nature communications

Perspective

https://doi.org/10.1038/s41467-026-69414-z

# The overlooked yet critical role of catholyte composition in microbial electrosynthesis

Received: 8 October 2025

Accepted: 30 January 2026

Published online: 13 February 2026

Check for updates

Dimitri van der Lee[1,2,3], Mungyu Lee[1,2,3] ✉, Minke Gabriëls[1], Robbert Kleerebezem[1] & Ludovic Jourdin[1,2] ✉

The circular economy should include $CO_2$ valorization, which could be achieved via microbial electrosynthesis (MES). In MES, the catholyte supplies all nutrients, yet its composition has been adopted from other biotechnologies, overlooking specific needs of MES. In this Perspective, we examine how catholyte design impacts MES performance at microbial, electrochemical, and process levels. We highlight mismatches in metal availability, electrode interactions, and medium origins. We propose reframing the catholyte as a key design parameter and introduce a decision-making framework for tailored formulation. This strategy has the potential to improve MES performance and serve as model for optimizing media in broader biotechnological applications.

The utilization of $CO_2$ as a primary carbon source is increasingly recognized as a key strategy to mitigate climate change and support the transition to a circular bioeconomy[1,2]. Among the technologies with the potential to enable this transformation, microbial electrosynthesis (MES) has emerged as a promising platform for converting $CO_2$ into value-added products using microbial catalysts and renewable electricity as the sole energy source[3,4]. Since its introduction by Nevin et al. in 2010[4], microbial electrosynthesis of soluble organics has advanced significantly in areas such as electrode design, microbial catalyst development, and elucidation of electron transfer mechanisms (Fig. 1). These advancements have significantly improved system productivity and broadened the range of applicable microbial species, laying the foundation for scalable $CO_2$-reducing bioprocesses[5–7].

While these developments have brought MES closer to practical implementation, it is now essential to re-examine system components that have received relatively little attention despite their potential impact on performance and stability. Many components of an MES system critically influence performance metrics such as energy efficiency, current densities, and faradaic efficiencies. These include, among others, electrode materials, electrode spacing, reactor hydrodynamics, and electrolytes[8]. Of the electrode and electrolytes, the anode and anolyte are often viewed as necessary complementary materials that do not need much attention, although these components can influence system-level performance (Box 1). However, the catholyte is also an oftentimes overlooked component, which functions simultaneously as the growth medium and the electrolyte. Beyond supporting microbial growth, the catholyte plays a central role in conductivity, generally increasing conductivity with increasing salinity, buffering of pH, and $CO_2$ delivery, making it a crucial element that directly influences the overall performance of MES. While MES studies have explored diverse microbial species, such as *Sporomusa ovata*, *Clostridium ljungdhalii* and *Thermoanaerobacter kivui*, as well as mixed microbial cultures, most of the attention has been directed toward the electrochemical properties of the catholyte, with far less focus on its formulation as a growth medium tailored to microbial requirements[9–11].

As MES transitions toward implementation in real-world conditions, the need to reconsider catholyte composition becomes increasingly important. To date, no published study has specifically addressed the catholyte as a potentially limiting component in $CO_2$-reducing MES systems (Fig. 1). However, the catholyte governs multiple key parameters, including ionic strength, gas–liquid mass transfer, pH buffering, and the availability of trace metals. It also influences the efficiency of downstream separation processes through its chemical composition. These interconnected features highlight that the catholyte is not merely a passive background medium, but rather an active design variable that links microbial physiology, electrochemical performance, and process integration.

In this Perspective, we trace the evolution of catholyte composition in MES, analyze its role as a central link between microbial

[1]Department of Biotechnology, Delft University of Technology, 2629 HZ Delft, The Netherlands. [2]e-Refinery Institute, Delft University of Technology, Leeghwaterstraat 39, 2628 CB Delft, The Netherlands. [3]These authors contributed equally: Dimitri van der Lee, Mungyu Lee. ✉e-mail: M.Lee-4@tudelft.nl; l.jourdin@tudelft.nl

https://doi.org/10.1038/s41467-026-69414-z

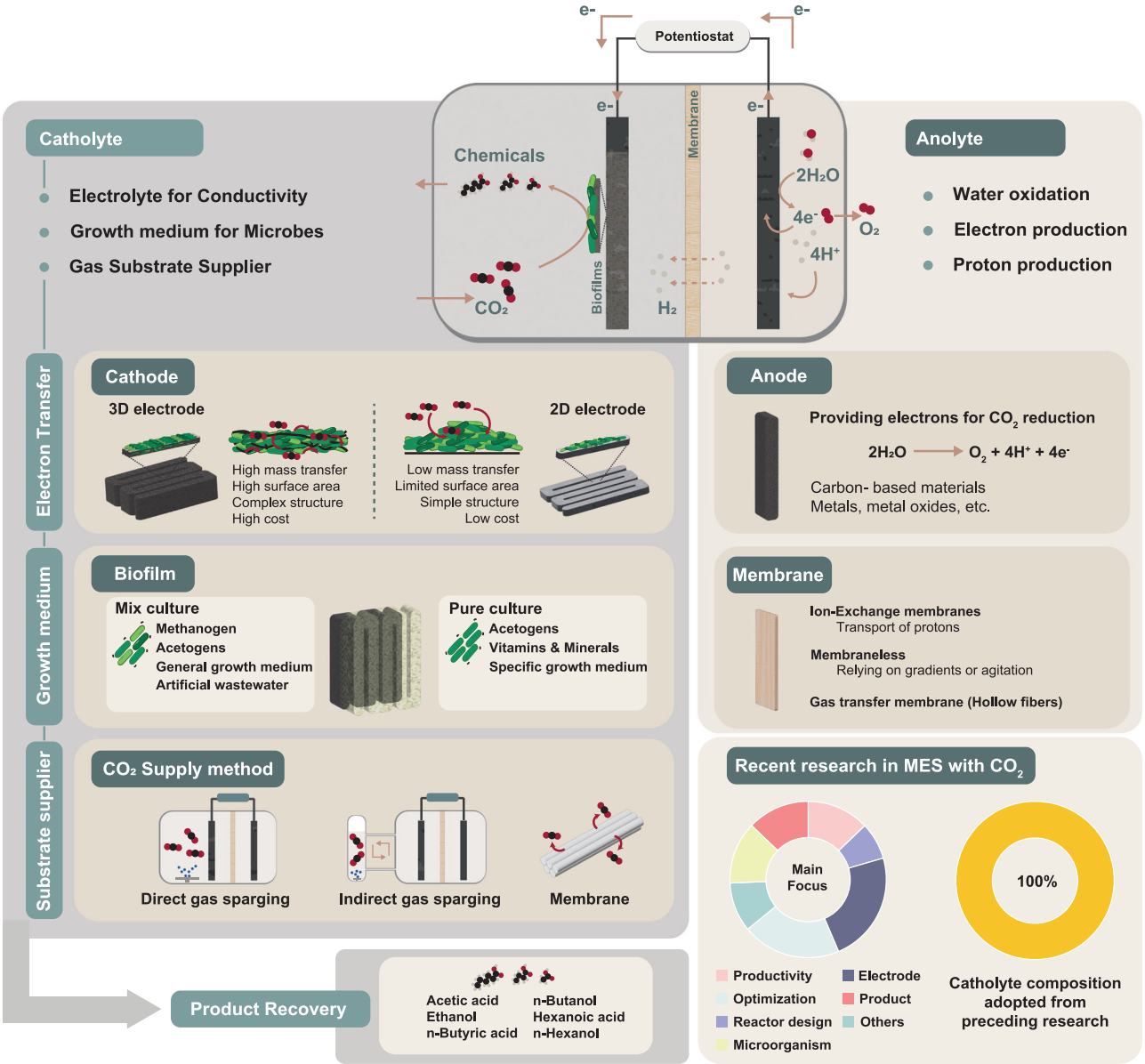


**Fig. 1 | Schematic overview of $CO_2$-driven MES systems and current research landscape.** The circular chart on recent MES research with $CO_2$ was generated based on an analysis of 39 recent publications, as detailed in Supplementary Table 1.

metabolism and target product formation, and identify key mismatches between widely adopted formulations and the specific requirements of MES. Based on this analysis, we propose a structured framework to guide catholyte design that aims to improve system-level performance and establish generalizable principles applicable to other bioelectrochemical systems.

