is fostered by a synthetic extracellular polymeric substance (EPS) matrix or a functionalization of the cell surface. The latter process can lead to drastically increased maximum power outputs of up to 11.8-fold[82–84]. For instance, a study functionalized individual cells of a model organism for anodic electron transfer with graphene oxide and silver nanoparticles. This functionalization led to an increase in biofilm formation and also to increased cell–cell electron-transfer rates so that the known limitations of power generation could be easily overcome.

So far, reactor technologies for bioelectrochemical processes that possess high adaptability, scalability and space-time yields are generally missing. Nevertheless, several reactor concepts, including rotating disk reactors and fluidized bed reactors, have already been successfully developed[85–88] (Fig. 1). Obviously, electrodes with a high surface area must be integrated into the reactors, but this electrode surface must be hydrodynamically accessible to avoid dead volumes.

Like electrodes, membranes can also be used as active substrata in gas fermentation applications. In this context, gaseous substrates, such as hydrogen, oxygen or syngas are provided via the membrane while other substrates are available to the biofilm via the bulk phase[89]. This technology is already applied in wastewater treatment, whereas its use for gas fermentation in the production of bulk and fine chemicals is still in its infancy[90,91]. The major benefit of these systems is that gases with low water solubility do not need to dissolve in the bulk aqueous phase, thus circumventing a key problem[78]. Owing to low solubility, standard reactor systems such as stirred tank reactors would have to be operated with a rather high energy input to decrease gas bubble size and consequently increase mass transfer rates. In membrane biofilm reactors, the gaseous substrates are depleted by the biocatalyst as they diffuse from the membrane through the biofilm towards the bulk phase. Thus, dissolution in the bulk phase is not necessary[58,91–93]. In wastewater treatment, these systems can enhance the removal of organic carbon by applying membrane-aerated biofilms or for denitrification by adding hydrogen via the membrane[94]. Similarly, it is possible to use membrane biofilm reactors for gas fermentations with autotrophic organisms thriving either in the presence of hydrogen and CO_2 or syngas. Competitive space-time yields in these reactors were revealed using laboratory-scale experiments when compared with established gas-fermentation technologies[91]. Although the energy input in these systems will be lower and scalability seems to be provided by the modular architecture of membrane modules, it will most

probably be the future costs of these modules that determine whether the technology will be reserved for more expensive fine chemical production or will be extended to bulk chemicals. Apart from the potential benefits of reduced energy input and higher mass transfer coefficients, which have been deduced using laboratory-scale systems to be around threefold higher, operation safety should also be mentioned. Continuous efforts to establish carbon dioxide as a substrate for biotechnology have also led to a renaissance of studies using Knallgas bacteria (aerobic hydrogen-oxidizing bacteria) for fermentation[95,96]. The higher metabolic energy that these organisms obtain from the oxidation of hydrogen with oxygen is accompanied by a higher degree of freedom regarding the engineering of the metabolism of the organisms to produce valuable compounds. Nevertheless, safe operation of Knallgas fermentations is cumbersome. Establishing membrane-assisted biofilm reactor systems supplying hydrogen and carbon dioxide from the membrane side and oxygen from the bulk phase would allow this limitation to be overcome.

Phototrophic biofilms in green biotechnology and bioengineering

Phototrophic organisms are appealing for whole-cell-based biocatalysis owing to their capability of utilizing light as an energy supply. Among this highly diverse group of organisms, cyanobacteria are especially attractive because they perform oxygenic photosynthesis and so use H_2O as an electron donor. Furthermore, they are autotrophic and exploit CO_2 as a carbon source. As described above, light transmission is a challenge when cultivating phototrophic organisms, necessitating transparent reactor materials. Furthermore, light transmission through the biofilm may pose a problem owing to self-shading of the outer biofilm layer. Nevertheless, biofilm thicknesses up to 1,500 µm in artificial systems have been reported[97]. In nature, phototrophs contribute heavily to the development of microbial mats, which can be regarded as large, highly complex biofilms reaching thicknesses of several millimetres to centimetres[98]. Although numerous proof-of-principle studies showing the synthesis of a wide range of diverse non-natural and natural products for planktonically cultured phototrophic microorganisms have been published[99], only a few studies considering phototrophic biofilms as production system are available. However, the field is developing towards understanding the biofilm formation of phototrophic organisms. One of the few examples in which this process has been investigated more closely is *Synechocystis* sp. PCC 6803. Here, modification of an ABC transporter system involved in O-antigen transport promotes bacterial adherence to hydrophobic surfaces and the formation of cell aggregates[100]. Furthermore, cyclic-di-GMP can also be involved in biofilm development in cyanobacteria[101]. Apart from these examples obtained from basic research, only a handful of studies on the application of phototrophic biofilms can be found. Examples include wastewater treatment, bioelectrochemistry, biofuel production, biotransformation and green fertilizers.

Photo-microbial fuel cells are microbial fuel cells in which electrogenic microorganisms transfer their surplus electrons over the cell membrane onto an electrode instead of reducing oxygen or other electron acceptors, and thereby produce an electric current[102,103]. A classical photo-microbial fuel cell typically utilizes non-oxygenic photosynthetic microorganisms (such as purple bacteria) or mixed-trophies consortia in which the phototrophic organisms use light energy to either drive the electron flux towards an external electrode or to synthesize organic compounds, which are released to feed the chemotrophic electrogenic microorganisms of the consortium for current production. An emerging field in this context is biophotovoltaic cells, which solely use oxygenic photoautotrophic organisms, such as cyanobacteria, and therefore are completely independent of organic carbon compounds[104]. Of the organisms investigated, *Synechococcus* sp. WH 5701 growing as a biofilm directly on the electrode achieved the highest biomass combined with superior current production among all tested strains[105]. Photosynthetic biofilms have also been described for biofuel production, mainly biodiesel[106]. Such biofilms are complex consortia of multiple strains comprising phototrophic and chemotrophic inhabitants. Because separating biomass and water causes a substantial increase in the total costs of biofuel production, biofilms are a potential strategy for facilitating harvesting or dewatering, and thus can save on production costs. A suitable example for the enhanced stability of biofilm catalysts was reported for the conversion of cyclohexane to the corresponding alcohol[56]. In this biotransformation process, a biofilm containing the cyanobacterium *Synechocystis* sp. PCC 6803 and *Pseudomonas* sp. VLB 120 was employed. The reaction stability could be prolonged from 2 hours in planktonic cultures to several weeks in the biofilm. The reaction was catalysed by the recombinant cyanobacteria, while *Pseudomonas* sp. served as a biofilm-supporting strain.

Biofilms in agriculture

Utilizing biofilms in agriculture is a young research field and the interactions between biofilms and their associated plants are not yet well understood in many cases. Although not precisely in line with the productive biotechnology applications discussed here so far, the beneficial impact biofilms have on growth and yield of crops for food and feed is remarkable. In agriculture research, the potential of biofilms to serve as a fertilizer or to enhance the water-binding capacity of the soil, the latter becoming more and more important in times of climate change, is being explored. In particular, species capable of fixing atmospheric nitrogen are of interest in this context[96]. Furthermore, biofilm-forming, self-sustaining microorganisms like filamentous cyanobacteria are used to support the colonization of the rhizosphere by other organisms like plant-growth-promoting rhizobacteria. Upon inoculation, soil parameters like nitrogen, phosphorus and carbon content increased, leading to faster plant growth and enhanced grain weight as well as to a substantial reduction in artificial fertilizer[6] (Fig. 5). To investigate the impact of cyanobacteria as ammonium fertilizers on higher plants, hydroponic systems have often been used. In hydroponic systems, the cyanobacteria are cultivated while submerged, and the plants are fixed on the medium surface in special holders whereby the roots are growing into the liquid phase. The roots serve as an attachment surface for the cyanobacterial biofilm and therefore a close interaction of the nitrogen-supplier (biofilm) and the nitrogen-consumer (plant) is ensured. These systems simplify the investigation of co-cultures because the exchange of nutrients and secondary metabolites is facilitated, and the liquid phase can easily be analysed[107,108]. The increase in surface nitrogen content in combination with the production of growth-promoting substances (such as S and P[109,110]) and other growth-regulating substances (such as amino acids, sugars and vitamins[111,112]) can positively influence plant growth and thus be an alternative to artificial fertilizers[107]. Thanks to the beneficial properties of cyanobacteria, these bacteria are already important microbial components in rice fields in Japan, helping to improve fertility, soil structure and crop yields[113–115]. Beneficial effects of cyanobacteria have also been demonstrated for growth of wheat[116,117], tomato[118–120] and maize[121,122].

Interestingly, most of the introduced examples utilize microbial consortia, wherein at least one phototrophic organism is teamed up

with at least one chemotrophic partner. Employing undefined microbial consortia is an emerging area in biotechnology and has potential for biofilm applications because spatially defined localization of the different partners is possible. A milestone for the design of such consortia was the development of surface display systems, which enable controlled microbial interactions[123,124].

Applied biofilms growing without substrata: aerobic granular sludge

All of the biofilms and applications discussed so far are based on the interactions of cells with active or inactive substrata. However, one of the major contributions we have seen in the field of biofilm application is aerobic granular sludge, which is an innovation in the field of wastewater treatment[125,126]. Although this kind of biofilm process was initially intended only for wastewater treatment, current research reveals its role as a starting point for biological resource recovery that can be applied to polyhydroxybutyrate and EPS biopolymer, phosphorus and nitrogen recovery[127,128]. Similar applications for resource recovery might also be developed for anaerobic granules used in nitrogen elimination from wastewater (see above) in future. Aerobic granular sludge is composed of a dense population of microorganisms possessing different metabolic properties that aid in carbon, nitrogen and phosphorus elimination from wastewater. The biofilms consist of a very dense diverse microbial community. The granules are a result of selective process conditions including high shear forces, short settling times in sequencing batch reactors, and a feast–famine regime. This feeding regime is characterized by a feast phase in which organic carbon is added under anoxic conditions, followed by the famine phase, in which oxygen is added as an electron acceptor while the available organic carbon concentration is low. The microbial activity in the granules leads to sharp gradients, allowing both nitrification and denitrification under oxic conditions, with nitrifying organisms in oxic parts of the granules and denitrifying organisms in the anoxic centre. Better performance can be achieved if the conditions are switched between feast and famine such that denitrifying organisms are supported by a higher concentration of electron donors[129]. However, the differential feeding regime also facilitated phosphate elimination with the help of phosphate-accumulating organisms. These organisms construct polyhydroxyalkanoates as polymeric carbon and energy storage compounds in the anoxic feast phase and use polyphosphate as an energy source. In the oxic phase, the polyhydroxyalkanoates are metabolized using oxygen as an electron acceptor while polyphosphate is produced from dissolved phosphate in wastewater[125]. A further developmental goal could be the integration of photosynthetic organisms to aid in providing oxygen in a famine phase associated with the addition of sunlight[130]. However, greening of granular sludge was associated with a decrease in EPS production in some studies[129].

Biofilms as engineered or functionalized materials and coatings

Biofilms are not only of interest because of their potential role in biocatalytic retentostat systems. They have additional interesting properties as biomaterials or as biocatalytic supports for functionalization[131] (Fig. 6). One study reported on the production of bacterial cellulose based on a biofilm reactor operated with *Acetobacter xylinum*[132]. The study revealed far higher rates of bacterial cellulose production compared with a control reactor with planktonic cells. Also, the material properties changed. For instance, the Young's modulus of the bacterial cellulose increased by almost a factor of 10 to 2.4 GPa,

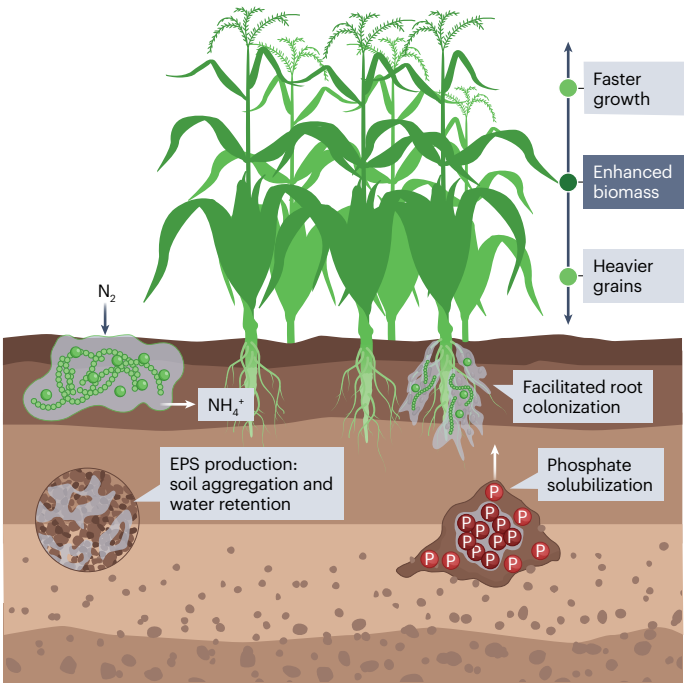

Fig. 5 | Impact of biofilms on crops and soil procurement. Biofilms increase the water-binding capacity of the soil, and support the supply of nitrogen compounds, phosphorus and carbon. They also facilitate the colonization of the rhizosphere by other, symbiotic microorganisms. Plant biomass is enhanced, growth is accelerated and grain weight is enhanced. EPS, extracellular polymeric substance. Adapted with permission from ref. 6, Elsevier.

a value similar to that of polycarbonate (Fig. 6a). Similarly, bacterial biofilms have been developed with even higher Young's moduli. By advancing the EPS matrix through the overproduction of CsgA amyloid fibrils, it was possible to develop ultralight *E. coli* biofilms with a Young's modulus of 10 GPa that could be increased threefold by introducing carbon nanotubes[7,8] (Fig. 6b). As the EPS matrix in the developed strain was engineered to a predictable biochemical composition, functionalization of the material based on the addition of several binding epitopes to CsgA became available. To this end, it was not only possible to tailor the cells to specific surfaces but also to covalently link arbitrary proteins or enzymes to the biofilm. Also, conductivity could be obtained by the addition of gold nanoparticles[131]. Similarly, engineered living materials composed of *Caulobacter crescentus* cells were introduced recently. The whole surface of the cells was engineered by producing semisynthetic proteins with genetically tailored elastic purposes and functionalization was also possible using the addition of a so-called Spy-tag that specifically interacts with the Spy-Catcher[8] (Fig. 6c). Of note, by tailoring the EPS matrix using different polymer-forming units, it is likely that the material properties can be fine-tuned in a variety of directions given that these biomaterials can be produced based on regenerative carbon or even CO_2, and it seems reasonable to assume that this process will be an interesting avenue for future material research and applications.

Another research direction for applying biofilms in the field of material research is the production of bioconcrete. The surface of microorganisms is normally negatively charged, which leads to binding of cations such as Ca^{2+}. The alkalizing activity of microorganisms owing

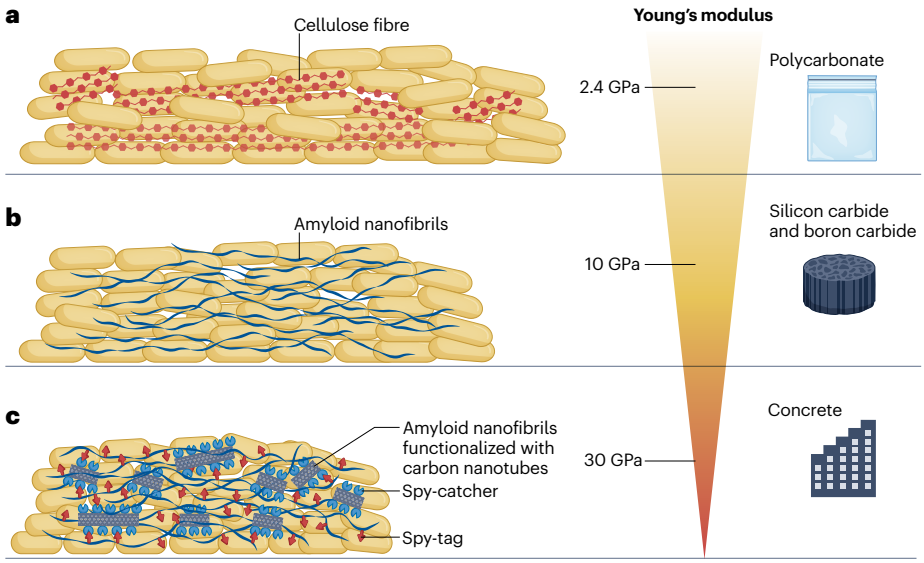

Fig. 6 | Different approaches to using biofilms as engineered materials. a, Production of cellulose (red) and incorporation into the extracellular polymeric substance matrix by *Acetobacter xylinum* biofilm leads to an increased Young's modulus[132]. **b**, Engineered *Escherichia coli* biofilm through the overproduction of CsgA amyloid (dark blue), advancing the extracellular polymeric substance matrix and thereby leading to an increase in Young's modulus[8]. **c**, Further engineering of the *E. coli* biofilm by functionalization of amyloid nanofibrils with Spy-tag (red) and adding Spy-catcher (blue) functionalized carbon nanotubes (grey)[8].

to urease export, deamination[133,134] of amino acids, or autotrophic growth can lead to an oversaturation of calcium carbonate and consequently to its precipitation. Targeting biofilms composed of organisms with this capability to surfaces will establish a layer of bioconcrete. If the activity of the microorganisms within the biofilm is controlled, it is even possible to discriminate between different calcium carbonate forms that are produced by the organisms[135].

Along these lines, stable calcium-carbonate-precipitating biofilms were also revealed as corrosion inhibitors on steel surfaces. Steel corrosion is a major problem for the construction industry, especially in marine environments. Major factors that positively influence corrosion are the high concentrations of Cl^- ions and oxygen in addition to hydrogen sulfide precipitation triggered by sulfate-reducing bacteria[136]. More and more studies have revealed that covering steel surfaces with some biofilm-forming microorganisms can be used as a strategy to prevent corrosion[137]. Therefore, the production of bioconcrete might act as a diffusion barrier to Cl^- (ref. 138). Nevertheless, calcite precipitation is not necessary to reach biofilm-driven corrosion inhibition, as one study reported[139]. The organic EPS-material of some strong biofilm-forming organisms seems to be sufficient for steel protection. Research is needed to reveal whether it is possible to establish a robust and sustainable steel coating with corrosion-preventing microorganisms. The latter would potentially have major implementation in the field of environmental protection because toxic coatings would no longer be necessary.

Conclusions and future directions

The field of biofilm research has substantially expanded over the past several decades, and has evolved from focusing on mere prevention and destruction strategies, especially in the medical field, to concepts aiming at utilizing biofilms for productive purposes. These approaches have been expanded to exploit beneficial features of biofilms in general, such as protecting surfaces or serving as functionalized materials. In addition, more and more examples for investigating not only single-species biofilms, but artificial consortia combining different metabolisms, are being reported. Furthermore, spatially defined consortia are being designed, taking advantage of the capability of immobilizing organisms in defined locations and thereby creating reaction cascades that involve multistep enzymatic pathways with whole-cell biocatalysts but also with a combination of cells and isolated immobilized enzymes. This field is just beginning to open up to new ideas, and the possibilities of making use of the biofilm-forming capability of microorganisms seems almost endless. Despite these novel developments in biofilm research, the field is suffering from the lack of real improvements regarding biofilm reactor development. This area does not seem to be making much progress. The reactors have looked much the same for decades apart from variations on the same theme, the scaling problem remains unsolved, and case studies focusing on bringing biofilm-driven processes to pilot scale are lacking. Such studies, together with industry initiatives, are needed to bring biofilm research to a point where such technologies can be implemented on a large scale. Furthermore, new reactor types would enable the cultivation of other organisms that are not accessible in submerged systems in vitro. It can be assumed that the large discrepancy between the total number of microorganisms and the microorganisms that can be cultivated is also due to the need to grow them as biofilms. A more intensive study of biofilm reactors could thus enable access to the synthesis performance of many microorganisms. The pending climate and resource crises ahead of us provide ideal incentives to foster such developments.

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