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Biofilms for Production of Chemicals and Energy

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Abstract

The twenty-first century will be the century of biology. This is not only because of breakthrough advances in molecular biology tools but also because we need to reinvent our economy based on the biological principles of energy efficiency and sustainability. Consequently, new tools for production routines must be developed to help produce platform chemicals and energy sources based on sustainable resources. In this context, biofilm-based processes have the potential to impact future production processes, because they can be carried out continuously and with robust stationary biocatalysts embedded in an extracellular matrix with different properties. We review productive biofilm systems used for heterotrophic and lithoautotrophic production and attempt to identify fundamental reasons why they may be particularly suitable as future production systems.

1. INTRODUCTION

Biofilms are aggregates of microorganisms that adhere to surfaces or to each other. Some evidence suggests that most microorganisms in nature occur in the form of these aggregates (1, 2). Interest in the analysis of biofilms was spurred originally by the medical sector because of the increased resistance and tolerance of microorganisms living in biofilms to antibiotics, which pose a significant threat to human health. However, biofilms have also been used in the technical sector for decades. Here, it was initially the water sector that began using biofilms, for example, in sand filters (3). Since then, the water sector has been at the forefront of developments in applied biofilm technology. Of several reasons for this, two seem particularly obvious. The water sector is largely dependent on autotrophic, slow-growing organisms, and process conditions, especially in wastewater treatment, must change dramatically to efficiently eliminate carbon and nitrogen. Biofilms are well suited to these tasks because slow-growing organisms are not diluted out of the system, and conditions in biofilms can change between oxic and anoxic within a few microns due to the metabolic activity of the microorganisms. In contrast, biotechnology has recognized the benefits associated with biofilm-based processes, but these are still in the developmental stage, and few processes have been developed to the point of industrial applicability. One hypothesis as to why biofilms are not used more widely in this field is that there is simply a gap in knowledge regarding reactor technology, process predictability, and biocatalyst stability. This could change as bio-based processes gain momentum to develop more sustainable production routines.

In biofilms, microbes are embedded in a matrix consisting of a complex composition of carbohydrates, proteins, and nucleic acids called extracellular polymeric substances (EPS) (4). These EPS can account for 50–90% of the total organic carbon in the biofilm (5). EPS properties and composition not only differ from species to species but also can vary greatly within a species, depending on external influences. The EPS matrix provides the biofilm with stability and resistance to external influences such as fluctuating conditions, pollutants, and shear stress. The EPS acts as an adhesive that holds the cells together, but it also contains several active sites and catalytic proteins and provides a certain surface charge. All these factors contribute to the biofilm's robustness, in addition to its function as a diffusion barrier. This robustness, the high cell density of up to 500 mg dry biomass per milliliter of biofilm, and the fact that the cells can be easily retained in a flow-through system are major advantages of biofilms over planktonic cells in industrial production processes (6).

We briefly review the necessary fundamentals of biofilm formation and then highlight various directions of applied biofilm research and typical applications for this biocatalytic material. For this purpose, we categorize the applications and systems into heterotrophic, autotrophic, and bioelectrochemical.

2. REGULATION OF BIOFILM FORMATION

Biofilms are not static but dynamic living systems. Biofilm architecture and composition are a response of the microbial community to process conditions, which include the surface on which they grow (called the substratum), but also shear stress and medium composition. Understanding biofilm growth is critical to the development of biofilm-based processes, because it provides the opportunity to design the biology and overall process for biofilms with tailored characteristics. These characteristics certainly include biofilm height and density, as these could limit mass transfer. In addition, EPS composition could be an interesting parameter for biofilm engineering, because it could be used to adjust the concentration of different functional groups in the biofilm.

Biofilm formation is a complex process that can be divided into different stages. Until recently, a five-stage biofilm model was widely used to visualize biofilm formation and spreading. The first

phase of the five-stage biofilm model describes the initial, reversible attachment to a substratum. This may be followed by irreversible attachment. The latter is followed by the first maturation phase, in which EPS are formed and cell clusters are built. The fourth biofilm phase describes the mature biofilm, in which microcolonies are formed. Finally, the mature biofilm dissolves and releases single cells and cell clusters. However, this biofilm model has been criticized because it considers only aggregates that adhere to surfaces and have a specific shape. Therefore, Sauer et al. (7) recently published a revised model of biofilm formation that extends the previous model. In addition to surface-associated biofilm formation, the new model also considers biofilm formation in solution and transitions between states.

Within these stages, microorganisms undergo significant changes at the gene-expression level compared with continuous planktonic growth (8, 9). Regulation during the different stages of formation is a very complex process and varies greatly among organisms. However, some regulatory elements have been observed in many organisms and play a crucial role in biofilm formation. According to numerous studies, initial biofilm formation is triggered by an increase in the messenger molecule cyclic diguanylate (c-di-GMP) (10–15). Synthesis and degradation of c-di-GMP in bacteria are regulated by two antagonistic classes of proteins (16). In principle, c-di-GMP synthesis is catalyzed by diguanylate cyclases containing a GGDEF domain, whereas degradation is catalyzed by phosphodiesterases containing an EAL or HD-GYP domain. According to the current hypothesis, various environmental factors can stimulate c-di-GMP production, which in turn triggers the production of adhesin and EPS compounds (11). For example, in the biofilm model organism *Pseudomonas aeruginosa*, the membrane-bound protein WspA recognizes signals associated with contact to surfaces (10). These signals are then transmitted to the histidine kinase WspE, which transfers a phosphate group to the diguanylate cyclase WspR. WspR eventually catalyzes the production of c-di-GMP, which in turn leads to increased secretion of adhesins and exopolysaccharides (11). c-di-GMP signaling is associated with biofilm formation in many other organisms in addition to *P. aeruginosa*. Nevertheless, this factor rarely has been used to develop whole-cell biocatalysts in biofilm processes. An example of such an approach is biofilm engineering using *Shewanella oneidensis*, a model organism for extracellular electron transfer (EET) in bioelectrochemical systems (BES) (see Section 6), showing the influence of c-di-GMP levels on biofilm formation. Here, researchers altered the intracellular c-di-GMP concentration to influence biofilm properties. Heterologous expression of the diguanylate cyclase *ydeH* from *Escherichia coli* in *S. oneidensis* increased biofilm formation and current density in a microbial electrolysis cell (MEC) by 5.3-fold and 3.4-fold, respectively (12).

In addition to surface recognition, quorum-sensing mechanisms significantly influence biofilm formation. Quorum sensing is a process that triggers physiological responses in a cell concentration–dependent manner. Gram-positive bacteria mostly synthesize autoinducing peptides as signaling molecules for quorum sensing. When the extracellular concentration of autoinducing peptides is high, the peptides can trigger a two-component system consisting of a membrane-bound histidine kinase and a transcription factor. Once the autoinducing peptide binds to the histidine kinase, the histidine kinase is activated and transfers a phosphate group to the transcription factor. The now-phosphorylated transcription factor in turn activates the transcription of genes of the quorum-sensing regulon (17). Gram-negative bacteria use small molecules, mostly acyl-homoserine or other molecules made from *S*-adenosylmethionine, as autoinducers that can diffuse through membranes (18). Once autoinducer concentration is high enough, they bind to cytoplasmic transcription factors that activate the transcription of quorum-sensing gene clusters (17). Several studies have demonstrated the influence of quorum sensing on biofilm formation. As early as 1998, Davies et al. (19) published a study showing that *P. aeruginosa* strains with quorum-sensing defects formed a flat and undifferentiated biofilm, whereas the wild type formed large

fungal-like structures under similar conditions. Moreover, quorum sensing plays an important role in the release of large amounts of extracellular DNA, a major component of EPS. Therefore, at least some microorganisms with a mutation in the quorum-sensing mechanism form only small and unstructured biofilms with little extracellular DNA, indicating that quorum sensing is essential for the formation of structured and differentiated biofilms at least in some microorganisms (20, 21). In other microorganisms, quorum-sensing mechanisms are less well studied. However, Zhu et al. (22) studied the quorum-sensing mechanism of *Shewanella baltica* and showed that the organism relies on alternative quorum-sensing molecules called diketopiperazines. These peptides are a small class of cyclic peptides previously known to communicate between species (23). In a closely related organism, *S. oneidensis*, behavior similar to quorum sensing has been observed. Biofilm formation of *S. oneidensis* is stimulated by external riboflavin in a concentration-dependent manner. However, the exact mechanism of the interaction of riboflavin and *S. oneidensis* has not been elucidated fully (24).

3. ADVANTAGES AND LIMITATIONS OF BIOFILMS IN PRODUCTION PROCESSES

Biofilm-based processes have several advantages for both heterotrophic and autotrophic processes. However, their complexity also comes with some disadvantages. These advantages and disadvantages are highlighted in this section.

3.1. Achieving Higher Biomass Concentrations and Continuous Production

A major advantage of biofilm systems is that they can easily retain larger amounts of biomass in the reactors, allowing continuous fermentation at high dilution rates. This can contribute to a more stable and efficient production process that offers significant improvements in yield and productivity and is sometimes the only way to implement processes with slow-growing organisms, such as many anaerobic bacteria (25). In this way, fermentation can be stable over long periods of time, as shown, for example, in a study in which constant propionic acid production was observed over a four-month period with a biofilm of *Propionibacterium acidipropionici* (26). For some organisms, it is even not possible to achieve planktonic cell densities similar to those in biofilm systems. For example, in a recent study, biofilm-based production of lactic acid was achieved using *Lactobacillus delbrueckii* in a tubular biofilm system that was operated for three weeks. The cell density that could be achieved in the biofilms was tenfold higher compared to the planktonic control. Consequently, six- to eightfold higher productivity (10 g L^{-1} h^{-1}) was observed in the biofilm system (27).

3.2. Resistance to Inhibitory Substances

In addition, the developing matrix in biofilm systems provides greater tolerance to inhibitory effects at high concentrations of substrates or products, making them more stable and resistant under changing conditions (28). This increased resistance makes biofilms particularly advantageous for challenging fermentations with unfavorable factors such as pH fluctuations, contaminants, antibiotics, or toxic substances. An example demonstrating the benefit of biofilm resistance is ethanol fermentation, in which the increased ethanol tolerance of *Zymomonas mobilis* cells enabled 25–100-fold higher production rates compared with planktonic control systems in stirred tank reactors (29, 30). This correlated well with ethanol tolerance tests, which revealed 2.3- to 15-fold higher survival rates of biofilm-derived cells compared with planktonic cells (31). Along the same lines, Li et al. (32) reported higher tolerance of *Z. mobilis* biofilms to benzaldehyde for benzyl alcohol production in a continuous biofilm reactor compared with free-floating cells of the same strain.

Styrene, a toxic solvent that causes membrane degradation in prokaryotes, was oxidized to (*S*)-styrene oxide using *Pseudomonas* cells as biocatalysts. A comparison between biofilm and planktonic cells revealed that 65% of biofilm cells were not permeabilized by the solvent, and only 7% of planktonic control cells survived the treatment (33). Taking advantage of this increased resistance, an engineered hyperadherent strain of the same organism showed significantly higher final concentrations of the product (34). The increased ability to resist inhibitory factors opens up possibilities for challenging fermentations, such as the use of inhibitory hydrolysates as substrate (35) or the fermentation of cyclohexanone to ε-caprolactone (36). In addition, the matrix encapsulating the organisms provides them with increased mechanical resistance to environmental stresses and higher survival rates at high temperatures for short periods of time. The composition of the matrix, particularly its polysaccharide content, may be associated with these effects (37, 38).

3.3. Decoupling of Growth and Productivity

Some physiological changes have been observed in biofilm cells that also contribute to improved performance of biofilm-based processes. Studies comparing immobilized and suspended growth of different microorganisms have shown that biomass yield decreases and production of valuable compounds increases upon immobilization (39, 40). This decoupling of growth and productivity could be crucial to achieving higher product yields and more efficient conversion of substrates. For example, different strains of *Z. mobilis* grown as biofilms catalyzed ethanol production with rates of 13.4 g L^{-1} and 9 g L^{-1}, whereas the same strains grown planktonically showed rates of only 0.4 g L^{-1} and 0.09 g L^{-1}, with percent theoretical ethanol yields of 72.5% compared with 3.7% and 48.4% compared with 2%, respectively (41).

3.4. Contamination Resistance

Interestingly, EPS production and confluent surface coverage may be a mechanism to protect a habitat from competitors or even reduce the potential impact of phages (42, 43). This could mean that biofilm-based processes may also be more resistant to contamination than processes based on planktonic cells. Evidence for the latter is provided by reports of non-sterile fermentations with biofilm systems showing stable production and stable microbial communities over long periods of time. A glucose conversion efficiency of 99% was achieved with *Z. mobilis* from non-sterile hydrolyzed B-starch at a substrate concentration of 120 g glucose L^{-1}. This resulted in the production of ethanol at a concentration of 50 g L^{-1} and an ethanol space-time yield (STY) of 13 g L^{-1} h^{-1} over 120 days. This yield was three times higher than that obtained from ethanol fermentation of sterilized substrate in a continuous stirred tank reactor (44). In another non-sterile experiment, a biofilm composed of *Caldicellulosiruptor saccharolyticus* showed stable hydrogen production over a period of 81 days (45).

3.5. Beneficial Evolution in Biofilm Systems

Under stable process conditions, biofilms develop predictable concentration gradients, and microniches are formed to which microorganisms can adapt. Consequently, the metabolic activity of cells within the biofilm also varies (46). Accordingly, biofilms of *P. aeruginosa* have been found to exhibit significantly increased phenotypic heterogeneity compared with planktonic cultures (47). Heterogeneity and stable process conditions may be the basis for adaptive evolution to process conditions. In an adaptive evolution approach, biofilm growth of *Kyrpidia spormannii* on a cathode was optimized, resulting in four times higher biofilm accumulation and faster coverage of the electrode surface, improving the overall process (48). In aiming to achieve higher current output with a similar approach, cells of *Geobacter sulfurreducens* were adapted in an experiment over 5 months,

resulting in more than fivefold-higher current and power output (7.6 A m^{-2} and 3.9 W m^{-2}) compared with the progenitor strain (1.4 A m^{-2} and 0.5 W m^{-2}). The adapted cells exhibited lower internal resistance and a higher number of electrically conducting nanowires (49). Interestingly, it was even revealed that phage resistance can be increased over time in biofilm systems (50).

3.6. Working with Gaseous Substrates

Gas fermentations can be carried out in stirred tank or gas upflow reactors. To overcome the limitations of gas–liquid mass transfer, microbubbles are generated, for example, by high stirring speeds. This is a good solution, but the energy requirement is significant. Although gas–liquid mass transfer associated with low diffusion rates is also a problem in mature biofilms, several approaches exist to circumvent this. In a comparative study of acetic acid production by *Clostridium aceticum*, productivity in a packed bed biofilm reactor was independent of the dilution rate due to the low gas–liquid mass transfer of H_2 ($\leq$17 mmol H_2 L^{-1} h^{-1}). In contrast, a trickle bed reactor, in which biofilms are kept only moist but surrounded by the gaseous substrate, showed a threefold-higher productivity with gas–liquid mass transfer rates of up to 56 mmol H_2 L^{-1} h^{-1} and a conversion rate of up to 96% of H_2 (51). In addition to these solutions, membrane biofilm reactors could be a good strategy for gas fermentations because they avoid the problem of low gas solubility. In these reactors, biofilms grow on the membrane surface exposed to the medium/bulk phase, and the gases are fed through the membrane. If the gas addition is controlled properly, complete depletion can be achieved during diffusion through the biofilm. In a synthesis gas fermentation with *Clostridium carboxidivorans*, a hollow-fiber membrane biofilm reactor showed a higher mass transfer coefficient in the abiotic state than stirred tank reactors, which achieved an ethanol production of 24 g L^{-1} (STY: 1 g L^{-1} h^{-1}), a doubling of the amount compared with a suspension culture system (52). **Figure 1** depicts the most common biofilm reactors.

3.7. Limitations of Biofilm-Based Processes

Despite all the advantages of biofilm systems, they are still poorly represented in industrial biotechnological processes. Mainly due to the higher complexity of biofilm reactors compared to conventional stirred tank reactors, there are challenges in reactor control, operation, and scale-up from laboratory- to large-scale systems (6). Especially for industrial-scale processes, the start-up time is prolonged until sufficient biomass is accumulated and a stable process is possible. On the other hand, effective methods to degrade biofilms remain lacking, resulting in extensive reactor cleaning procedures. Further research and development are needed to gain sufficient experience with biofilm reactor operation and predictability in terms of control and start/stop procedures (53). Because biofilm reactors are often more complex and sometimes rely on membranes as biofilm supports, the initial cost of biofilm reactors is generally higher compared with stirred tank systems. Due to long operating times and flow-through systems, products are often highly diluted, indicating a need for optimized purification protocols, especially for large-scale systems (54). In addition, longer operating times and the use of membranes can lead to material fouling, so strategies for the materials used and cleaning procedures must be developed to reduce the cost of part replacement due to fouling (54, 55). However, because biofilm reactors have been used successfully in the water sector for decades, it might be only a matter of time before more and more processes for biofilm reactors are developed.

4. REACTOR SYSTEMS: ACTIVE AND INACTIVE SUBSTRATA

At least in biotechnology, most biofilm reactors contain a substratum on which the organisms settle and form catalytic biofilms. This differs from water treatment systems, where aerobic and

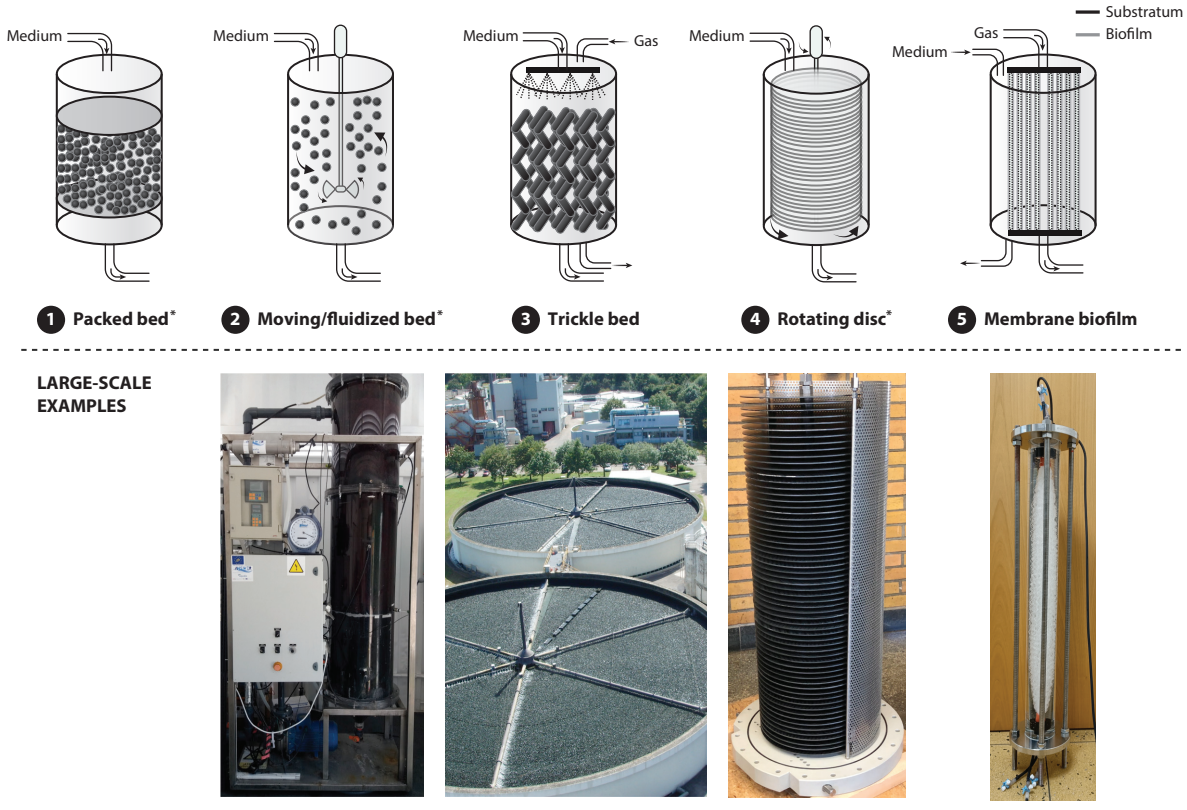

Figure 1

Most common biofilm reactor designs as schematic representations (*upper part*) or as large-scale examples (*lower part*). Asterisk indicates these designs are also used as bioelectrochemical system reactors. Images were kindly provided by Dr. Abraham Esteve-Núñez (University of Alcalá; ②), Stadtwerke Karlsruhe GmbH (Germany; ③), and Dr. Harald Horn (Karlsruhe Institute of Technology; ④). Figure adapted from images created with BioRender.com.

anaerobic granular sludge systems have already found application. Here, the biocatalytic microorganisms form dense granules that quickly sink to the bottom of the reactors and thus are easily retained in the reactor systems (56). To operate with the highest possible percentage of catalytic biomass, biofilm reactors attempt to include as much substratum surface area as possible and at the same time avoid dead volumes. In general, a distinction can be made between active and inactive substrata. Inactive substrata provide only a surface on which the microorganisms grow, whereas active substrata also provide a substrate. Examples include membrane biofilm reactors, in which gaseous substrates are added to the biofilm, which grows directly on the membrane, or BES, in which the substratum is used by the organisms as either an electron donor or acceptor.

To date, several biofilm reactor configurations have been developed, as excellently described by Schmeckebier et al. (53). Briefly, in fixed-bed and fluidized bed reactors, the microorganisms form biofilms on granular substrata that either can be fluidized by the medium flow or are fixed. At lower concentrations, the submerged substrata can also be integrated into stirred tank reactors to retain biomass. In rotating disc or drum reactors, in contrast, the biofilms grow on rotating surfaces, which reduces the limitation of mass transfer with minimal energy input. As mentioned earlier, membrane biofilm reactors can be an excellent solution for gas fermentation processes.

Membranes can be purchased as thin bundles, allowing for a very large surface area within the reactor volume (57).

5. PRODUCTION OF CHEMICALS USING HETEROTROPHIC AND AUTOTROPHIC BIOFILM SYSTEMS

On the way to industrial application, biofilm-based processes must meet certain criteria in terms of productivity (STY; g L^{-1} h^{-1}), product concentration (g L^{-1}), yield (g g^{-1} or %), and operating time (h) to be viable and attractive alternatives for industrial processes. Depending on the product, a STY of 0.1–1 g L^{-1} h^{-1} is a minimum requirement for microbial production to be of interest (58). Still, the possibility to use cheap substrates, such as bio-based waste streams or waste gases, can strongly influence the minimum requirements, because using a waste product as a substrate in combination with a high conversion rate lowers the overall cost. The operating time makes biofilm systems very interesting, because they are generally considered as long-term stable processes without the need for many repeated cycles of reinoculation, thus avoiding frequent process restarts (26). Achievable product concentrations certainly depend on the product itself, and available downstream processing methods might even allow for continuous depletion from the fermentation broth. In the following, we highlight some advances in heterotrophic and autotrophic biofilm-based processes. **Table 1** provides a more thorough overview.

5.1. Heterotrophic Fermentations

In the past decades, promising results have been reported for heterotrophic biofilm processes in terms of substrates, production rates, and run times. In 2018, Ferone et al. (59) reported the highest ever mean STY of 22 g L^{-1} h^{-1} in a succinic acid fermentation with maximum productivities of 35 g L^{-1} h^{-1} over a 5-month period using a mixture of glucose, arabinose, and xylose as substrates and *Actinobacillus succinogenes* as biocatalyst. As early as 1996, the highest achieved productivity in ethanol fermentation was reported with a STY of 536 g L^{-1} h^{-1} using *Z. mobilis* and glucose as substrate over a two-month period (30). Other interesting approaches to ethanol fermentations were reported using rice bran hydrolysate as substrate with *Z. mobilis* as the producing organism, showing a productivity of 0.14 g L^{-1} h^{-1} for more than 6 months (41). This showcases the effective use of waste products containing toxic inhibitors for microorganisms in biofilm fermentations in combination with a long system run time. Whey permeate, another abundant byproduct of industrial processes, was used for nisin production with *Lactococcus lactis* over a period of more than 6 months in a stable process (60). Using the same substrate, 2,3-butanediol production by *Klebsiella pneumoniae* showed a STY of 2.3 g L^{-1} h^{-1} in a packed column reactor with stable production for 7 weeks compared with similar studies using planktonic cells that showed productivities of 0.1–1 g L^{-1} h^{-1} (61). Here, the authors noted that low flow rates are critical for the start-up time of these systems. In 2002, 100% substrate utilization was achieved in a butanol fermentation using *Clostridium beijerinckii*, with a STY of 16.2 g L^{-1} h^{-1} achieved by circulating the medium through the biofilm system, demonstrating the effectiveness of a stable, static biomass and a flexible media phase (62). Hydrogen, a byproduct of metabolism, can also be produced effectively by fermentation, as demonstrated in a fermentation using xylose as an abundant substrate to produce 0.05 g L^{-1} h^{-1} hydrogen with stable production over 2 months (63). An interesting approach to hydrogen production is photoheterotrophic fermentation with biofilms. In 2017, Zhang et al. (64) used light and synthetic wastewater as medium to culture biofilms of *Rhodospirillum rubrum*, *Rhodobacter capsulatus*, and *Rhodopseudomonas palustris* to produce 8 mg L^{-1} h^{-1}. In 2018, scientists cultured a biofilm of *R. capsulatus* that used sucrose from sugar beet molasses as a carbon source to produce 1.6 mg L^{-1} h^{-1} hydrogen. Interestingly, the reactor was placed outdoors for 40 days and still showed stable production by the biofilm (65).

Table 1 Excerpt of fermentations of different chemicals[a]

	Organism	Product	Substrate	Involved pathways[b]	Productivity ($g\ L^{-1}\ h^{-1}$)	Year	Source
Heterotrophic							
Organic acids	*Lactobacillus delbrueckii*	Lactic acid	Glucose	1, 2	7–10	2019	27
	Rhizopus oryzae	Lactic acid	Corn starch	1	1.65	2002	73
	Actinobacillus succinogenes	Succinic acid	Glucose, Xylose, Arabinose	1, 2, 8 (reverse, interrupted)	35	2018	59
		Succinic acid	Xylose	1, 2, 8 (reverse, interrupted)	1.5–2.6	2016	74
	Rhizopus arrhizus	Fumaric acid	Glucose	1, 2, 8 (reductive, interrupted), 9	1.335	2013	75
	R. oryzae	Fumaric acid	Glucose	1, 2, 8 (reductive, interrupted), 9	4.25	1997	76
	Aspergillus niger	Citric acid	Glucose	1, 2, 8 (interrupted)	2.26	2018	77
		Citric acid	Glucose	1, 2, 8 (interrupted)	0.344	2000	78
	Acetobacter pasteurianus	Acetic acid	Glucose + Ethanol	1, 2, 4 8 (side products)	6.5	2000	79
	Clostridium thermolacticum and *Moorella thermoautotrophica*	Acetic acid	Lactose	1, 2, 4	0.18–0.54	2008	80
	Propionibacterium acidipropionici	Propionic acid	Lactate	1, 2, 8, 10	1.621	1992	26
Alcohols and solvents	*Zymomonas mobilis*	Ethanol	Rice bran hydrolysate	3, 4	0.14	2014	41
		Ethanol	Glucose	3, 4	536	1996	30
		Ethanol	B-starch	3, 4	13	1993	44
	Pseudomonas putida	1-Octanol	Octane	11	0.054	2012	81
	Synechocystis sp. and *Pseudomonas taiwanensis*	Cyclohexanol	Cyclohexane	11	0.2	2019	82
	Clostridium acetobutylicum	Butanol (ABE)	Glucose	1, 2, 6	4.4	2010	83
	Clostridium beijerinckii	Butanol (ABE)	Glucose	1, 2, 6	16.13	2004	84
		Butanol (ABE)	Glucose + Corn steep liquor	1, 2, 6	2.01	2004	85
		Aceton (ABE)	Glucose	1, 2, 6	15.8	2000	86
	C. acetobutylicum	Aceton (ABE)	Whey permeate	1, 2, 6	5.1	1987	87
	Gluconobacter oxydans	Dihydroxyacetone (DHA)	Glycerol	11	3.7	2007	88
Antimicrobial	*Pediococcus acidilactici*	Pediocin	Glucose	ND	0.133	2008	89
	Lactococcus lactis	Nisin	Sucrose	ND	3[c]	2007	90
		Nisin	Whey permeate	ND	ND	2003	60
	Cephalosporium acremonium	Cephalosporin-C	Sucrose	ND	0.0071	1999	91
	Kluyveromyces lactis	Lysozyme	Lactose	ND	ND	2021	92
Gas	*C. beijerinckii*, *Clostridium pasteurianum*, and *Enterobacter* sp.	Hydrogen	Glucose	1, 2, 4, 6 (side products)	0.135	2021	93
	Fermenter sludge from xylose fermentation mainly *Clostridium* sp., *Thermoanaerobacterium* sp., and *Caloramator* sp.	Hydrogen	Xylose	1, 2, 4, 6 (side products)	0.0498	2018	63

(*Continued*)

Table 1 (*Continued*)

	Organism	Product	Substrate	Involved pathways[b]	Productivity ($g\ L^{-1}\ h^{-1}$)	Year	Source
	Thermoanaerobacterium thermosaccharolyticum	Hydrogen	Sucrose	1, 2, 4, 6 (side products)	0.304	2008	94
	Rhodospirillum rubrum, *Rhodobacter capsulatus*, and *Rhodopseudomonas palustris*	Hydrogen (photo-heterotroph)	Synthetic wastewater	1, 3, 8, 11	0.000786	2017	64
	Rhodobacter sphaeroides	Hydrogen (photo-heterotroph)	Volatile fatty acids	1, 3, 8, 11	0.091	2015	95
		Hydrogen (photo-heterotroph)	Malic acid	1, 3, 8, 11	0.053	2013	96
	Rhodopseudomonas faecalis	Hydrogen (photo-heterotroph)	Acetate	1, 8, 11	0.00295	2012	97
	R. palustris	Hydrogen (photo-heterotroph)	Glucose	1, 8, 11	0.0035	2010	98
Other	*Pseudomonas* sp.	(*S*)-styrene oxide	Styrene	11	1	2010	99
		(*S*)-styrene oxide	Styrene	11	1.167	2010	100
	Blakeslea trispora	Carotene	Glucose + corn steep liquor	1, 2, 7	0.0575	2017	66
	Cupriavidus necator	PHB	Glucose	3	0.0378	2004	101
	Escherichia coli	5-Halotryptophan	5-Haloindole	11	ND	2013	102
		5-Halotryptophan	5-Chloroindole, 5-fluoroindole, 5-bromoindole	11	ND	2011	71
	Klebsiella pneumoniae	2,3-Butanediol	Whey permeate	1, 2	2.3	1986	61
Autotrophic							
Alcohols	*Clostridium carboxidivorans*	Ethanol	Syngas	5, 4	0.279	2017	70
	Clostridium ragsdalei	Ethanol		5, 4	0.16	2017	68
Gas	Sump liquid of a trickle bed reactor for biological methanation	Methane	H_2, Co_2	ND	0.05	2021	57

[a]Shown are the products, the fermenting organisms, the used substrates, the year of publication, the calculated volumetric productivities, and the sources.
[b]See **Supplemental Figure 1** for a description of pathways.
[c]Indicates calculation of STY without specific numbers.

Supplemental Material >

Last but not least, Roukas (66) reported in 2018 the production of carotene from corn steep liquor by a biofilm of the fungus *Blakeslea trispora*. A productivity of 0.06 g L^{-1} h^{-1} was reached, a value six times higher than what was achieved in conventional stirred tank reactors.

5.2. Autotrophic Fermentations

In autotrophic fermentations, organisms use inorganic sources to meet their energy and carbon requirements and produce desired products. Syngas has emerged as a promising substrate, consisting of different ratios of CO, CO_2, and H_2, which can be converted by acetogens into organic acids (typically acetic acid) and, in a second step, into solvents (mainly ethanol) by solventogenesis. The latter process is reversible depending on specific process conditions (67). In the continuous production of ethanol from syngas in a trickle bed reactor using *Clostridium ragsdalei*, the process achieved a peak productivity of 0.16 g L^{-1} h^{-1} at a molar ratio of ethanol to acetic acid of 4:1. The gas flow rates investigated in this study showed that a gas flow rate between 1.5 and 2.8 standard cubic centimeters per minute resulted in H_2 and CO conversion rates above 90% (68). In a laboratory evolution study that lasted over 69 days, the team could demonstrate that the same

organism achieved a threefold increase in CO and H_2 uptake rates (69). To address the gas–liquid mass transfer limitation, a horizontally oriented rotating fixed-bed reactor with *C. carboxidivorans* cells was used, with the liquid and gas contact alternatingly provided through the rotations. Using this approach, the researchers achieved an ethanol STY of 0.28 g L^{-1} h^{-1}, which is more than three times higher than the value obtained in stirred tank reactors under identical conditions (70). Another type of autotrophic cultivation is hydrogenotrophic methanation, which uses hydrogenotrophic organisms to produce methane from a mixture of H_2 and CO_2. The authors demonstrated a methane content of 97% using a custom membrane biofilm system and achieved a maximum production per reactor volume of 0.05 g L^{-1} h^{-1}. Pratofiorito et al. (57) predicted a significant increase in productivity by using smaller-diameter capillary fiber membranes, resulting in a larger specific membrane surface area and reducing the hydrogen solubility problem.

The interest in studying biofilm processes has increased greatly in the last two decades, with reports and investigations on the production of low- and high-value products as well as fine chemicals (71, 72). **Table 1** shows an excerpt of different chemicals produced by biofilms as an example for interesting results with unusual substrates, processes, or high productivities. The graph in **Supplemental Figure 1** depicts the active metabolism pathways involved in chemical production, as mentioned in **Table 1**.

Supplemental Material >

6. BIOFILMS IN BIOELECTROCHEMICAL SYSTEMS

Not only has interest in conventional biofilm production processes increased, but interest in biofilm processes in BES has also grown tremendously. In BES, microbes serve as biocatalysts for production of biofuels and value-added compounds, as well as electricity and hydrogen.

Exoelectrogenic organisms capable of EET using electrodes as terminal electron acceptors can act as electrochemically active biocatalysts in these systems. They couple intracellular redox reactions with EET (103). These organisms catalyze oxidative reactions on the anodic side and reductive reactions on the cathodic side. **Figure 2** provides an overview of different BES systems.

6.1. Anodic Bioelectrochemical Systems

To use anodes as extracellular electron acceptors, microorganisms must be able to transport electrons across the cell membrane and transfer them to insoluble electron acceptors. To facilitate this electron transfer, microorganisms usually have a network of *c*-type cytochromes spanning the membranes and periplasm (104). Various mechanisms have evolved to further transfer electrons from the cell envelope to insoluble electron acceptors. Because the anodic surface is often the limiting factor for productivity in BES, the formation of thick, conductive biofilms is a promising approach to achieve high STYs. Consequently, only two mechanisms are important for bacterial electron transfer to the anode in biofilms. The first is direct electron transfer, in which electrons are transferred directly from the cell surface to the anode. Crucially, the distance between the cells and the anode must be less than 15 Å. Therefore, direct electron transfer is suitable only for monolayers on the anode (105). Second, many microorganisms can form a conductive biofilm several cell layers thick. In these biofilms, electron transfer is achieved by embedding nanowires composed of cytochromes or other electron-transferring proteins or molecules into the extracellular matrix (106–108).

In general, anodic BES can be divided into microbial fuel cells (MFCs) and MECs (**Figure 2**). In MFCs, electrons transferred to the anode are passed through a resistor to the cathode, where they react with oxygen and protons diffusing from the anode compartment to form water. Thus, in MFCs, chemical energy from the substrate is converted into electrical energy. In contrast, MECs are designed not to generate electricity but to produce hydrogen at the cathode. Here, the potential

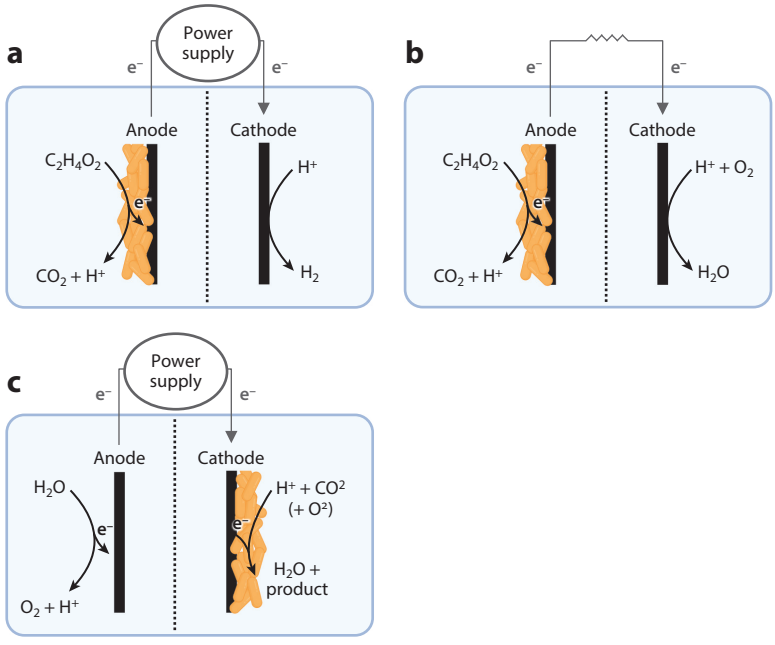

Figure 2

Overview of different bioelectrochemical systems. (*a*) A microbial electrolysis cell is shown. Here anode potential is controlled via a potentiostat. The electrons and protons at the cathode side react to hydrogen. (*b*) A microbial fuel cell is shown. The electrons go through an external resistor, producing electrical energy. (*c*) A microbial electrosynthesis cell is shown. Microbes take up electrons or hydrogen at the cathode side and use CO_2 as a carbon source for the formation of a product of choice. Figure adapted from images created with BioRender.com.

of the anode is controlled by, for example, a potentiostat, whereas the cathodic region is kept anoxic. The electrons transferred to the anode move via the potentiostat to the cathode, where they can react with the protons diffusing from the anode region to form hydrogen. In contrast to conventional water electrolysis, the required cell voltage can be reduced by a factor of 10 by using MECs (109).

6.2. Enhanced Electron Transfer Rates in Anodic Biofilms

EET of Gram-negative microorganisms begins in the inner membrane. There, electrons are transferred from the quinone pool to a membrane-associated *c*-type cytochrome. From there, electrons are transferred to periplasmic *c*-type cytochromes, which transport electrons through the periplasm to a membrane complex that spans the outer membrane and usually consists of three to four proteins. The first of these proteins is located on the inside of the outer membrane and accepts electrons from the periplasmic *c*-type cytochromes. Direct contact is made with a third protein via a β-barrel protein that forms a pore in the outer membrane. This protein acts as a terminal reductase and transfers electrons to the extracellular electron acceptor. Often attached to the terminal reductase is a fourth protein that is also capable of terminal electron transfer. Philipp et al. (110) provide an overview of EET in the two model organisms *S. oneidensis* and *G. sulfurreducens*.

The first target for an increase in electron transfer rate and thus increased productivity is intracellular NADH concentration. Increased intracellular NADH concentration accelerates the

electron transfer rate to the quinone pool and subsequently to *c*-type cytochromes. Thus, Li et al. (111) increased NAD(H/+) biosynthesis and consequently observed a threefold increase in maximum power output (0.1058 W m^{-2}) along with increased coulombic efficiency. In another approach, the effect of overexpression of the gene encoding the *c*-type cytochrome of the inner membrane (CymA) in *S. oneidensis* was investigated. This *c*-type cytochrome facilitates electron transfer from the quinone pool to the periplasm. Vellingiri et al. (112) could increase power output by 20% and growth rate by 100% by overexpressing *cymA* in an MFC. To improve periplasmic electron transfer, Delgado et al. (113) overexpressed the periplasmic *c*-type cytochrome CctA by replacing the *nrfA*, *ccpA*, *napB*, and *napA* genes with additional copies of *cctA*. Overexpression resulted in a 1.7-fold increase in iron reduction rate and a 23% increase in current generation. To increase the electron transfer rate across the outer membrane, Min et al. (114) overexpressed the genes of the outer membrane spanning complex consisting of MtrA, MtrB, and MtrC in *S. oneidensis*. The group coupled overexpression of *mtrABC* with overexpression of the riboflavin biosynthetic gene cluster and achieved a 110% increase in maximum current density. In summary, the described studies investigated the individual steps of electron transfer to the cell surface, and overexpression of the respective genes positively affected the electron transfer rate. No group has yet linked the individual approaches to increase cellular electron transfer. However, combining the individual approaches could dramatically increase the electron transfer rate and thus productivity.

Along with the cellular electron transfer rate, the electron transfer from the cell surface to the anode is also crucial. Especially in thicker biofilms, this requires the conductivity of the entire biofilm to transfer electrons from the outer biofilm layers to the electrode. For example, Leang et al. (115) studied biofilms of *G. sulfurreducens*, focusing on PilZ domain proteins. These proteins are activated upon binding of c-di-GMP and regulate downstream protein activities at the post-translational level (116). By deleting genes containing PilZ domains, a strain was identified that showed increased production of pili and exopolysaccharides by deletion of GSU1240. In addition, the corresponding biofilms that grew on electrodes were sixfold more conductive than wild-type biofilms. Overall, a 70% increase in power density was achieved (115). Recently, Wang et al. (117) demonstrated that overexpression of nanowire proteins resulted in a 2.6- to 3-fold increase in electrical power. Moreover, biofilm formation was increased by 74–93%, suggesting that a bottleneck for the formation of thick biofilms in BES is their conductivity. In addition to overexpression of native conductive proteins, biofilm conductivity can also be enhanced via integration of other conductive particles, which can be either formed intracellularly or added externally. In a study by Chen et al. (118), *G. sulfurreducens* intracellularly formed gold nanoparticles from the precursor $NaAuCl_4$. These gold nanoparticles increased the current density by 40% and the substrate turnover rate by 2.2-fold. In another approach, use of a hydrogel matrix composed of agarose fibers promoted biofilm formation of *S. oneidensis*. Further addition of carbon nanotubes (CNTs) functionalized with riboflavin resulted in a 9.1-fold increase in current density (119).

6.3. Modification of the Anode or Biofilm in Anodic Bioelectrochemical Systems

In addition to altering the biofilm itself, changes at the anode or cathode can also have positive effects on electron transfer. In contrast to direct modification of bacterial properties, this approach can also be pursued when the organisms are less well characterized and genetically inaccessible. Mier et al. (120) provide a good overview of recent studies on anode and cathode materials and modifications. In general, anode modifications can be divided into modifications based on metal/metal oxides, conductive polymers, and CNTs. Zhang et al. (121) pursued a very promising approach for a metal/metal oxide–doped anode. The group achieved a maximum power density of 3.6 W m^{-2} with a mixed community by coating the anode with MnO_2. They also showed that the

flow mode of the MFC reduced the anode mass transfer resistance by 41.4%. The combination of MnO_2 coating and a top layer of conducting poly 3,4-ethylenedioxythiophene also provided a high maximum power density of 1.5 W m^{-2} (122). Although the absolute power density in the study by Liu and colleagues (122) is lower than that in the study by Zhang and colleagues, they are not entirely comparable, because the power density is also significantly affected by the reactor design. A common approach to increase the anode surface area is to deposit CNTs. However, modifying anodes with CNTs often hinders the attachment of microorganisms due to biotoxicity and hydrophobicity. Nevertheless, numerous studies demonstrate an increase in productivity in BES with the use of CNTs. Often, CNTs are coated with polymers to achieve better bioavailability. This was the case in the study by Cui et al. (123), who coupled CNTs onto a graphite felt and then coated them with polyaniline. This anode modification resulted in a 343% increase in output voltage and a 0.26 W m^{-2} increase in maximum power density.

When choosing a reactor system for biofilm-based anodic BES, a large anode surface area–to–volume ratio is critical. This can ensure efficient electron transfer in the system and increase STY. A biofilm-based reactor type originating from wastewater treatment that has been used successfully in anodic and cathodic BES is the rotating disc reactor. **Figure 1** presents an illustration and photograph of a rotating disc reactor. In this reactor, a surface-to-volume ratio of 100 m^2 m^{-3} and current densities up to 1.13 A m^{-2} could be achieved with a coculture of *G. sulfurreducens* and *S. oneidensis* (124). Another promising reactor type for biofilm-based production processes in anodic BES is the packed bed reactor. Here, a carbon-based granule is used as anode and biofilm substrate, achieving a power density of up to 0.32 W m^{-2} (125).

6.4. Production of Platform Chemicals in Anodic Bioelectrochemical Systems

In addition to hydrogen and energy production, some studies are focusing on the production of platform chemicals using anodes as a nondegradable electron acceptor. Use of anodes as electron acceptors can keep production costs low because no gassing of the process is required to introduce oxygen, and energy in the form of electrical power or hydrogen is produced as a valuable byproduct. However, platform chemical production in BES is limited to genetically accessible microorganisms or the natural products of electroactive microorganisms. Moreover, the reported STYs remain comparatively small. A commonly targeted end product is the platform chemical acetoin. The US Department of Energy ranked acetoin as one of the 30 most promising platform chemicals in 2004 (126–128). It can be produced from the key metabolic intermediate pyruvate via acetolactate by the two enzymes acetolactate synthase (AlsS) and acetolactate decarboxylase (AlsD). Bursac et al. (129) synthesized acetoin from lactate via heterologous expression of *alsS* and *alsD* from *Bacillus subtilis* using *S. oneidensis* as the production strain. They achieved remarkable carbon yields of up to 86%. However, only 0.273 g L^{-1} of acetoin was produced within 72 h. Kong et al. (130) pursued another approach for producing acetoin by biofilms in anodic BES using glycerol as substrate. The authors used a genome-level metabolic network to predict the key players in the process and increase power density and acetoin yield. Using this approach, they achieved acetoin production of 0.313 g L^{-1} and a power density of 0.149 W m^{-2}.

In addition to acetoin, ethanol was also produced in an anodic BES using *S. oneidensis* as the production organism. For this purpose, pyruvate decarboxylase and alcohol dehydrogenase from *Z. mobilis* were heterologously expressed. Again, glycerol was used as a substrate and made available via expression of the *glpF*, *glpK*, *glpD*, and *tpiA* genes from *E. coli*. Using this approach, a carbon efficiency for the conversion of glycerol to ethanol of 80% was achieved (131). **Table 2** provides an overview of the presented production processes.

Although production rates in BES for platform chemical production remain quite low, the process could be worthwhile due to savings in process costs and generation of valuable byproducts.

Table 2 Overview of anodic bioelectrochemical systems and approaches for improved production

Organism	Modification	Increase in current/ power output	Product	Reference
Shewanella oneidensis	Enhanced intracellular NADH regeneration	300% increase in max power density	Power: 0.11 W m^{-2}	111
	Overexpression of the membrane bound *c*-type cytochrome CymA	20% increase in max power output	Power[a]: 0.07 W m^{-2}	112
	Overexpression of the periplasmatic *c*-type cytochrome CctA	23% increase in max current density	Current[a]: 0.23 A m^{-2}	113
	Overexpression of *mtrC-mtrA-mtrB* and flavin biosynthesis cluster	110% increase in max current density	Current: 0.19 A m^{-2}	114
Geobacter sulfurreducens	Deletion of gene GSU1240 (PilZ domain protein)	70% increase in power density	Power[a]: 1.2 W m^{-2}	115
	Overexpression of genes for nanowire proteins	262–297% increase in power density	Power: 1.39–1.58 W m^{-2}	117
	In situ formation of gold nanoparticles	40% increase in current density	Current: 7.3 A m^{-2}	118
S. oneidensis	Agarose gel matrix to enhance biofilm formation + addition of riboflavin functionalized CNT	910% increase in current density	Current: 1.32 A m^{-2}	119
Mixed culture	Electrodeposition of manganese oxide on the anode	24.7% increase in maximum power density	Power: 3.58 W m^{-2}	121
	Vapor polymerization of PEDOT and manganese oxide onto carbon felt anode	57.7% increase in maximum power density	Power: 1.53 W m^{-2}	122
Shewanella putrefaciens	Electropolymerization of polyaniline on graphite felt followed on electrophoretic deposition of CNTs	186% increase in maximum power density	Power: 0.26 W m^{-2}	123
S. oneidensis	Acetoin production via expression of *alsS* and *alsD* from *Bacillus subtilis*, enhanced current density by deletion of lambda phage, and increase of optical density	380% increase in current density	Current: ~0.19 A m^{-2} Acetoin production: 0.237 g L^{-1}	129
	Construction of strain capable of using glycerol for production of acetoin and bioelectricity	508% increase in power density, onefold increase in acetoin production	Power: 0.149 W m^{-2} Acetoin production: 0.313 g L^{-1}	130
	Genetical engineering for conversion of glycerol to ethanol	Not mentioned	Not mentioned	131

[a]Indicates the calculation without specific numbers.

In addition, Philipp et al. (110) calculated that a conductive biofilm with a thickness of 40 μm (comparable to *G. sulfurreducens* biofilms) on an anode with an area of 1 cm^2 would hold approximately 3.6×10^9 cells. The current per cell for *G. sulfurreducens* and *S. oneidensis* was reported to be 75–204 fA. Taken together, the 40-μm conductive biofilm could achieve a current density of 0.27 and 0.72 mA/cm^2. This corresponds to an increase in current density of 24.5 to 65.5 times for *S. oneidensis* and 1.5 to 4 times for *G. sulfurreducens*.

6.5. Cathodic Bioelectrochemical Systems

In addition to using the anode in the BES, several approaches also use the cathode of the BES to grow microorganisms and produce valuable compounds. These BES are called microbial electrosynthesis cells (MES) (**Figure 2**). In MES, electrons or hydrogen is released from the cathode and used by the microorganisms as an energy source, and CO_2 is usually used as a carbon source that is converted into value-added compounds in the process. In general, most microorganisms capable of autotrophic growth can be used as biocatalysts in MES. A major advantage of MES over conventional autotrophic cultivation is that hydrogen is continuously generated in the process and used by the cathodic biofilm. Hence, the low solubility of hydrogen in aqueous solutions is negligible. The vast majority of applications described for cathodic BES involve acetate or methane production. Therefore, acetate and methane production, as well as cathodic modifications to improve production yields in MES, are discussed in more detail below.

6.6. Cultivation and Production in Anoxic Microbial Electrosynthesis Cells

To increase the surface area, create a positively charged surface, improve hydrophilicity, and increase conductivity, cathode surface modifications are a promising approach to achieve high STYs. Carbon is often used as a starting material for cathodes in MES because it is highly biocompatible, chemically stable, and inexpensive. However, because the commonly used carbon materials have low surface area, Flexer & Jourdin (132) proposed the use of deposited CNTs on 3D-structured reticulated glassy carbon as the 3D-structured carbon material. The resulting extremely large surface area not only increased biofilm formation and current density but also led to an acetate production rate of 55.42 $g\ m^{-2}\ h^{-1}$. Another promising approach is metal nanoparticle deposition on the cathode, which can increase conductivity and provide a more positive surface charge and a larger surface area for biofilm growth. In a study by Wang et al. (133), deposition of a nickel-phosphide catalyst increased the acetate production rate by 1.7-fold, resulting in a final production rate of 7 $mg\ L^{-1}\ h^{-1}$. In the same process, butyrate production was also increased 2.5-fold to 4 $mg\ L^{-1}\ h^{-1}$. In addition to modifying the cathode surface itself, bioconjugation of conductive nanoparticles and microorganisms can also be used to increase the active area on a cathode. Ye et al. (134) pursued an interesting approach in which a streptavidin tag is coupled to magnetic Fe_3O_4 particles. These particles can then bind to microorganisms and are directed to the cathode using a magnet. By using *Methanosarcina barkeri* as the biocatalyst and the magnetic bioconjugation approach, methane yields were increased 33.2-fold.

In addition to modifying cathode surfaces, MES can also be used to enhance growth and production under autotrophic conditions. Due to low hydrogen solubility, growth under autotrophic conditions is often limited. Therefore, MES can serve as a direct, nondepletable hydrogen source. In a study with *Clostridium ljungdahlii*, 0.46 $g\ L^{-1}\ h^{-1}$ acetate could be produced together with glycine and ethanolamine (135). Another commonly targeted end product in anoxic MES is methane. Methane and acetate production is often carried out with mixed cultures. However, in 2019, Mayer et al. (136) compared different methanogenic pure cultures and their STYs in MES. The highest methane yield of 6 $mg\ m^{-2}\ h^{-1}$ was obtained with *Methanococcus maripaludis*.

In addition, methanogens are widely used to upgrade biogas from anaerobic digestion processes and to increase methane content. Recently, Ning et al. (137) compared different scenarios for reduction of biogas-based CO_2 to methane. The authors compared a power-to-gas anaerobic digestion in which hydrogen is produced via classical water splitting with an approach in which the biogas from an anaerobic digestor is injected into a second vessel for external microbial electrosynthesis. Their scenario calculation is based on 100 t of feedstock, which is first converted into 2.1 t of methane and 3.8 t of CO_2. This CO_2 is then reduced to methane using either hydrogen or electricity in microbial electrosynthesis systems. Although the overall energy output in the bioelectrochemical scenario is 23.533 kWh, the power-to-gas-based system can deliver only up to 6,830 kWh. This is due to the significantly lower amount of electricity/voltage necessary for the bioelectrosynthetic reduction of CO_2 compared to first producing hydrogen that is then used by hydrogenotrophic organisms (137).

For efficient reaction processes, MES reactors must meet microbial and electrochemical requirements hand in hand. These special conditions require optimized reactors with special attention to ohmic losses, electrode overpotential, gas–liquid transfer, biofilm attachment, and surface-to-volume ratio. Therefore, to date, no standardized bioelectrochemical reactor meets all requirements to the same degree. In particular, regarding production rates rather than characterization of individual process parameters, literature is scarce. However, modular stacked reactors are a promising approach. Here, two flat chambers are separated by a membrane to form an anode chamber and a cathode chamber. This approach allows for a short distance between the anode and cathode and a large membrane area relative to volume, reducing the internal resistance of the system. A stacked reactor was used to produce 0.41 g L^{-1} h^{-1} acetate as well as 0.13 g L^{-1} h^{-1} *n*-butyrate and 0.04 g L^{-1} h^{-1} *n*-caproate in a continuous system (138). Other applied reactor technologies are based mostly on already-established bioreactors. For example, fixed-bed reactors have been used not only as anodic BES but also as MES. By using conductive filler material such as graphite granules, high biofilm density and excellent surface-to-volume ratio as well as acetate production rates of 0.041 g L^{-1} h^{-1} could be achieved (139). However, most so-far-developed reactor systems do not exceed a volume of a few liters and thus are limited in CO_2 fixation rate. This is why in future larger plants will be needed to deliver industrially relevant quantities of products. The most popular reactors for MES are shown in **Figure 1**.

6.7. Cultivation and Production in Oxic Microbial Electrosynthesis Cells

Although the most popular microorganisms for cathodic BES are acetogens and methanogens, recent work has also been conducted on oxic MES to broaden the range of achievable products, because the higher energy yield of the biocatalysts can be associated with more complex end products. However, most of the studies for higher-yielding products are still very recent, and much work has been done to characterize new biocatalysts and their potential products. The first oxic MES was studied as early as 1965 by Schlegel & Lafferty (140), who used *Cupriavidus necator* as the biocatalyst. The authors showed that H_2 and O_2 production via electrolysis in the reactor and the addition of CO_2 were sufficient for organism growth. This was the first known work on the cultivation of oxyhydrogen bacteria in MES. The organism has proven to be one of the most promising production hosts for industrial applications, particularly because of its rapid growth and genetic accessibility. The first product to attract interest is the hydrophobic storage polymer polyhydroxybutyrate (PHB), which is natively produced by *C. necator* and can be used for bioplastics production. By using a cathode of N-doped CNTs, Li et al. (141) achieved PHB production of up to 9.94 g m^{-2} h^{-1}. Moreover, PHB accounted for 42.61% of the cell dry weight. However, due to the easy genetic accessibility of *C. necator*, the production of non-native end

products is also possible. For example, Torella et al. (142) achieved isopropanol production of up to 0.002 g L^{-1} h^{-1} by deleting the genes for PHB synthesis, overexpressing a ketothiolase and an acetoacetyl-CoA transferase, and heterologously expressing an acetoacetate decarboxylase and an alcohol dehydrogenase from *Clostridium* spp. Another approach by Krieg et al. (143), using *C. necator* as the production strain, was to heterologously express the mevalonate pathway from *Myxococcus xanthus*. This led to the production of up to 0.83×10^{-4} g L^{-1} h^{-1} α-humulene. Production of lycopene using *C. necator* with a STY of 0.167×10^{-4} g L^{-1} h^{-1} was also demonstrated (144). Another recently isolated organism for the production of higher carbon compounds is *K. spormannii*. This thermophilic organism was isolated in 2018 from hydrothermal sediment samples from the Azores (Portugal) and is characterized by its optimal growth temperature of 55°C and optimal pH of 5.5 (145). In 2020, Reiner et al. (146) showed that the organism can also be cultured on cathodes in oxic MES, making it an interesting candidate for further research as a natural PHB producer. Pillot et al. (147) investigated the optimal carbon-fleece cathode pretreatment and reactor design. Pretreating the cathode with isopropanol and sonication and reducing the distance between the cathode and anode resulted in a fivefold increase in PHB production, with a maximum production of 0.005 g m^{-2} h^{-1}. **Table 3** lists the mentioned productivities.

Table 3 Overview of cathodic bioelectrochemical systems and approaches for improved production

Organism	Modification	Product and production rate (increase)	Reference
Mixed culture	Deposition of carbon nanotubes on 3D-structured reticulated vitreous carbon	55.42 g m^{-2} h^{-1} acetate[a]	132
Clostridium ljungdahlii	Electrochemical deposition of nickel phosphide on cathode	0.007 g L^{-1} h^{-1} acetate (170% increased) 0.004 g L^{-1} h^{-1} (250% increase)	133
Methanosarcina barkeri	Conjugation of magnetic nanoparticles with microorganisms; directed to cathode via magnet	0.1×10^{-3} g L^{-1} h^{-1} methane (332% increased)	134
C. ljungdahlii	Growth of *C. ljungdahlii* using cathodic hydrogen	0.0046 g L^{-1} h^{-1}	135
Methanococcus maripaludis	Methane production with different pure cultures in comparison	0.0058 g m^{-2} h^{-1} methane[a]	136
Mixed culture	Use of stacked reactor for production of acetate, butyrate, and caproate	0.399 g L^{-1} h^{-1} acetate 0.13 g L^{-1} h^{-1} n-butyrate 0.04 g L^{-1} h^{-1} n-caproate	138
Mixed culture	Use of fixed-bed reactor for production of acetate	0.043 g L^{-1} h^{-1} acetate	139
Cupriavidus necator	Nickel nanoparticles embedded in N-doped carbon nanotubes as catalyst on cathode—no nickel leaching	0.058 g L^{-1} h^{-1} polyhydroxybutyrate (PHB)	141
	Genetically engineered strain for isopropanol production	0.002 g L^{-1} h^{-1} isopropanol	142
	Production of terpenes via heterologous expression of mevalonate pathway and α-humulene synthase	0.83×10^{-4} g L^{-1} h^{-1} α-humulene	143
	Production of lycopene	0.167×10^{-4} g L^{-1} h^{-1}	144
Kyrpidia spormannii	Optimization of growth and PHB production by using different cathode materials and different distances between anode and cathode	0.005 g m^{-2} h^{-1} PHB (500% increase)[a]	147

[a]Indicates that the values are normalized to the cathode area.

Because oxic MECs are a very new field of research, only one investigation on suitable reactor techniques has been carried out. Hackbarth et al. (124) showed that a rotating disc reactor can be used successfully to cultivate biofilms in oxic MECs. Another promising reactor type for oxic MES is the trickle bed reactor. Like the rotating disc reactor, it originates from wastewater treatment. Because the biofilm is only trickled, the additional oxygen input can be omitted, which lowers the process costs. However, to enable efficient production processes in oxic MES, the reactors must be optimized specifically for the process conditions.

7. CONCLUSION

Production of chemicals and energy using biofilms offers greater independence from fossil fuels as the main source of energy and platform chemicals. Although microbial fermentation offers the opportunity to produce much-needed platform chemicals from waste materials or low-cost substrates, biofilm systems can further simplify production and increase efficiency. Productivity is often increased because the self-sustaining cells are more robust to fluctuating process conditions or contaminants, allowing the establishment of long-term and continuous production processes (5, 25, 26). Artificially produced biofilms have also shown promise as catalysts for biotransformation of fine chemicals, in some cases outperforming both purified proteins and cell-free lysates (71).

On the other hand, biofilm systems present new challenges for process engineering and industrial applications because of the long start-up times required for sufficient biomass growth, high initial capital costs for equipment and environmentally friendly materials, and lack of standardized procedures for biofilm degradation during reactor cleaning. In addition, the products are often highly diluted, indicating the need for optimized purification protocols, and process controls remain severely limited while the systems are running (148–150), indicating the need for further research in these areas.

As mentioned earlier, there are several approaches to understanding biofilms and controlling production processes. One commonly observed phenomenon is the emergence of cellular heterogeneity within biofilms. Last year, Jo and colleagues (150) published a comprehensive study that not only focused on individual elements leading to microniches in biofilms but also examined the crosslinks between physical gradients and physiological patterns, taking a first step toward elucidating the correlations in these systems. Another interesting area of research is biofilm degradation, with a focus on medical applications and potential use in productive biofilms when the need for control is considered. Economically viable use of biofilms requires achieving a steady state of optimal productivity while preventing system clogging. Traditionally, this has been attempted via flow rate control, but the use of native dispersion mechanisms of biofilms appears to be a more promising approach, especially with respect to reactor cleaning (151). Reactor setups, designs, and material studies themselves are a broad research topic with the goal of improving process stability and productivity while considering the intended end product. Numerous reviews illustrate the various approaches to bioconversions (148), fermentations (54), value-added products (152), improved electrode interactions (153), gas fermentations (154), and imaging techniques (155, 156).

Recent advances provide insight into what can be achieved with productive biofilms. However, to achieve the necessary improvements in industrial production, biofilms must be optimized and researched to reach their full potential. This requires better management of the unclear boundaries between terms such as biofilms, self-/passively immobilized cells, solid-state, and static culture. Although these terms refer to specific cultivation types or conditions, they regularly diffuse and overlap in the relevant research areas, hindering overall progress.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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