## Roles of catholyte in microbial electrosynthesis for $CO_2$ reduction

### Nutrient functions and microbial support of the catholyte

The catholyte (growth medium) plays a pivotal role in MES systems by providing essential nutrients such as carbon and nitrogen sources, vitamins, minerals, and buffering agents like phosphate or bicarbonate. In general, most MES studies assume only carbon or electron donor limitations. The components in the catholyte are critical for maintaining pH stability, facilitating $CO_2$ utilization and electron transfer, and supporting microbial functionality and activity overall. However, variations in catholyte composition can also impact key parameters such as conductivity and may lead to undesirable effects like metal electrodeposition on cathodes, which can reduce the bioavailability of nutrients and thus the overall system performance. Therefore, focusing solely on nutrient supply can be misleading and even detrimental. To address these challenges and ensure maximum efficiency and stability, optimizing the catholyte composition is essential.

### Gas–liquid mass transfer affected by the catholyte

The catholyte (mass transfer medium) plays a critical role in mass transfer coefficient ($k_La$)[12], which determines the gas-liquid mass transfer rate of $H_2$ and $CO_2$, and is relatively hard to predict as it is dependent on many interactions between the compounds present. Since substrate availability for microbial uptake is directly linked to $CO_2$ availability[13], catholyte composition indirectly influences substrate availability. For instance, electrolyte concentration can modify physicochemical properties such as diffusion coefficients and, in bubble columns, bubble behavior, resulting in different effects on mass transfer depending on system configuration. One study under multiphase bubble flow conditions showed that high-charge-density

## BOX 1

# Anode and anolyte constraints in MES

In MES, the anode and anolyte play a supporting role by sustaining charge balance and enabling continuous electron flow through anodic oxidation reactions, most commonly water oxidation. Alternative anodic reactions, including the oxidation of organic substrates or sulfide, as well as chloride oxidation under specific conditions, have also been reported. Although these components are not directly involved in microbial $CO_2$ conversion, their electrochemical behavior can impose constraints on overall system performance[2].

The composition of the anolyte determines which anodic reactions may occur. The presence of electrochemically active ions, such as chloride (Box 2), can lead to competing anodic reactions and the formation of reactive species, which may accelerate electrode degradation, damage membranes, and reduce long-term operational stability[105,106]. For this reason, anolytes are often formulated to minimize side reactions while maintaining sufficient conductivity.

Ion-exchange membranes are not perfectly selective, and partial crossover of ions or reaction products between the anode and cathode compartments can occur. These effects are generally secondary relative to catholyte composition, they may influence local pH, redox conditions, or microbial performance under specific operating conditions[18,84,107]. To minimize crossover of ions other than protons, vapor-phase or gas-fed anode configurations have been explored in MES systems, as they reduce direct liquid-phase ionic contact between compartments and thereby limit unintended ion transport across the membrane[48].

In addition, the conductivity and buffering capacity of the anolyte contribute to the overall internal resistance and cell voltage of the MES system. These parameters influence cathodic pH stability and energy efficiency by affecting ohmic losses and proton transport, but they do not directly govern product selectivity or microbial metabolic pathway selection[2,86,101].

electrolytes like $MgSO_4$ inhibit bubble coalescence, thereby increasing the gas–liquid interfacial area and leading to a substantial enhancement of the mass transfer coefficient, which rose from 103 to 711 $h^{-1}$ compared to pure water[14]. In contrast, in a static system involving single $CO_2$ bubbles rising through a solution, increasing NaCl concentration (0 to 14 wt%) led to an approximately 21% decrease in the diffusion coefficient of $CO_2$, which correspondingly caused a gradual reduction in the $k_La$[15]. Thus, the design of an MES system requires a comprehensive understanding of how catholyte composition interacts with gas–liquid transport phenomena under specific reactor conditions.

### Influence of catholyte on product recovery

The catholyte (solvent) composition must also account for its impact on the downstream process of product recovery in MES systems. During this stage, the catholyte acts as a solvent carrying the products from the biocathode to the recovery process. For instance, in adsorption-based recovery methods, high concentrations of chloride, sulfate, and phosphate ions can co-adsorb onto amine-functionalized resins as their corresponding mineral acids. Under conditions of high ionic strength, these inorganic anions compete with volatile fatty acid anions for active adsorption sites, which has been reported to reduce VFA adsorption capacity by approximately 50–80% and to alter resin selectivity[16,17]. In electrodialysis (ED) systems, residual calcium and magnesium can form precipitates with carbonate and sulfate, causing membrane scaling, fouling, and reduced ion transport efficiency. For example, Ca and Mg precipitated as $CaCO_3$ and $Mg(OH)_2$, respectively, causing current efficiency to drop from 85% to 49% and significantly hindering ion migration[18]. Therefore, optimizing the catholyte composition from an integrated process perspective is essential for the successful commercialization of MES.

## Constraints emerging from catholyte roles in MES

### Essential metals with toxic effects

The addition of (heavy) metals is necessary to maintain microbial activity and ensure stable operation in MES systems. The addition of bioavailable metal ions can cause productivities to increase, especially in cases of previous underfeeding. However, when metal concentrations are added in excess, toxicity can also occur. For example, Cd exhibited inhibitory effects on methanogenic granules, with 50% reduction in gas production observed at a concentration of 0.5 mM. Similarly, Co caused complete inhibition of gas production at 16.1 mM in anaerobic sludge, indicating strong toxicity[19–21]. Lastly, zinc and copper were showed to inhibit microbial activity in methane-producing MES, with concentrations above 1 mM for zinc and 0.5 mM for copper significantly reducing gas productivity[22].

### Impacts of electrodeposition in MES

Although the required metals by microorganisms in MES systems is well known from metabolic studies in other environments, the use of electrodes and the application of external voltage introduce practical challenges during operation, particularly electrodeposition. This process involves the electrochemical reduction of metal ions at the cathode surface, resulting in the formation of solid metal particles. As a consequence, these metals become immobilized on the electrode surface and are no longer available in the medium, thereby limiting their bioavailability for microbial metabolism[23,24]. In addition, organic compounds present in the catholyte, among which the produced carboxylic acids, can interact with carbon-based electrodes, thereby altering electrode surface chemistry which influences electrochemical reactions and interactions taking place on the electrode surface[25].

Nevertheless, at the system level, electrodeposition has been reported to enhance MES performance. For example, in situ electrodeposition was induced by adding $NiSO_4{\cdot}6H_2O$ and $FeSO_4{\cdot}7H_2O$ to the cathode at a concentration of 0.2 g $L^{-1}$, resulting in a 38% increase in $CH_4$ production. These results have been attributed to improved electrode conductivity due to metal deposition on the electrode surface[26]. Similarly, addition of concentrated trace mineral solution prior to inoculation has been shown to result in an increase in current as well as production compared to control experiments, probably because of increased catalytic activity toward the hydrogen evolution reaction[27,28]. Despite these positive effects, metals acting as catalyst on the cathode surface are no longer bioavailable. Conversely, when metal ions are imported into the cell membrane, the ions are no longer able to be electrodeposited, as they can no longer reach the cathode surface. Indeed, there is constant competition between metal electrodeposition and metal incorporation into biomass in MES reactors. Interestingly, no systematic research has been performed on the long-term bioavailability of metal ions in MES reactors.

Moreover, under changing medium compositions or long-term operation, the bioavailability of metals may also vary. Therefore, it becomes difficult to determine whether performance improvements are due to physical enhancements of the electrode or changes in metal availability. To accurately interpret MES performance, it is essential to

https://doi.org/10.1038/s41467-026-69414-z

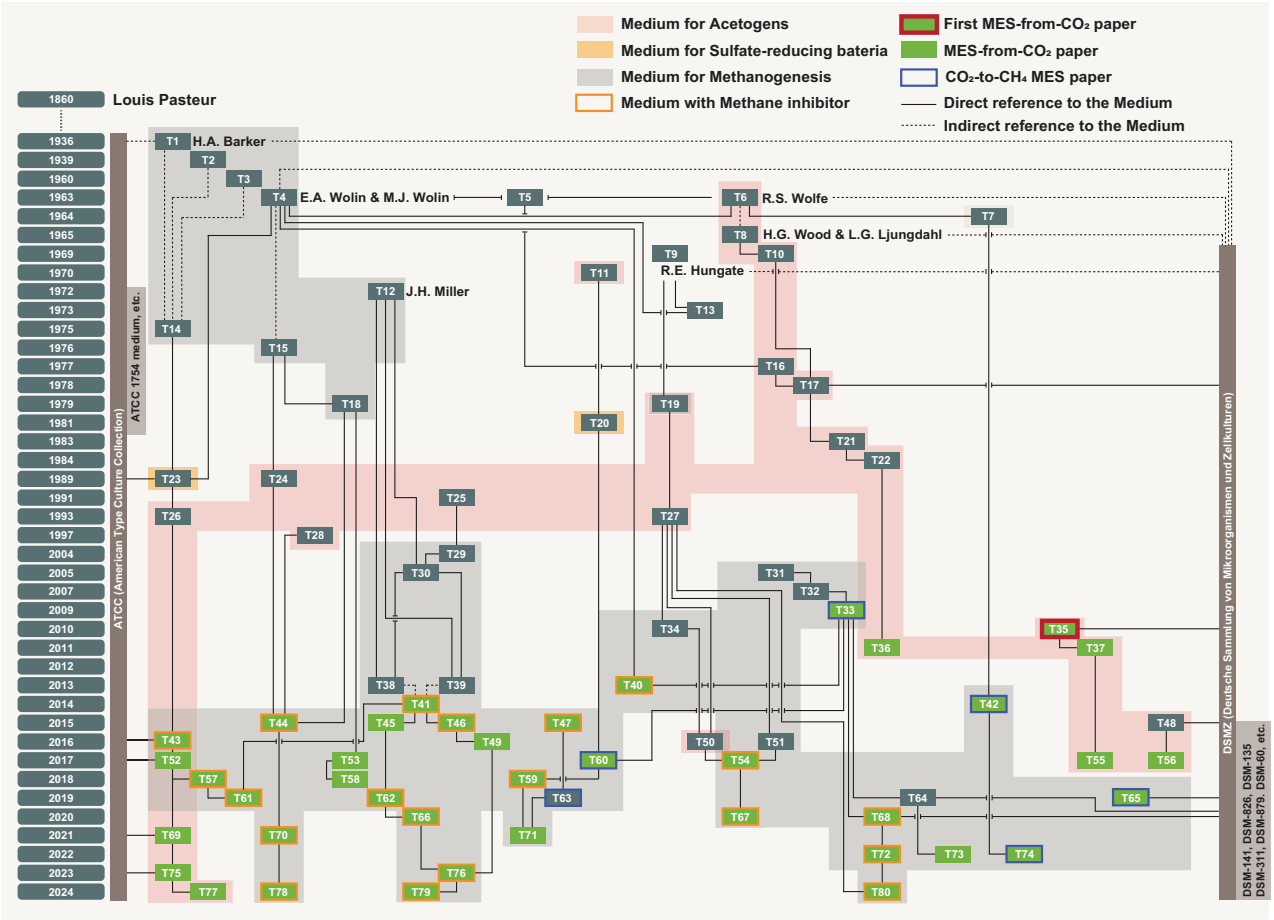


**Fig. 2 | Catholyte origin tree, organized chronologically by publication year from top to bottom, depicting historical dependencies in MES research.** Medium compositions were included if they were either directly referenced in the papers (solid lines) or indirectly inferred from studies with similar research objectives or shared authorship (dashed lines). Boxes not connected to earlier studies did not include specific information on catholyte composition. The border colors of the boxes represent different study characteristics: yellow borders indicate studies that used a methane inhibitor, red borders denote the first MES studies utilizing $CO_2$, defined here as $CO_2$-driven microbial electrosynthesis systems producing soluble organics (T35), and blue borders mark studies aiming primarily at methane production. To distinguish these references from those in the main text, each paper is labeled with a "T" (Tree) followed by a number. Detailed references (T1–T80) are provided in the supplemental information. Source data are provided as a Source Data file.

consider the dynamic changes in actual metal concentrations within the reactor, for example by considering the effect of continuous nutrient feed versus batch operation.

## Tracing the evolution of catholyte compositions in MES research

To investigate the reasons behind the selection of catholyte compositions in existing studies, we constructed a growth medium history tree based on compositions and references cited in MES-related papers (Fig. 2). This tree traces the progression of catholyte formulations, focusing on recent $CO_2$-driven MES studies and extending backward to identify their foundational references.

Interestingly, studies originating from different research groups often converged on similar medium formulations when traced back to their foundational references. In addition to citing well-established repositories like ATCC and DSMZ (T75,T65)[29,30], researchers frequently adapted medium compositions from earlier works (T74,T42)[31,32]. This practice highlights the interconnectedness of research communities in shaping medium development.

We further categorized the primary objectives of the medium components by their relevance to acetogens (pink background) or methanogens (gray background). A clear trend emerged, early studies predominantly focused on methanogens, and their medium compositions served as the foundation for developing acetogen-specific media (T1,T3,T2,T4)[33–36]. In more recent studies, MFC (microbial fuel cell) and MES (microbial electrosynthesis) systems reverted to methanogen-based medium formulations, even if the aim was to avoid methanogenesis (T33,T42,T41,T44,T29)[3,32,37–39].

This reliance on methanogen-oriented medium appears to stem from two factors: (1) the use of inoculum directly sourced from wastewater treatment processes, which inherently contain methanogenic communities (T33,T40,T45,T43,T60,T63,T71)[3,40–45], and (2) the adaptation of microbial community from prior methanogen research for non-methane-producing MES applications (T39,T64,T73)[46–48]. Consequently, most of the MES experiments using $CO_2$ as a major substrate employed methanogen-based artificial wastewater media as their starting point, which is generally based on low nutrient concentrations by default.

In studies where acetogens were the dominant strains (T68,T69,T78)[49–51] or where single-culture MES systems (T35,T37,T55,T77)[4,52–54] were employed, researchers often referenced syngas fermentation medium as the foundation for catholyte composition (T36,T52)[55,56]. However, excluding the studies that specifically aimed for methane production (highlighted with a blue border), the majority of 39 $CO_2$-driven MES studies (green boxes) referenced methanogen-based medium composition (gray background). Most of these studies were intended to suppress methane formation, yet they still relied on methanogen-based media, accounting for 80% of all

https://doi.org/10.1038/s41467-026-69414-z

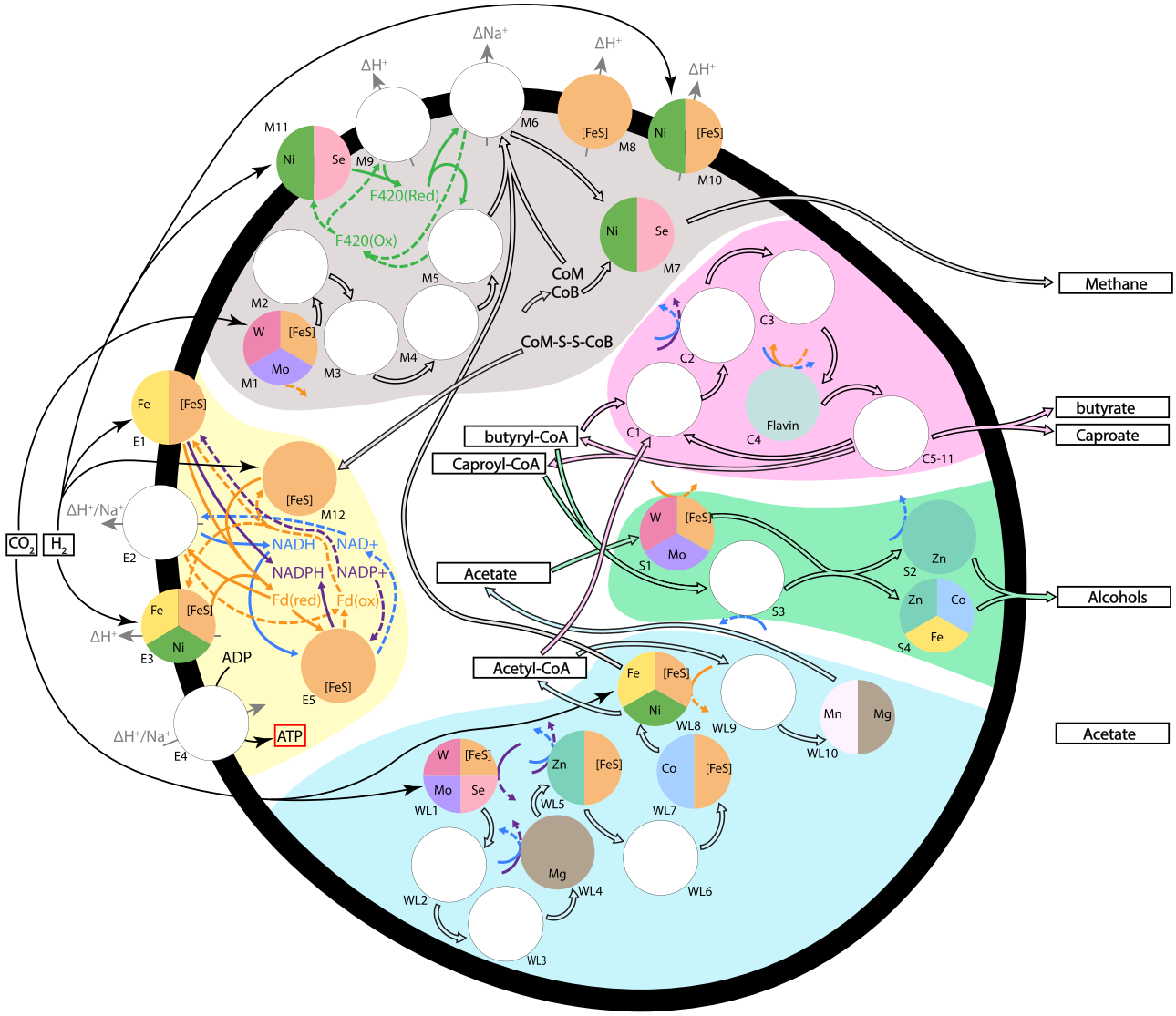


**Fig. 3 | The metabolic pathways involved in MES from $CO_2$.** The different pathways are divided into energy metabolism and cofactor regeneration (yellow), methanogenesis (gray), Wood-Ljungdahl pathway (blue), chain elongation (pink), and solventogenesis (green). Enzymes are indicated as circles, in which colors indicate metal atoms where applicable. White circles indicate no metals are required for this enzyme. Enzyme numbers can be cross-referenced in Supplementary Table 1. Bordered arrows indicate the flow of metabolites in the direction of products; some of these reactions could be reversed as well. Arrows without a border indicate the production or consumption of cofactors, with solid and dashed arrows indicating the reduced and oxidized form, respectively. Orange: ferredoxin; purple: NADP(H); blue: NAD(H); [FeS]: iron-sulfur clusters. Source data are provided as a Source Data file.

cases. These studies generally used the original media without further modifications. Among these, 63% of research introduced a methane inhibitor to the existing medium, such as bromoethanesulfonic acid, without further modifications, highlighted with a yellow border (T80,T78,T79,T46,T62,T67,T76)[5,7,10,51,57–59]. While this approach offered practical simplicity, it underscores the missed opportunity to direct efforts toward developing catholyte compositions specifically tailored to maximize acetogen performance, while minimizing methanogenesis. The remaining 20% of studies designed catholytes with acetogen-specific considerations, such as cultivating *Sporomusa* species in single-culture systems or accommodating dominant acetogen strains (T69,T77,T56)[50,54,60].

Notably, most studies described catholyte formulations as 'modified' without providing clear justifications for these modifications. This lack of detailed reasoning raises concerns about the reproducibility and optimization of catholyte formulations in MES research. Such trends indicate that the catholyte compositions used in $CO_2$-reducing MES are not optimized but instead selected and operated based on historical practices or interpretative results. If future catholyte formulations continue to rely on such reused catholytes without sufficient justification, this may perpetuate a cycle of poorly optimized medium usage. To break this cycle, an optimized catholyte should be defined as one that (i) maintains stable microbial activity and electrocatalytic performance, (ii) is tailored to the target product, and (iii) is adapted with the envisioned production process in mind. Building on this definition, we aim to identify the essential medium components required for converting $CO_2$ to chemicals and to clarify their functional roles in biocatalysts, based on medium formulations reported to date.

## Metabolic pathways and microorganisms

The first step towards a systematic approach for electrolyte design is to identify the microbial metabolic requirements. For the conversion of $CO_2$ to chemicals via MES, the most active pathway regarding $CO_2$ fixation is the Wood-Ljungdahl Pathway (WLP)[61]. Some of the key

enzymes that play a role in the WLP operate via the concept of electron bifurcation, which couples an endergonic with an exergonic reaction[62]. Many of these enzymes contain metal catalysts to reduce the required activation energy[63,64]. Consequently, the supply of necessary metal cofactors for key enzyme production is essential in MES. The differing metal requirements of pathways make it possible to selectively promote products of interest, albeit to varying extend depending on the pathway. In this section, the pathways required to produce several MES products are analyzed, with special attention to the most commonly used metal cofactors (data retrieved from Uniprot and KEGG databases)[65,66]. Nutrients that were found to be not directly contributing to the main pathways, for example vitamins, are not discussed here, even though they may be necessary for other metabolic pathways. All findings are shown in Supplementary Table 1 and visualized in Fig. 3.

### Acetogenesis

The WLP is the most predominant pathway for $CO_2$ fixation in MES, also known as acetogenesis. Although the pathway was first named in 1986, the thermodynamics only became clearer after linking it with electron bifurcation in 2012[67–69]. This couples reactions with a high redox potential, with reactions with a lower redox potential, maximizing metabolic energy use. As the WLP has already been described elaborately elsewhere, we will mainly focus on the metal requirements of metalloenzymes[70]. The individual enzymatic steps are shown in Fig. 3.

There is a high demand for several metal ions in the WLP. Many of the enzymes rely on one or multiple iron-sulfur clusters, either incorporated in the enzyme or in ferredoxin as cofactor, to function[71]. Furthermore, there are 5 more enzymes in the WLP that require one or more metal atoms to function. This includes the hydrogenases (E1), which are responsible for converting the energy in hydrogen to reducing factors, shown in the energy metabolism section in Fig. 3. Hydrogenases oftentimes require nickel and iron to function, next to the iron-sulfur clusters present in both the enzyme and the cofactor ferredoxin.

Two other enzymes which are highly dependent on metal ions are involved in $CO_2$ capture: CO dehydrogenase (CODH) and formate dehydrogenase (FDH) (WL1 and WL8). Though the exact requirements may vary between different organisms, these facultative electron bifurcating metalloenzymes are dependent on iron sulfur clusters, selenium, tungsten, cobalt, nickel, and molybdenum. One of the main reasons for this is that the reactions that fix $CO_2$ take place close to thermodynamic equilibrium. As such, these enzymes incorporate metals as catalysts in order to minimize the amount of energy required[72,73].

### Methanogenesis

Though methanogens and acetogens are often found in similar environments, their metabolisms are vastly different[74,75]. While acetogens dependent on the WLP often use hydrogen as an electron donor, methanogens are divided into three different classes: hydrogenotrophic, methylotrophic, and acetoclastic methanogens[68,70,76,77]. The first class uses hydrogen as an electron donor and fixes $CO_2$ via several subsequent steps. Comparable to acetogenesis, the enzyme responsible for $CO_2$ capture is a metalloenzyme (M1), relying on iron-sulfur clusters, molybdenum, and tungsten. The steps following $CO_2$ fixation are comparable, yet different from acetogenesis in terms of metal dependency, likely because methanogenesis is thermodynamically more favorable.

Except for the last step leading up to methane generation, none of the intermediate enzymes are metalloenzymes. Furthermore, the flavin-dependent cofactor F420 is mainly used as an electron carrier throughout the methanogenesis pathway. Compared to nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) and ferredoxin, the main cofactors used in acetogenesis, F420 is equal or lower in reduction power depending on the physiological conditions[71,78,79]. This may allow for better energy conservation.

The last step for methane release is a metalloenzyme that relies on the availability of selenium (M7). Furthermore, though it is not technically direct metal incorporation, the enzyme also uses the cofactor F430, which requires a nickel atom. After the release of methane, CoM and CoB need to be regenerated. This is done by an often membrane-bound enzyme, which relies on iron-sulfur clusters (M8, M12). In addition, this step is also linked to proton gradient generation.

For growth, methanogens use a CODH-acetyl-CoA synthase (CODH-ACS) enzyme complex (WL8) for the generation of acetyl-CoA from the intermediate compound before the methyl group is transferred to CoM (M6). This also implies that methanogens require the same metal cofactors for biomass growth regarding this enzyme when compared to acetogens. The same reaction can run in reverse direction to allow for acetoclastic methanogenesis. Furthermore, different methyl transferases (M6) exist, which enables methylotrophic methanogenesis, e. g. using methanol as substrate.

### Energy metabolism and cofactor generation

A conservative energy metabolism is critical for microorganisms inhabiting environments close to thermodynamic equilibrium. Acetogens and methanogens have evolved distinct strategies to cope with these constraints, as previously reviewed[75,80–82]. For both groups, ATP generation and regeneration of cofactors are interconnected. For example, the Rnf complex couples the regeneration of NADH with the creation of an ion gradient. Interestingly, most enzymes involved in the energy metabolism of both organisms are dependent on iron-sulfur clusters. One clear difference between acetogens and methanogens is the dependence on methanophenazine as a membrane-bound electron carrier for some methanogens, especially when using carbon sources other than $CO_2$[75]. This links the reactions performed by various membrane-bound proteins with proton translocation, thus indirectly with ATP synthesis.

### Reverse beta oxidation (RBO)

The production of medium-chain carboxylic acids (MCCAs) can proceed via multiple pathways. In MES it is mostly accepted that the synthesis of MCCAs follows the reverse beta oxidation (RBO) pathway[83]. None of the enzymes in the RBO pathway has been characterized as a metalloenzyme to date. However, an indirect metal use lies in the electron bifurcating enzyme (C4) that links one of the reaction steps with energy generation by coupling this reaction to the reduction of ferredoxin. This ferredoxin can in turn be used by the Rnf complex for the generation of a proton/sodium gradient. As ferredoxin has at least one iron-sulfur complex, metal cofactors should also be considered for the RBO pathway.

### Solventogenesis

The biosynthesis of alcohols from acyl-CoA, solventogenesis, usually occurs via dehydrogenases. Starting from acyl-CoA, they convert the carboxylic acids to alcohols, often with an aldehyde intermediate produced by a preceding enzyme (S3). Alternatively, aldehyde dehydrogenase (S1) can convert acetate directly to aldehyde, but requires molybdenum and tungsten to function. Alcohol dehydrogenases (S2 + 4) always require a zinc ion to function.

## Medium composition in MES

Comparing the media used in MES studies should give an overview of the variation that can be found in the current literature. Catholyte compositions from 28 recent MES studies (listed in Supplementary Table 2)[4,5,7,10,11,29–32,38,40,45,49,51–56,59,60,84–88], also used in Fig. 2, were collected and compared. Within these studies, 4 focused on methane

production (T33,T42,T65,T74), 4 on the production of alcohols (T35,T37,T40,T55), while the remaining studies focused on the production of carboxylic acids.

Omission of selenium and tungsten was observed in 12 media, while these metals are required for all of the pathways used (Fig. 3). This may result in severe underfeeding of the microbial culture, yet metabolite production was still observed in these experiments. The production could have several alternative reasons. First, molybdenum could substitute tungsten due to similar chemical properties[89]. Furthermore, tungsten and selenium could still be present in trace amounts, originating from the inoculum, glassware, or water, depending on the water source used. However, it is unclear whether these trace amounts would be sufficient to completely satisfy the requirements of the culture, though some examples show that adding or altering selenium and tungsten in the catholyte significantly influences productivity and product spectrum[90,91].

To investigate further, we analyzed the media used in 8 CO-containing gas fermentation studies, which utilized microbial strains similar to those in MES and represent a commercially established bioprocess[92–99]. Interestingly, there is a nearly 10x higher concentration of zinc in the gas fermentation media, significantly higher than in MES media (student $t$-test, $P \leq 0.05$) (Fig. 4; Supplementary Fig. 1 and 2). In addition, ammonium concentrations were 4x increased in syngas fermentation. Furthermore, ferrous iron, boron, copper, and manganese were all found to be significantly increased in gas fermentation media, while potassium and phosphate were significantly decreased. Notably, though selenium and tungsten were found to be 67 and 33 times increased in gas fermentation media, this difference was not significant, likely due to the large variations present. Summarizing the analysis above, even though these gas fermentation studies used microorganisms comparable to MES, which would lead to comparable nutrient requirements, many medium component concentrations were increased to some extent. In general, gas fermentation studies report higher microbial growth rates and kinetics compared to MES, partially because CO is a very strong electron donor. Additionally, biofilm MES systems can rival or even exceed biomass concentrations compared to syngas fermentation[5,7]. Therefore, possible underfeeding of the microbial culture in MES experiments cannot be excluded. In optimal conditions nutrient feed should be matched with microbial growth rate and kinetics.

There are many differences between the media used in MES and other related biotechnologies. These differences cannot be solely explained by the requirements of the pathways, and it seems likely that media composition is generally at the bottom of the priority list. To improve on this, we would like to propose a general, quick-to-use flow sheet for determining and verifying the media used in experiments.

## Guideline for selecting catholyte composition in MES

This study proposes a basic guideline for optimizing catholyte composition in MES systems for $CO_2$ conversion (see Fig. 5). Rather than addressing the overall process design, the guideline aims to provide stepwise criteria for defining the composition of key catholyte components, including carbon, nitrogen, phosphorus, and minerals, based on the experimental objectives and the specific conditions of the system. As the requirements of different microorganisms, microbial communities, and reactor systems can vary significantly, quantitative determination of optimal nutrient concentrations is inherently system-specific and dependent on factors such as growth and kinetics. Accordingly, this guideline mostly focuses on qualitative attributes.

Once a starting growth medium is selected, it is important to determine whether the research is aimed at an industrial application or a laboratory-scale study. Accordingly, the medium should be examined for the presence of non-defined components or potentially inhibitory substances (vitamins, antifoam, yeast extract, use of tap water, and antibiotics), which can often create challenges for scalability by applying a financial burden, reproducibility, and regulatory compliance. For studies targeting industrial applications, it is advisable to exclude or replace potentially limiting components with alternatives[100]. Undefined components such as yeast extract, tap water, vitamins, antibiotics, or antifoam agents may vary in composition depending on their source, location, and time of use, thereby introducing batch-to-batch variability and limiting reproducibility. In addition, the use of such components may complicate scale-up by increasing operational costs and introducing additional requirements for quality control or compositional consistency. Accordingly, catholyte composition can only be considered fully defined if these components are avoided or if sufficient chemical characterization of undefined inputs is provided, ensuring a reproducible and scalable catholyte composition.

The next step is to set the target amount of biomass, based on the desired productivity and calculate the required concentrations of carbon, nitrogen, and phosphorus to verify the medium composition. Various empirical formulas are used to approximate microbial biomass. Among them, the formula $C_5H_7O_2N$ is widely accepted as a standard in wastewater treatment and environmental processes[101]. For more precise phosphorus estimation, the formula $C_1H_{1.8}O_{0.5}N_{0.2}P_{0.01}$, which includes contributions from nucleic acids and phospholipids, can be used; this is particularly useful in high-density cultures or phosphorus-limited conditions[102]. Sulfur is also an essential element, with microbial requirements typically 2-5 mmol of Sulfur per mol of carbon[103].

For mineral components, selection depends on the inoculum source chosen according to the experimental objectives and varies based on the types of microbial strains present. Specifically, the microorganisms to be targeted differ according to the desired products such as methane, acids, or alcohols (Supplementary Tables 3, 4). Methanogenesis requires enzymes containing nickel, tungsten, and selenium, with iron being essential for the activity of key enzymes. Organic acid biosynthesis additionally requires zinc, cobalt, magnesium, and manganese to activate the main pathways. Of these, zinc is of even more importance when aiming for alcohols, in addition to tungsten, molybdenum, cobalt, and iron. Chain elongation, however, does not directly require metal ions to function, although iron-sulfur clusters are used in ferredoxin; a cofactor that is used in all discussed pathways. Note that metal ions may also be required for undiscussed pathways. The concentrations of these metal ions are also a critical factor. However, due to the complexity and interconnection of microbial metabolic networks, a detailed analysis of metal ion concentration effects will require further investigation.

Once the required metal components are identified, appropriate chemical compounds containing these metals and their corresponding counter-ions can be selected to finalize the medium formulation (Box 2). After precisely tailoring the catholyte composition with a focus on metabolism, it is essential to consider that both the $CO_2$ and $H_2$ mass transfer coefficients as well as the medium conductivity, presence of unwanted side reactions like electrodeposition, pH buffer (process 11) and the product recovery rate (process 12) in MES systems are influenced by the composition of the catholyte. For example, chelating agents like ethylenediaminetetraacetic acid (EDTA) have proven to be effective in minimizing electrodeposition in MES, however, EDTA has also been proven to interact with anion exchange resins[27,104]. As such, while EDTA addition may have benefits, it can also influence downstream processing depending on the methods used. Therefore, all factors discussed above should be carefully considered, leading to a fully optimized and complete medium formulation.

https://doi.org/10.1038/s41467-026-69414-z

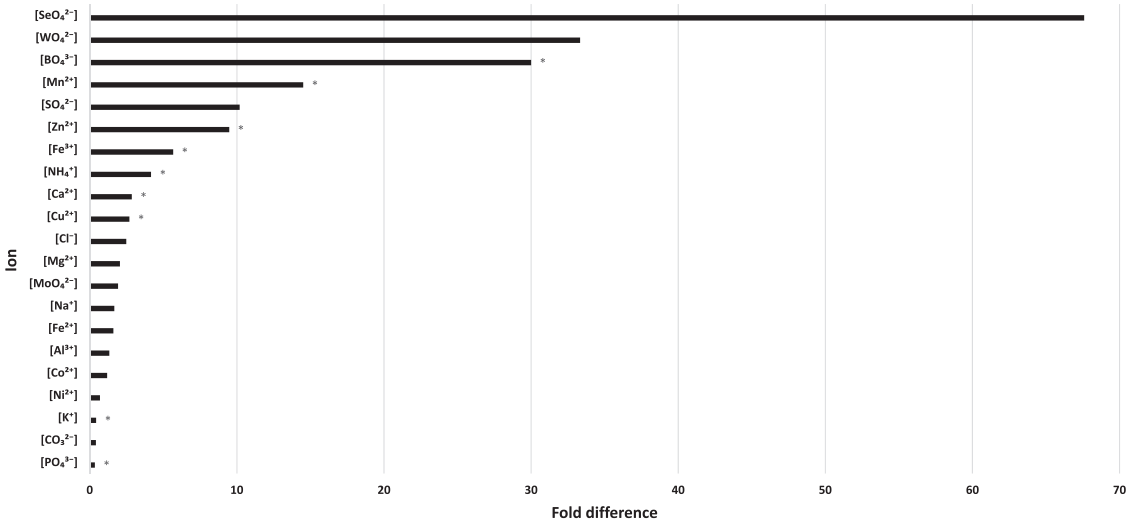


**Fig. 4 | Fold differences of MES compared to gas fermentation media.** Asterisk indicates significant difference (Student's *t* test, $P \leq 0.05$). Data process methods are detailed in Supplementary Method 1.

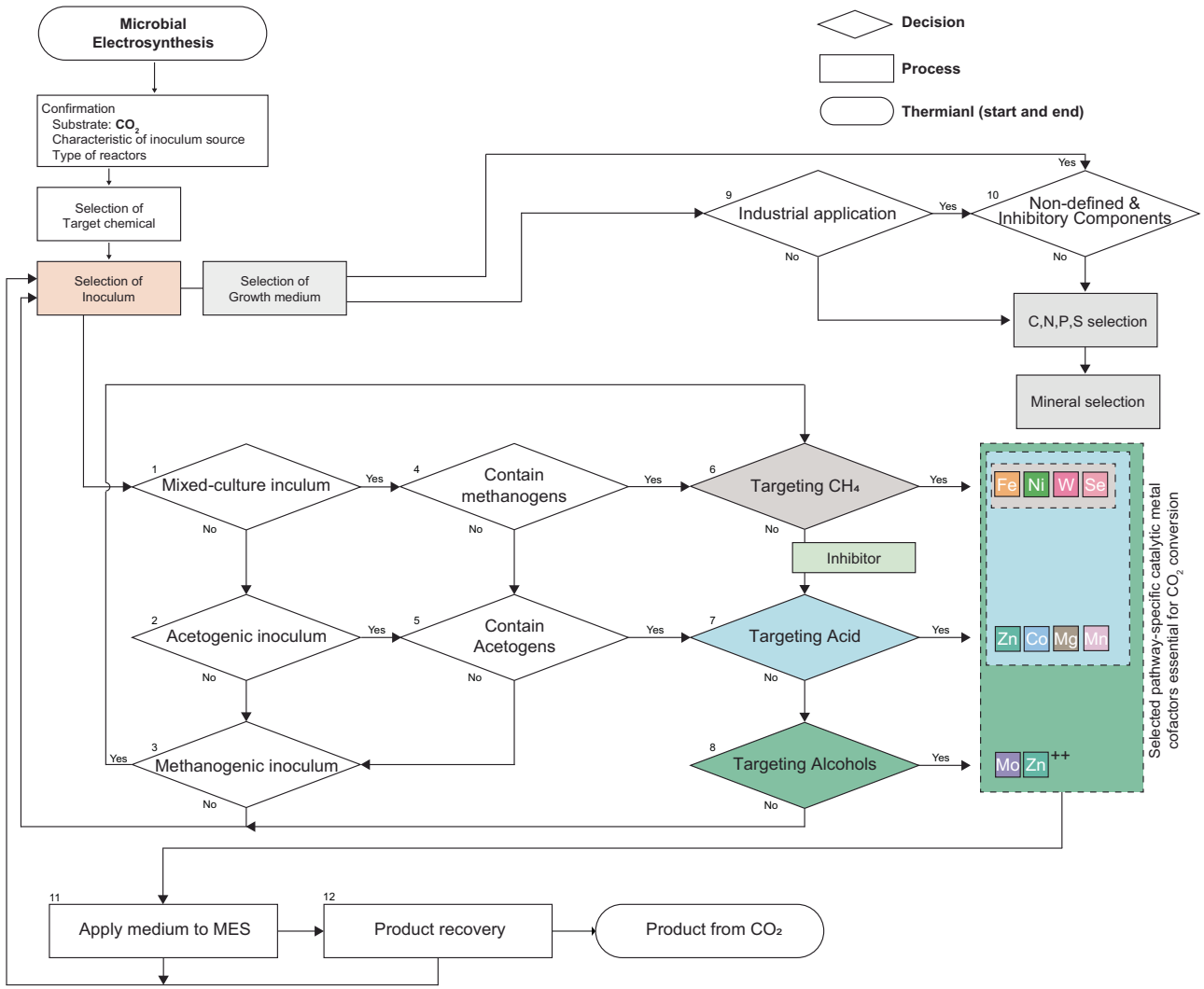


**Fig. 5 | Flow diagram of $CO_2$ conversion to products via MES, highlighting catholyte selection.** This flow chart focuses on catholyte-related decision, assuming that all other limiting factors in the process have been addressed. "$^{++}$" indicates that the corresponding step requires additional attention and is considered particularly important.

## BOX 2

# Role of inorganic ions as counter ions in medium composition

Among cations, $Na^+$ serves as a major counterion in MES medium, thus it usually has one of the highest concentrations among solutes (Box Table). It plays a crucial role in maintaining osmotic balance and, in certain microbes, supports sodium-dependent ATP synthesis. While essential, excess $Na^+$ can induce osmotic stress, negatively affecting microbial viability and performance. Sodium is commonly introduced not only through the base medium but also during pH adjustment steps (e.g., via NaOH addition). $K^+$ may partially substitute $Na^+$, depending on the tolerance of the microbial strain.

The chloride anion is one of the most commonly used counterions in MES. Even if only used in the catholyte, the concentration gradient leads to diffusion to the anode side. At the operating potentials used in MES, chloride can be oxidized at the anode to produce chlorine gas ($Cl_2$), along with reactive species such as chlorine radicals and hypochlorous acid[105,106,108–110]. These activated chlorine species exhibit strong bactericidal properties and are especially prominent during early system startup or when chloride-containing anolyte is replenished. Since these compounds can diffuse through the cation exchange membrane (CEM), they may inhibit microbial activity and compromise system performance. Moreover, chloride radicals can react with ammonium to form nitrogen gas, possibly interfering with mass balancing[111]. Additionally, chloride ions and their oxidation products have been found to be highly corrosive to electrochemical systems, including any catalysts present, which can have lasting effects on system performance[112].

To address these concerns, sulfate and phosphate are often adopted as alternative counter anions as they are generally more electrochemically stable under typical operating conditions. However, due to membrane selectivity, their diffusion to the anode compartment cannot be fully excluded, and under specific operating conditions or with certain anode catalyst materials, surface adsorption or unintended electrochemical interactions may interfere with optimal anode activity. While sulfate can serve as a sulfur source for microbial metabolism and phosphate contributes to ionic balance and pH buffering, both should be evaluated carefully in the context of system electrochemistry. Nitrate, on the other hand, has a risk of reducing at the cathode at the potentials typically used in MES reactors, and is therefore advised not to be used in the catholyte as nitrogen source[113]. These examples illustrate the need to examine the electrochemical properties of compounds before they are added to electrolyte solutions.

**Box Table** Roles and potential drawbacks of major inorganic ions in catholyte

| Element/ Ion | Primary role | Drawbacks when in excess |
|---|---|---|
| $Na^+$ | Major counter cation: maintains osmotic balance and drives sodium dependent ATP synthesis in some microbes | Osmotic stress can occur, potentially reducing microbial viability[111]. |
| $K^+$ | Counter cation; also functions as enzyme activator in some microbes | Excess $K^+$ may inhibit specific microbial strains and cause ionic toxicity[114]. |
| $Cl^-$ | Major counter anion; maintains intracellular charge balance | $Cl^-$ can undergo oxidation to form chlorine gas ($Cl_2$), which are toxic and exhibit bactericidal effects in MES systems.$2Cl^- \rightarrow Cl_2(g) + 2e^-$ $E^o = 1.36$ V vs SHE $Cl_2(g) + H_2O \rightleftharpoons HCl + HClO$ |
| $SO_4^{2-}$ | Counter anion; serves as sulfur source | Excess sulfate can promote the activity of sulfate-reducing bacteria (SRB), resulting in the production of $H_2S$[115,116]. $SO_4^{2-} + 2\ CH_2O \rightarrow H_2S + 2\ HCO_3^-$ |
| $PO_4^{3-}$ | Buffer and counter anion; also supplies phosphorus | Over-supplementation can cause precipitation with $Mg^{2+}$ or $Ca^{2+}$, reducing metal ion availability and disrupting medium balance[107]. |

## Concluding remarks

In this Perspective, cultivation media in MES systems were found to be generally adopted without system-specific optimization, and systematic studies tailored to the specific characteristics of $CO_2$-reducing MES systems remain limited. The catholyte plays a vital role in supporting microbial growth as well as in facilitating substrate and product transport. Therefore, its optimization requires an integrated and multidisciplinary approach beyond empirical adjustments.

To design a catholyte composition, it is essential to identify the enzymes affected and the factors influencing their activity. It is also important to determine the appropriate concentration ranges based on this understanding.While experimentally validating all possible combinations is impractical, bioinformatic tools can accelerate the prediction of optimal compositions when supported by sufficiently comprehensive datasets. However, MES systems expose microbes directly to electrons supplied from electrodes, altering redox conditions and metabolic pathways. As there may be metabolic differences between MES systems and conventional cultivation, predictions based solely on conventional cultivation data may be insufficient, necessitating the incorporation of datasets generated under electrotrophic conditions for accurate modeling.

Additionally, ensuring stable and scalable MES operation requires systematic investigation into how applied voltage and electrode materials influence the accumulation of inhibitory factors such as electrodeposition or toxic intermediates. These effects can vary depending on reactor design, electrode surface properties, and electrolyte composition. Defining the optimal operational window for a given MES design through such investigations is critical to achieving long-term performance and scalability. Moreover, our analysis reveals a knowledge gap of metal ion bioavailability in MES reactors.

Ultimately, the catholyte serves as the key interface linking microbial and electrochemical processes in MES. A rationally designed catholyte that integrates both biological and electrochemical considerations will be crucial for enabling efficient, cost-effective, sustainable, and industrial-scale $CO_2$ conversion.

Moreover, the approach outlined in this Perspective may benefit fields beyond MES, as discrepancies between medium formulations and microbial requirements are unlikely to be unique to MES research. This strategy can identify potential target components for medium optimization, significantly reducing the experimental burden.

## References


1. Zegers, M. A. J., Augustijn, E., Jongbloed, G. & Jourdin, L. Novel miniaturised microbial electrosynthesis reactor: a study on replicability. *Chem. Eng. J.* **516**, 163881 (2025).
2. Jourdin, L. & Burdyny, T. Microbial electrosynthesis: where do we go from here? *Trends Biotechnol* **39**, 359–369 (2021).
3. Cheng, S., Xing, D., Call, D. F. & Logan, B. E. Direct biological conversion of electrical current into methane by electromethanogenesis. *Environ. Sci. Technol.* **43**, 3953–3958 (2009).
4. Nevin, K. P., Woodard, T. L., Franks, A. E., Summers, Z. M. & Lovley, D. R. Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. *MBio* **1**, 1128 (2010).
5. Winkelhorst, M., Cabau-Peinado, O., Straathof, A. J. J. & Jourdin, L. Biomass-specific rates as key performance indicators: A nitrogen balancing method for biofilm-based electrochemical conversion. *Front. Bioeng. Biotechnol.* **11**, 1096086 (2023).
6. Chatzipanagiotou, K.-R., Mesman, J., Jourdin, L., Bitter, J. H. & Strik, D. P. B. T. B. Combined influence of $CO_2$ supply and metal addition on the biocathode performance in microbial electrosynthesis. https://doi.org/10.2139/ssrn.4406058 (2023).
7. Cabau-Peinado, O. et al. Microbial electrosynthesis from $CO_2$ reaches productivity of syngas and chain elongation fermentations. *Trends Biotechnol.* **42**, 1503–1522 (2024).
8. Mirea, R., Popescu, E. & Zaharescu, T. Microbial electrosynthesis: the future of next-generation biofuel production—a review. *Energies* **18**, 5187 (2025).
9. Li, S. et al. Solid neutral red/Nafion conductive layer on carbon felt electrode enhances acetate production from $CO_2$ and energy efficiency in microbial electrosynthesis system. *Bioresour. Technol.* **363**, 127983 (2022).
10. Li, S. et al. Housing of electrosynthetic biofilms using a roll-up carbon veil electrode increases $CO_2$ conversion and faradaic efficiency in microbial electrosynthesis cells. *Bioresour. Technol.* **393**, 130157 (2024).
11. Vassilev, I. et al. Microbial electrosynthesis of isobutyric, butyric, caproic acids, and corresponding alcohols from carbon dioxide. *ACS Sustain. Chem. Eng.* **6**, 8485–8493 (2018).
12. Volger, R., Puiman, L. & Haringa, C. Bubbles and broth: A review on the impact of broth composition on bubble column bioreactor hydrodynamics. *Biochem. Eng. J.* **201**, 109124 (2024).
13. Cabau-Peinado, O., Straathof, A. J. J. & Jourdin, L. A general model for biofilm-driven microbial electrosynthesis of carboxylates from $CO_2$. *Front. Microbiol.* **12**, 669218 (2021).
14. Kim, K. et al. Enhancement of methane-water volumetric mass transfer coefficient by inhibiting bubble coalescence with electrolyte. *J. Ind. Eng. Chem.* **33**, 326–329 (2016).
15. Hori, Y., Hayashi, K., Hosokawa, S. & Tomiyama, A. Mass transfer from single carbon-dioxide bubbles in electrolyte aqueous solutions in vertical pipes. *Int. J. Heat Mass Transf.* **115**, 663–671 (2017).
16. Reyhanitash, E., Kersten, S. R. A. & Schuur, B. Recovery of volatile fatty acids from fermented wastewater by adsorption. *ACS Sustain. Chem. Eng.* **5**, 9176–9184 (2017).
17. Lee, M., Jankovic, T., Caparrós-Salvador, F., Jourdin, L. & Straathof, A. J. J. Sustainable production of pure hexanoic acid from $CO_2$ using microbial electrosynthesis with in-situ product recovery. *Chem. Eng. J.* **524**, 169412 (2025).
18. Casademont, C., Pourcelly, G. & Bazinet, L. Effect of magnesium/calcium ratios in solutions treated by electrodialysis: Morphological characterization and identification of anion-exchange membrane fouling. *J. Colloid Interface Sci.* **322**, 215–223 (2008).
19. Paulo, L. M., Stams, A. J. M. & Sousa, D. Z. Methanogens, sulphate and heavy metals: a complex system. *Rev. Environ. Sci. Biotechnol.* **14**, 537–553 (2015).
20. Lin, C.-Y. & Chen, C.-C. Toxicity-resistance of sludge biogranules to heavy metals. *Biotechnol. Lett.* **19**, 557–560 (1997).
21. Bhattacharya, S. K., Uberoi, V., Madura, R. L. & Haghighi-Podeh, M. R. Effect of cobalt on methanogenesis. *Environ. Technol.* **16**, 271–278 (1995).
22. Matyakubov, B., Hwang, Y. & Lee, T.-J. Evaluating interactive toxic impact of heavy metals and variations of microbial community during fermentative hydrogen production. *Int. J. Hydrogen Energy* **47**, 31223–31240 (2022).
23. Semple, K. T. et al. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. *Environ. Sci. Technol.* **38**, 228A–231A (2004).
24. Bockris, J. O. Book review: Electrochemical phase formation and growth. *J. Electroanal. Chem.* **433**, 227–228 (1997).
25. Moreno-Castilla, C. Adsorption of organic molecules from aqueous solutions on carbon materials. *Carbon* **42**, 83–94 (2004).
26. Vidales, A. G., Bruant, G., Omanovic, S. & Tartakovsky, B. Carbon dioxide conversion to C1-C2 compounds in a microbial electrosynthesis cell with in situ electrodeposition of nickel and iron. *Electrochim. Acta* **383**, 138349 (2021).
27. de Smit, S. M. et al. Integration of biocompatible hydrogen evolution catalyst developed from metal-mix solutions with microbial electrosynthesis. *Bioelectrochemistry* **158**, 108724 (2024).
28. de Smit, S. M., van Mameren, T. D., Xie, Y., Strik, D. P. B. T. B. & Bitter, J. H. Trace metals from microbial growth media form in situ electro-catalysts. *Electrochim. Acta* **462**, 142722 (2023).
29. Romans-Casas, M. et al. Boosting ethanol production rates from carbon dioxide in MES cells under optimal solventogenic conditions. *Sci. Total Environ.* **856**, 159124 (2023).
30. Mayer, F., Enzmann, F., Lopez, A. M. & Holtmann, D. Performance of different methanogenic species for the microbial electrosynthesis of methane from carbon dioxide. *Bioresour. Technol.* **289**, 121706 (2019).
31. Baek, G., Shi, L., Rossi, R. & Logan, B. E. Using copper-based biocathodes to improve carbon dioxide conversion efficiency into methane in microbial methanogenesis cells. *Chem. Eng. J.* **435**, 135076 (2022).
32. Siegert, M. et al. Comparison of nonprecious metal cathode materials for methane production by electromethanogenesis. *ACS Sustain. Chem. Eng.* **2**, 910–917 (2014).
33. Barker, H. A. On the biochemistry of the methane fermentation. *Arch. Mikrobiol.* **7**, 404–419 (1936).
34. Johns, A. T. & Barker, H. A. Methane formation; fermentation of ethanol in the absence of carbon dioxide by *Methanobacillus omelianskii*. *J. Bacteriol.* **80**, 837–841 (1960).
35. Barker, H. A. Studies upon the methane fermentation. IV. The isolation and culture of *Methanobacterium omelianskii*. *Antonie Van Leeuwenhoek* **6**, 201–220 (1939).
36. Wolin, E. A., Wolin, M. & Wolfe, R. S. Formation of methane by bacterial extracts. *J. Biol. Chem.* **238**, 2882–2886 (1963).

37. Jourdin, L. et al. A novel carbon nanotube modified scaffold as an efficient biocathode material for improved microbial electrosynthesis. *J. Mater. Chem. A* **2**, 13093–13102 (2014).
38. Patil, S. A. et al. Selective Enrichment establishes a stable performing community for microbial electrosynthesis of acetate from $CO_2$. *Environ. Sci. Technol.* **49**, 8833–8843 (2015).
39. Jang, J. K. et al. Construction and operation of a novel mediator- and membrane-less microbial fuel cell. *Process Biochem.* **39**, 1007–1012 (2004).
40. Zaybak, Z., Pisciotta, J. M., Tokash, J. C. & Logan, B. E. Enhanced start-up of anaerobic facultatively autotrophic biocathodes in bioelectrochemical systems. *J. Biotechnol.* **168**, 478–485 (2013).
41. Jourdin, L., Freguia, S., Donose, B. C. & Keller, J. Autotrophic hydrogen-producing biofilm growth sustained by a cathode as the sole electron and energy source. *Bioelectrochemistry* **102**, 56–63 (2015).
42. Batlle-Vilanova, P., Puig, S., Gonzalez-Olmos, R., Balaguer, M. D. & Colprim, J. Continuous acetate production through microbial electrosynthesis from $CO_2$ with microbial mixed culture. *J. Chem. Technol. Biotechnol.* **91**, 921–927 (2016).
43. Choi, K. S., Kondaveeti, S. & Min, B. Bioelectrochemical methane ($CH_4$) production in anaerobic digestion at different supplemental voltages. *Bioresour. Technol.* **245**, 826–832 (2017).
44. Lee, M., Reddy, C. N. & Min, B. In situ integration of microbial electrochemical systems into anaerobic digestion to improve methane fermentation at different substrate concentrations. *Int. J. Hydrogen Energy* **44**, 2380–2389 (2019).
45. Noori, M. T., Mohan, S. V. & Min, B. Microbial electrosynthesis of multi-carbon volatile fatty acids under the influence of different imposed potentials. *Sustain. Energy Technol. Assess.* **45**, 101118 (2021).
46. Flexer, V., Marque, M., Donose, B. C., Virdis, B. & Keller, J. Plasma treatment of electrodes significantly enhances the development of anodic electrochemically active biofilms. *Electrochim. Acta* **108**, 566–574 (2013).
47. Ragab, A., Katuri, K. P., Ali, M. & Saikaly, P. E. Evidence of spatial homogeneity in an electromethanogenic cathodic microbial community. *Front. Microbiol.* **10**, 1747 (2019).
48. Baek, G., Rossi, R., Saikaly, P. E. & Logan, B. E. High-rate microbial electrosynthesis using a zero-gap flow cell and vapor-fed anode design. *Water Res.* **219**, 118597 (2022).
49. Li, S. et al. Bioelectrosynthetic conversion of $CO_2$ using different redox mediators: electron and carbon balances in a bioelectrochemical system. *Energies* **13**, 2572 (2020).
50. Romans-Casas, M., Blasco-Gómez, R., Colprim, J., Balaguer, M. D. & Puig, S. Bio-electro $CO_2$ recycling platform based on two separated steps. *J. Environ. Chem. Eng.* **9**, 105909 (2021).
51. Roy, M., Kumar, S. & Patil, S. A. Brewery $CO_2$ conversion into acetic acid at an optimized set of microbial electrosynthesis process parameters. *J. Environ. Chem. Eng.* **12**, 114436 (2024).
52. Nevin, K. P. et al. Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. *Appl. Environ. Microbiol.* **77**, 2882–2886 (2011).
53. Cui, M., Nie, H., Zhang, T., Lovley, D. & Russell, T. P. Three-dimensional hierarchical metal oxide–carbon electrode materials for highly efficient microbial electrosynthesis. *Sustain. Energy Fuels* **1**, 1171–1176 (2017).
54. Romans-Casas, M. et al. Selective butyric acid production from $CO_2$ and its upgrade to butanol in microbial electrosynthesis cells. *Environ. Sci. Ecotechnol.* **17**, 100303 (2024).
55. Song, J. et al. Microbes as electrochemical $CO_2$ conversion catalysts. *ChemSusChem* **4**, 587–590 (2011).
56. Batlle-Vilanova, P. et al. Microbial electrosynthesis of butyrate from carbon dioxide: production and extraction. *Bioelectrochemistry* **117**, 57–64 (2017).
57. Jourdin, L. et al. High acetic acid production rate obtained by microbial electrosynthesis from carbon dioxide. *Environ. Sci. Technol.* **49**, 13566–13574 (2015).
58. Jourdin, L., Winkelhorst, M., Rawls, B., Buisman, C. J. N. & Strik, D. P. Enhanced selectivity to butyrate and caproate above acetate in continuous bioelectrochemical chain elongation from $CO_2$: steering with $CO_2$ loading rate and hydraulic retention time. *Bioresour. Technol. Rep.* **7**, 100284 (2019).
59. Raes, S. M. T., Jourdin, L., Buisman, C. J. N. & Strik, D. P. Bioelectrochemical chain elongation of short-chain fatty acids creates steering opportunities for selective formation of n-butyrate, n-valerate or n-caproate. *ChemistrySelect* **5**, 9127–9133 (2020).
60. Aryal, N., Tremblay, P.-L., Lizak, D. M. & Zhang, T. Performance of different Sporomusa species for the microbial electrosynthesis of acetate from carbon dioxide. *Bioresour. Technol.* **233**, 184–190 (2017).
61. Ibrahim, I. et al. Role of microbial electrosynthesis system in $CO_2$ capture and conversion: a recent advancement toward cathode development. *Fron. Microbiol.* **14**, 1192187 (2023).
62. Peters, J. W., Miller, A. F., Jones, A. K., King, P. W. & Adams, M. W. W. Electron bifurcation. *Curr. Opin. Chem. Biol.* **31**, 146–152 (2016).
63. Glass, J. B. & Orphan, V. J. Trace metal requirements for microbial enzymes involved in the production and consumption of methane and nitrous oxide. *Front. Microbiol.* **3**, 61 (2012).
64. Alfano, M. & Cavazza, C. Structure, function, and biosynthesis of nickel-dependent enzymes. *Protein Sci.* **29**, 1071–1089 (2020).
65. Bateman, A. et al. UniProt: the universal protein knowledgebase in 2025. *Nucleic Acids Res.* **53**, D609–D617 (2025).
66. Kanehisa, M. & Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30 (2000).
67. Buckel, W. & Thauer, R. K. