Microbial Electrosynthesis—An Inventory on Technology Readiness Level and Performance of Different Process Variants

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The objective of microbial electrosynthesis (MES) is to combine the advantages of electrochemistry and biotechnology in order to produce chemicals and fuels. This combination enables resource-efficient processes by using renewable raw materials and regenerative energies. In the last decade, different MES processes have been described, for example, MES based on biofilms or mediators, electro-fermentation, and secondary MES. This review compares the MES technologies with regard to the reached process performances in terms of key process indicators (i.e., coulombic efficiency (CE), product titre, productivity) and technology readiness level (TRL). Often the underlying mechanism of electron transfer in biofilm-based processes has not been elucidated and can therefore not be optimized. Similarly, technical aspects of electro-fermentation processes and processes with soluble mediators are under investigation and techno–economic assessments are missing. In contrast, the electrochemical production of microbial substrates in secondary MES or hybrid systems show high key process indicators and TRLs up to 7. In summary, the different types of MES processes offer options for today's industrial use, as well as an exciting and future-oriented technology that can be applied in a medium-term perspective.

1. Introduction

Mankind is facing global challenges that arise with a growing world population and rising living standards. Among those challenges are an increase in CO₂ concentration in the atmosphere, finite natural resources, and instability of their supply. Thus, we are at the end of an era characterized by economic and societal changes to which the chemical production has to adjust. Therefore, it is consensus that a new biobased economy—bioeconomy—has to be formed which is based on renewable resources and eco-efficient processes for chemical production. A bioeconomy has not only to be based on renewable raw materials and renewable energies, but also has to assure circular utilization of resources and the interlinking of (bio)chemical transformations with the storage and utilization of electric energy.[1] Renewables in the electric energy sector like photovoltaics face temporal fluctuations, as well as a spatial separation of source and sink that creates a demand for storage and conversion technologies. However, creating a link between the chemical and energy sector can only to a very minor extent be achieved with established technologies. The concept “power-to-X” summarizes innovative technologies to transform (surplus) electric energy to other energy forms, any kind of storage compound, or bulk or fine chemicals, whereby “X” stands for “heat,” “chemicals,” “gas,”
“fuels,” or “molecules,” among others.[2] Electrobiotechnology strives for the concept of power-to-chemicals to narrow or even close the gap between the energy and the chemistry sector.[3] This technology is based on the combination of biotechnology and electrochemistry and promises to create a new path for the conversion of electric to chemical energy. In general, electrobiotechnology covers a wide range of potential applications ranging from electric energy generation from wastewater via removal of decontaminants to the synthesis of complex chemicals.[3] While electrobiotechnology for harvesting of electric power has been investigated for many decades, bioelectrosynthesis has received increasing attention only in recent years. In bioelectrosynthesis enzymes and microorganisms are exploited as catalysts for electrobiotechnological applications that would allow overcoming several of the fundamental challenges of a bioeconomy.

If microorganisms are applied, the process is called microbial electrosynthesis (MES). Different definitions for MES[4] do exist, however, here we refer to the most common and inclusive one being “MES is the execution of microbially catalyzed electrochemical reactions to transform a substance into a desired product.”[5] During the last decades numerous examples on MES were published. These can be classified into four basic types of processes: A) biofilm-based MES, B) MES based on soluble mediators, C) electro-fermentations, D) MES based on secondary microbial electrosynthesis technologies (MET) and hybrid technologies (Figure 1).

In biofilm-based processes a direct extracellular electron transfer (DEET) typically occurs (Figure 1A). Metal containing proteins and cellular appendages are the key compounds for the transfer of electrons across the cell barrier. DEET is enabled by physical contact of the cells with the electrode via the aforementioned proteins or cellular appendices (e.g., ref. [6]). In mediated extracellular electron transfer (MEET) soluble non-metabolisable shuttle molecules (mediators) reversibly transfer electrons between the electrode and the microorganism even over larger distances. MEET has been investigated with different model organisms such as *Shewanella*.[7] and it was recently shown that this type of electron transfer seems to be widely used, for instance in the gut.[8] Natural mediators, for example, flavins and phenazines, are secreted by several microorganisms.[9] Additionally, various microorganisms can transfer electrons by using artificial mediators added to the medium.[10] This transfer mechanism is the key to the processes described in section 3 “MES based on soluble mediators” (Figure 1B). In electro-fermentation, established fermentation processes are improved by electrochemical steering. An applied potential influences the fermentation environment and microbial metabolism in either a reductive or oxidative manner and often leads to an improved carbon yield.[11] In MES based on secondary MET, an electrochemical reaction is mainly used to produce metabolites (e.g., hydrogen or formate) or to upgrade a microbially synthesized product. In the first case, the metabolites are consumed and converted to a more valuable product in a subsequent microbial conversion. In contrast to the mediators used in MEET, the compounds undergo an irreversible redox process. Here, the process steps can be realized either in one reactor or in two separated reaction systems. In the
following, the latter configuration is referred to as a hybrid system, as it can be assumed that there is no mutual influence of the reactions. The above-mentioned classification does not cover all possible processes and certain overlaps do exist, but it serves as a valuable guide. Mediator regeneration, for example, can also be carried out in a second reactor, while the biotechnological production process takes place separately. Further, MEET is often used to improve productivity or yields, and thus overlaps with electro-fermentation.

Many excellent articles review the different processes and electron transfer mechanisms as well as industrial applicability of MES processes in general. The aim of this review is a quantitative comparison of the process performances using key process indicators, such as product titre, space-time yield, coulombic efficiency, etc. (see Box 1). Energy efficiency as key process indicator would undoubtedly be another suitable parameter to compare. However, data on energy efficiency of MES are rarely available which does not allow a meaningful comparison. Building upon key process indicators, technological maturity of the different reaction systems based on technology readiness levels (TRLs) is discussed. Unlike in the recently published work of Prévot et al., the different process designs present in MES are distinguished in regard of their technical maturity, opportunities, and obstacles yet to overcome. This “inventory” approach will highlight the advantages and disadvantages of the different processes in particular.

### Box 1. Definition of key process indicators in MES

| Indicator               | Formula                                      | Description                                                                 |
|-------------------------|----------------------------------------------|-----------------------------------------------------------------------------|
| CE                      | $CE = \frac{Q_{\text{measured}}}{Q_{\text{theoretical}}}$ | Coulombic efficiency (CE; based on substrate consumption): A measure of the ratio of theoretical available electrons from the substrate and the transferred electrons to the electrode; can be regarded as measure of metabolic efficiency. |
| Current density         | $J = \frac{i}{A}$                            | Maximum current density ($J_{\text{max}}$): Maximum current normalized for the electrode area. According to Faraday’s law, the amount of product which can be produced at the cathode is directly proportional to the conducted charge, thereby to the current density. |
| Reaction yield          | $Y = \frac{m_{\text{Product}}}{m_{\text{Substrate}}}$ | Reaction yield ($Y_{\text{P/S}}$): Mass of product obtained from the substrate. |
| Product concentration   | $c_{\text{Product}} = \frac{Q_{\text{Product}}}{V}$ | Product concentration ($c_{\text{P}}$): Amount of product per reaction volume. |
| Product rate            | $r_{\text{Product}} = \frac{Q_{\text{Product}}}{t}$ | Production rate ($r_{\text{P}}$): A measure of productivity of the described process; quantified as product concentration per time unit. |

2. Biofilm-Based MES

One major advantage of using biofilm-based production processes is preventing a washout of the bio(electro)catalyst, that is, the microorganism, in continuous reactors and thereby increasing the long-term stability of the production process. However, biofilm-based MES comes with two major bottlenecks, which are the microbe-electrode interaction and the mass transport in an electrode-biofilm-environment complex. Electrode engineering aims to overcome these challenges. Some strategies, for example, changing the surface chemistry or engineering the electrode topology by generating 3D electrode structures were summarized by Kerzenmacher. The electron transfer mechanism in biofilm-based processes is mainly DEET, whereby a cell layer attached to the electrode does not necessarily imply this mode of electron transfer. Instead, electrons are often transferred indirectly via $H_2$ (IET; indirect electron transfer) that is either produced bioelectrochemically (biotic) by electrode-attached enzymes or organisms or electrochemically (abiotic). In this case we still call it a biofilm-based process in this section since productive biomass is attached to the electrode even if the electron transfer mechanism might not be DEET. Since not every organism favorable for MES is capable of DEET, cocultures with biofilm-forming species are one approach to allow biofilm-based processes. Table 1 summarizes an exemplary selection of the recent studies on biofilm-based MES. The heterogeneity of process designs reflects the various approaches existing in order to enhance key process indicators, for example, varying the type of inoculum, the electrode topology or surface chemistry.

The main share of the presented processes includes production of acetate from CO$_2$ using mixed cultures from various waste water streams. An example for the production of more valuable compounds was presented by van Eerten-Jansen et al. who were the first to produce caproate and butyrate from acetate and electric current using a mixed culture. In the following, Ganigué et al. reported the production of butyrate from electricity and CO$_2$ for the first time. In acetate producing biofilm-based processes, Jourdin et al. provided an example in which altering cathode topology led to an improved process performance. In this work, cathode surface and conductivity were improved using conductive nanoparticles (e.g., carbon nanotubes or reduced graphene oxide (rGO)), thereby reaching increased acetate production rates. Song and coworkers also achieved a 50% increase in production rate when using in situ assembly of an rGO modified carbon felt cathode compared to non-treated carbon felt electrodes. Further possible electrode modifications include the enrichment of the electrode surface with positively charged molecules to increase biocompatibility. In addition to electrode topology and surface chemistry the composition of the microbial community plays an important role. Mostly mixed cultures from various sources come into use in biofilm-based MES; hence the actual process of electron transfer from the cathode to the microbial community is complex and remains unknown in many cases. Mixed cultures are generally more robust to changing conditions and have higher substrate flexibility. However, since different species compete for the electrons supplied, product specificity is often decreased. Possible solutions to this problem include enrichment of certain species using electrochemically driven selection in the long run or by adding supplements (e.g.,...
sulphate in Liu et al.\cite{21a} to enrich sulphate-reducers). Another approach demonstrated for instance by Deutzmann et al.\cite{16} is the use of an artificial coculture in which one strain is capable of CO₂ fixation. Even though the use of an artificial coculture in which one strain is capable of acetogenesis, LaBelle and May reached the highest acetate production rate relative to cathode surface of the studies (\textit{Bradyrhizobium sp.} and \textit{E. coli}). Finding a compromise between available electrode surface, electron and nutrient supply, and a potent inoculum sides were considered.

### Table 1. Biofilm-based MES: Comparison of process performances for biofilm-based MES.

| Microorganism | Mechanism | Substrate/product | Electrode material | Key process indicators | Literature |
|---------------|-----------|-------------------|-------------------|------------------------|------------|
| Mixed culture, pre-dominantly \textit{Clostridium kluyveri} | IET (via H₂) | Acetate/mainly butyrate and caproate | Graphite felt | \( \epsilon_{\text{caproate}} = 26 \mu\text{mol} \cdot \text{cm}^{-2} \) | \cite{14} |
| Carboxydotherophilic mixed culture (syngas-fermenting reactor) | IET (via H₂) | CO₂/acetate and butyrate | Carbon cloth | \( \epsilon_{\text{butyrate}} = 73 \mu\text{mol} \cdot \text{cm}^{-2} \) | \cite{17b} |
| Mixed culture | n.a. | CO₂/acetate | CNT on RVT foam | \( \epsilon_{\text{acetate}} = 194 \mu\text{mol} \cdot \text{cm}^{-2} \) | \cite{18} |
| Mixed culture (from waste water sludge) | n.a. | CO₂/acetate | rGO on carbon felt | \( \epsilon_{\text{acetate}} = 119 \mu\text{mol} \cdot \text{L}^{-1} \) | |\cite{19} |
| Pure culture of \textit{Sporomusa ovata} met | n.a. | CO₂/acetate | rGO-TEPA on carbon cloth | \( \epsilon_{\text{acetate}} = 4 \pm 2 \mu\text{mol} \cdot \text{L}^{-1} \cdot \text{cm}^{-2} \) | \cite{20} |
| Mixed culture, acetate enriched (from brewery waste water initially) | n.a. | CO₂/acetate | Graphite granules | \( \epsilon_{\text{acetate}} = 175 \mu\text{mol} \cdot \text{L}^{-1} \) (after 121 days) | \cite{22} |
| Coculture of \textit{Desulfofilla corrodens} (strain 154) and \textit{Methanococcus maripaludis} or \textit{Acetobacterium woodii} | DEET + interspecies | CO₂/methane | Graphite bars | \( \text{r}_{\text{methane}} = 4.6 \mu\text{mol} \cdot \text{L}^{-1} \cdot \text{cm}^{-2} \cdot \text{h}^{-1} \) | \cite{16} |
| Acetogenic mixed culture (mainly \textit{Acetobacterium}) | n.a. | CO₂/acetate | RVT foam | \( \epsilon_{\text{acetate}} = 275 \mu\text{mol} \cdot \text{L}^{-1} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \) | |\cite{23} |

\( ^{a} \)Calculated from values given in original publication \( ^{b} \)Electrode surfaces were calculated based on the given geometrical dimension. When 2D-electrodes were used, both sides were considered.

3. MES Based on Soluble Mediators

MEET can be used in anodic\cite{24} as well as cathodic MES.\cite{25} The major aim of the anodic MES (also called anode respiration) is to overcome the demand of a continuous oxygen supply by replacing the natural electron acceptor O₂ by a mediator and eventually the anode. In cathodic MES the main objective is improving yields and productivity by providing reducing equivalents in the form of electrons. Therefore, it may also be regarded as mediated electro-fermentation (see below). Here, the added carbon source is mainly or solely used for the formation of biomass and products, while the electrode and the mediator are used to drive the energy metabolism and hence biosynthesis. It must be mentioned that in several approaches the addition of mediators without applying a potential also led to an increase in productivity and/or yield. As an example, the influence of the addition of the mediator with and without applying a potential to enhance the butyrate productivity of a \textit{Clostridium} strain was investigated. The addition of methylviologen (MV) to the reaction media containing sucrose as carbon source led to a 1.3-fold increase of butyrate productivity and yield.\cite{26} The authors concluded that the electron flow was altered by MV addition and a higher amount of NADH was converted to NAD. It is apparent from Table 1 that the cathodic MES is not yet at its ceiling potential for a future bioeconomy.\cite{12i}

However, in a recent analysis on the value of bioelectrochemical systems for a circular bioeconomy, Jung et al. assessed biofilm-based processes used for acetate production from CO₂ (and similar reaction systems), and assigned them a general technological immaturity.\cite{12i} According to their analysis, the process immaturity is mainly caused by high production costs with at the same time low production rates. This is consistent with the key process indicators for biofilm-based processes presented in this work (Table 1). Nevertheless, Jung et al. also attest the technology potential for a future bioeconomy.\cite{12i}
was available leading to an increased butyrate production. In the same study a cathode was polarized (−0.2 V vs SHE) and neutral red was used as mediator for enhancing MES. Here, the butyrate concentration increased from 5 to 8.8 g L⁻¹ (factor 1.8) and the yield from 0.33 up to 0.44 g g⁻¹ (factor 1.3) compared to an unpolarized cathode. As mentioned above, two types of MEET for MES can be distinguished: i) the synthesis and secretion of the mediators in the production organism or ii) the addition of artificial mediators to the medium. So far, the second type is investigated, and likely because the naturally mediated electron transfer path can be easily overlooked. Schmitt et al. engineered a Pseudomonas putida strain that produces phenazine as redox-mediators from P. aeruginosa to allow partial redox balancing with an electrode under oxygen-limited conditions.[27] Nine genes from P. aeruginosa were over-expressed in P. putida KT2440 and thereby up to 45 mg L⁻¹ mediator (pyocyanin) were produced in 25 h. Table 2 summarizes the process performance in different mediator-based reaction systems.

4. Electro-Fermentation

Microbial production processes are determined by the availability of electron donors and acceptors, which for soluble components is reflected in the oxidation–reduction potential of the medium. In electro-fermentation systems, a supplementary electron source or sink by an electrode is added to a “classical” fermentation, whereby electrical current is not the main substrate or target product. Thereby, electro-fermentation allows the process to occur under unbalanced redox-conditions and with an improved carbon yield.[11] In contrast to other MES applications small current instead of high current densities can be used since the number of transferred electrons is not directly proportional to the amount of product.[11b] The exact way how the cell metabolism is influenced is not yet known. By changing the extracellular oxidation–reduction potential, the NADH/NAD⁺ balance inside the cell is also altered, which can impact the product spectrum of fermentations.[11b] The addition of mediators also showed positive effects, either by influencing the redox potential or by acting as electron shuttles.[24c,28] These processes lie in the grey area between MES based on soluble mediators (described above) and electro-fermentations and cannot be strictly distinguished. This is valid for the earliest works on mediated electro-fermentations, for example, by Hongo and Iwahara who increased the yield of L-glutamate production with Brevibacterium flavum by 10% when adding the redox dye neutral red to the fermentation[29] or by Emde et al. who used electro-chemically regenerated ferri-cyanide as electron acceptor in anaerobic glycerol fermentation to ethanol, acetate, and lactate with E. coli.[30] The shift of the product spectrum in glycerol fermentation is a prominent example for the application of electro-fermentations. In a glycerol fermentation the 1,3-propanediol (1,3-PDO) production yield is adversely affected by various metabolic processes in the cell that compete for reducing power from the NADH/NAD⁺ pool. This adverse effect is enhanced when mixed cultures are used.[31] Zhou et al. proposed that additional reducing equivalents supplied by an electrode could enhance NADH generation routes in the cell metabolism and thereby improve 1,3-PDO production. When applying −0.9 V versus standard hydrogen electrode (SHE) the yield of 1,3-PDO increased 2-fold to 0.5 mol[PDO] molC[glycerol]⁻¹ in comparison to applying no potential, where mainly propionate was produced which is the metabolically favored redox-neutral

### Table 2. Soluble mediators: Comparison of process performances for MES based on soluble mediators.

| Microorganism           | Substrate/product | Key process indicators | Remark                                                                 | Literature |
|-------------------------|-------------------|------------------------|------------------------------------------------------------------------|------------|
| Corynebacterium glutamicum | Glucose/lysine    | $Y_{YLD} = 0.56 \text{ g g}^{-1}$ | Anodic MES, $[\text{Fe(CN)}_6]^{3-}/4^-$ was used as mediator          | [24c]      |
|                         |                   | $\eta_{\text{pyocyanin}} = 2.9 \text{ mM}$ |                                                                        |            |
|                         |                   | $\eta_{\text{pyocyanin}} = 4 \mu\text{M L}^{-1} \text{ h}^{-1} \text{ cm}^{-2}$ |                                                                        |            |
|                         |                   | $\eta_{\text{pyocyanin}} = 0.5 \mu\text{M L}^{-1} \text{ h}^{-1} \text{ cm}^{-2}$ |                                                                        |            |
| Pseudomonas putida      | Glucose/2-keto-glucconate | $Y_{YLD} = 0.9 \text{ g g}^{-1}$, CE = 0.99 | The above mentioned system[24] was scaled up from 0.35 L to 2.4 L, specific consumption rates were comparable | [24b]      |
|                         |                   | $\eta_{\text{2-keto-glucconate}} = 0.46 \mu\text{M L}^{-1} \text{ h}^{-1} \text{ cm}^{-2}$ |                                                                        |            |
| Clostridium beijerinckii | Glucose/butyanol  | $Y_{YLD} = 0.25 \text{ g g}^{-1}$ (17% higher compared to a control) | Cathodic MES, neutral red as mediator, applied potentials without a mediator and addition of mediator without reduction show also an effect, but less than the reduced mediator, the mediator reduction affects the ATP content | [25b]      |
|                         |                   | $\eta_{\text{butanol}} = 92 \mu\text{M L}^{-1} \text{ h}^{-1} \text{ cm}^{-2}$ |                                                                        |            |
| Clostridium tyrobutyricum | Sucrose/butyrate  | $Y_{YLD} = 0.45 \text{ g g}^{-1}$ (35% higher compared to a control) | Neutral red was used as mediator, the electron flow increased the NADH formation | [26]       |
|                         |                   | $\eta_{\text{butanol}} = 33 \mu\text{M L}^{-1} \text{ h}^{-1} \text{ cm}^{-2}$ |                                                                        |            |
| Escherichia coli (resting cells) | Acetophenone/R-phenylethanol | $\eta_{\text{R-phenylethanol}} = 1.7 \mu\text{M L}^{-1} \text{ h}^{-1} \text{ cm}^{-2}$ | MV was used as mediator to regenerate NADPH and driven the alcohol dehydrogenase from Lactobacillus brevis | [47] |
|                         |                   | $Y_{YLD} = 0.39 \text{ g g}^{-1}$ |                                                                        |            |

**Provisional: Calculated from figure in the publication; b) Electrode surfaces were calculated based on the given geometrical dimension. When 2D-electrodes were used, both sides were considered.**
product. With respect to mediated electro-fermentations, Utesch et al. showed that various mediators have different effects on the product spectrum of glycerol fermentation. While brilliant blue increased 1,3-PDO production yields by 21%, the production of n-butanol was increased by 33% when neutral red was added to the medium (both in overlapping effects with added iron).

Additionally to the glycerol fermentation, electro-fermentation was used for the production of lysine from glucose by C. glutamicum, of para-hydroxybenzoate (pHBA) from citrate by P. putida and of butanol from glucose by Clostridium acetobutylicum, to name only a few examples. For pHBA production, a potential was applied to an anode introduced to the reactor to maintain the redox-potential at 0.425 V versus SHE under anaerobic conditions. A maximum yield of 9.91 mmol[pHBA] mol[citrate]⁻¹ was achieved, being 69% higher than during aerobic fermentation. During an acetonearbutanol–ethanol-based electro-fermentation, a cathode potential of −0.8 V versus SHE shifted the product spectrum towards butanol at an increased carbon efficiency (38%) compared to the fermentation without applied potential (23%). This study was done without a mediator, suggesting that the metabolic activity is influenced by different means compared to other electro-fermentations. Although several examples showed that electro-fermentation can increase productivity and selectivity for different products, still not all mechanisms were investigated and the process is not ready for industrial application.

5. MES Using Secondary MET and Hybrid Systems

Secondary MET for MES are based on the integration of microbial conversions into electrosynthesis, meaning that only abiotic electrochemical reactions take place. Secondary MES are rather new, even in the young field of MES, and two main strategies are followed: i) using electroorganic synthesis for providing chemical feedstock (i.e., substrate/ metabolites) for subsequent microbial conversions, and ii) upgrading of products gained by microbial processes using follow-up electrosynthesis. Here, the potential of electroorganic synthesis in aqueous reaction media—being often overlooked in classical electroorganic synthesis that uses organic solvents—is leveraged. Thereby MES by secondary MET can take place in one reactor, as well as in several reactors being connected so that the process components mutually determine their process environment. Furthermore, hybrid systems based on the integration of microbial and electrochemical conversions in a process line without mutual effect on the process conditions are summarized in the following. All process steps can be integrated with further physical, chemical and biological conversions, leading to the set-up of electrobiorefineries.

Table 3 summarizes some of the most recent exemplary studies. When using CO₂ as carbon source Haas et al. have recently demonstrated a promising example for the conversion of CO₂ to alcohols. Therefore, they combined the electrochemical reduction to CO₂/H₂-gas streams that were fed into bioreactors with the microbial fermentation. While the first phase of combining the CO₂ electrolyser with a fermenter was performed at lab-scale in a 1 L bioreactor, they entered the second project phase in 2019 with a 2.000 L reactor at Evonik’s plant in Marl (Press release from Siemens: https://press.siemens.com/global/en/pressrelease/research-project-reticulous). An alternative approach was introduced earlier by Liao and colleagues that demonstrated CO₂-reduction to formate in situ with its microbial conversion to C4-alcohols. Although the microbial performance was impressive, the electrochemical process-step was only poorly described and provided significant potential for engineering. Here, during the last years not only key process indicators like CE and rate of the CO₂ to formic acid conversion were significantly improved to values that can allow an integration of the abiotic electrosynthesis with the microbial conversion, but also its scaling to 1 L electrobioreactors was demonstrated successfully. With the concept of providing the microbial substrate via electrochemistry Krieg et al. achieved the production of terpenes from CO₂ using electrochemically generated H₂ as microbial energy source.

The power-to-gas sector covers many more solutions in the field of secondary MES of which the methanation process established by the company Electrochaea one of the most prominent. Their patented system was recently implemented to a 1MW power-to-gas-plant to supply a 25000 people town in Germany with regenerative energy while flexibly storing energy in the form of methane. A detailed overview on power-to-gas projects in Europe including their TRL is given by Wulf et al.

For the process lines which first utilize a microbial conversion yielding a product which is electrochemically upgraded, Urban et al. showed that a mixture of medium chain carboxylic acids (MCCA) can be converted into a mixture of the corresponding hydrocarbons using the Kolbe-reaction. Thereby the MCCA were gained by microbial conversion from a complex feedstock (corn beer) and the product that formed a second phase “swimming” on top of the aqueous reaction phase could be easily harvested. This product can serve as fuel compound since it is composed of C9-compounds which were gained recently by the non-Kolbe-reaction of 3-hydroxy decanoic acid (3-HDA) and other 3-(3-hydroxy-alkanoyloxy)alkanoates by Pseudomonas taiwanensis VLB120 from xylene. Additionally, the Tessonier group demonstrated the conversion of muconic acid, derived from glucose via fermentation using a genetically engineered Saccharomyces cerevisiae, to a monomer for a Nylon-like polymer. Furthermore, the production of methylsuccinic acid from glucose via microbial production of the intermediate itaconic acid using Aspergillus terreus DSM 23 081 that is subsequently electrochemically converted in situ into methylsuccinic acid—a building block for solvents and polymers—was demonstrated by Holzhäuser et al.

6. Conclusion—Ranking of MES in the TRL-Scale

In the previous sections several examples illustrate the specific advantages of different MES processes. In order to compare them, Table 4 summarizes advantages and open technical questions, as well as TRL of the different processes. Originally, the TRL concept was developed in 1988 by NASA for the evaluation of space technologies. Besides the maturity assessment of a particular technology, TRL was subsequently also used to compare between different types of technologies and can also be applied to electrobiotechnology. TRL analysis assesses nine different levels, starting form TRL 1 (basic principle observed)
Table 3. Secondary MET and hybrid systems: Comparison of process performances of MES using secondary MET and microbial-electrochemical hybrid systems.

| System and process description | Electrochemical reaction | Microbial reaction | Substrate/product of the overall process | Key process indicators | Literature |
|--------------------------------|--------------------------|--------------------|----------------------------------------|------------------------|------------|
| Hybrid system for converting CO₂ to fuel-like alkanes | Reduction of CO₂ to CO and electrolytic H₂ generation | Conversion of CO and H₂ to alcohols by Clostridium autoethanogenum and Clostridium kluyveri | CO₂/butanol, hexanol | CE = 100% a) | [35] |
| Secondary MET for converting CO₂ to terpene | Reduction of CO₂ to formate | Conversion of formate/CO₂ to isobutanol and 3-methyl-1-butanol by engineered Cupriavidus necator H16 (previouslyRalstoniaeutropha) | CO₂/C₄-alcohols | CE n.r. and YHEMAOH n.r., but for similar conditions CE = 90% a) and up to YHEMAOH = 2.5 mmol·h⁻¹·cm⁻² at η = 0.12 kWh mol⁻¹ formate⁻¹[37] | [36] |
| Secondary MES for converting CO₂ to terpenes | Water electrolysis to yield “Knallgas” (H₂ and CO₂) | Conversion of “Knallgas” and CO₂ to α-humulene by engineered C. neocallum | CO₂/α-humulene | CE = n.r. and YCO₂/CHOH = n.r. | [39] |
| Electrochemical upgrading of microbial products/metabolites | Kolbe electrolysis of MCCA yielding hydrocarbons (mainly n-alkanes) | Conversion of corn beer to MCCA (mainly caprylic acid) using a reactor microbiome | Corn beer/hydrocarbons | CE = 80% a) at 0.1 kWh mol⁻¹ of converted MCCA | [42] |
| Hybrid systems to yield fuel from xylose containing waste streams | Non-Kolbe reaction of 3-hydroxy decanoic acid (3-HDA) and other 3-(3-hydroxy-alkanoxy)alkanoates yielding C₈ compounds (e.g., 2-nonenone, nonanol, 1-nonanol, nonanal-dimethyl-acetal, methyl-nonanoate) | Production of 3-hydroxy decanoic acid (3-HDA) and other 3-(3-hydroxy-alkanoxy)alkanoates by P. taiwanensis VLB120 from xylose | Xylose/fuel-like mixture with cetane number of 63 | CE = 71% a) (for short reaction times and low Farad equivalents) | [43] |
| Hybrid systems for synthesis of polyamide brick from sugar | Electrochemical hydrogenation of muconic acid to 3-hexenedioic acid (being an brick for a Nylon6,6-like polymer) | Conversion of glucose to muconic acid by an engineered strain of S. cerevisiae | Glucose/3-hexenedioic acid (being further used as building block for a Nylon6,6-like polymer) | CE = 80% a) at P = 0.1 kWh mol⁻¹ of converted CA | [44] |
| Secondary MES for production of methylsuccinic acid from glucose | Electrochemical conversion of itaconic acid to methylsuccinic acid | Fermentation of glucose to itaconic acid by Aspergillus terreus DSM23081 | Glucose/methylsuccinic acid | CE = 93% a) YHEMAOH = 1 mol·h⁻¹·cm⁻² (for 1 h reaction time only of the fermentation broth) | [45] |

a) CE of the electrochemical reaction; b) Yield of the overall reaction in product (equivalent) per substrate (equivalent).

to TRL 9 (actual system proven in operational environment). The first three levels can be regarded as basic and applied research, levels 4 and 5 as industrial research. At TRL 6 (technology demonstrated in relevant environment) a functional prototype of the assessed technology is developed and run in an operational environment. The last three levels (TRL 7–9) can be described as commercial development. In order to illustrate the TRL of the different MES processes, they were evaluated with regard to scale, applied medium/electrolyte, and the industrial relevance of the key process indicators (e.g., applied current densities, productivities). Generally, TRL 6 is considered as the “launching pad” in the transition from research to industrial implementation and commercialization.[3] At the moment, levels 6 and 7 are only reached when combining microbial and electrochemical conversions in hybrid systems in an industrial consortium (e.g. Haas et al.[35]). The next lower level of technical maturity is reached by MES using secondary MET. These processes have the capacity to intensify reaction systems but currently suffer from small substrate spectra and the difficulty to integrate the electrochemical and microbial sub-processes into
Table 4. Comparison and TRL: Advantages, scientific questions, and TRL of different MES processes.

| Process Description                                   | Main Advantages                                                                 | Scientific Obstacles/Questions                                                                 | TRL  |
|-------------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|------|
| Biofilm-based MES                                     | • Continuous process based on self-immobilization of the microorganisms        | • EET mechanisms need to be revealed                                                            | Low 2–3 |
|                                                       | • Recovery of products is facilitated by intrinsic retention of the bioelectrocatalyst | • Resilience of microorganisms against higher current densities need to be investigated          |      |
|                                                       | • Exploitation of symbiotic communities and food-webs due to spatial proximity  | • Lack of suitable scale-up procedures to bring processes from the lab scale to reality           |      |
|                                                       | • Biofilm-based MES usually show high CE and j at low potentials resulting in lower energy requirements | • Limited space-time yield and hence need for high surface electrode to provide sufficient surface-to-volume ratios |      |
| MES based on soluble mediators                        | • Complete reactor volume can be used for bioconversion which simplifies scale-up of processes | • Mediators must be removed from the fermentation broth at the end of the process, which results in a higher expenditure for downstream processing | Low 2–3 |
|                                                       | • Applied potentials are higher compared to biofilm-based processes but lower in comparison to a process based on secondary MET | • Externally added mediators are often limited in turn-over number                               |      |
|                                                       | • In principle, higher current densities at relatively small electrodes can be used, which reduces scale-up issues | • Whether microbial CO₂ reduction/fixation can be driven by reduced mediators is still questionable |      |
|                                                       | • In some applications both the cathode and the anode reaction can be used (paired electrolysis) | • The point of interaction between electrode and microorganism is often not known, which makes targeted optimization more difficult |      |
| Electro-fermentation                                  | • Enhanced carbon yield and product specificity by sub-stoichiometric electron flow. Thereby more effective cost structure and resource consumption | • The technical aspects, e.g. electrode surface-to-volume ratio and scalability are not sufficiently addressed so far | 3–4  |
|                                                       | • Flexible and quick process adaption since established reactors can easily be equipped with electrodes | • Transferability of lab results to real production organisms and processes remains unclear until now |      |
|                                                       | • In principle, higher current densities at relatively small electrodes can be used, which reduces scale-up issues | • A detailed techno-economic assessment of the electro-fermentation is missing                   |      |
| MES using secondary MET                               | • Intensified reaction system due to the combination of electrochemical production and microbial conversion | • Small number of substrates (e.g., hydrogen, formate) can be generated by cathodes and organisms which are able to convert the substrates | 4–5  |
|                                                       | • Multifunctional reactors offer the possibility to reduce investment costs for the reaction system | • Integrating the electrochemical and microbial sub-processes into one reactor requires compromises regarding favorable conditions for the electrochemical and the microbial step (e.g., media, applied potential) |      |
|                                                       | • In principle, higher current densities at relatively small electrodes can be used, which reduces scale-up issues | • Integrating the electrochemical and microbial sub-processes into one reactor requires compromises regarding favorable conditions for the electrochemical and the microbial step (e.g., media, applied potential) |      |
| Hybrid systems of microbial and electrochemical conversions for MES | • Separate optimization of electrochemical and microbial process possible | • No major disadvantages, by-product formation in the individual processes and purification steps must still be taken into account | 5–7  |
|                                                       | • Existing plants and processes can be adapted, which results in reduced development and investment costs | • Innovation can be established only by valorizing new unused feedstock                          |      |

One reactor. Electro-fermentations already enhance carbon yields in established fermentation processes but currently lack techno-economic assessment and deeper understanding of how the microorganism interacts with the electrode. Therefore, TRL of electro-fermentations are assessed lower compared to MES using secondary MET. The mediator- and biofilm-based processes have the lowest TRL of the compared processes as they are far from application on industrial scale. Here, our assessment is in agreement with Prévot et al. in the way that hybrid processes currently have the greatest probability to produce “real-life products” in the near future. However, the progress made for example in electro-fermentation with regard to increased resource efficiency by steering product specificity cannot be neglected. The implementation of these processes, next to decoupling of electrolysis and microbial conversion, still opens the greatest opportunities for novel efficient reaction systems. Overall, the different MES processes offer great perspective for a future bioeconomy, albeit under different time frames.
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Conflict of Interest
The authors declare no conflict of interest.

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electrobiorefinery, electro-fermentation, mediated electron transfer, microbial electro-synthesis, technology readiness level

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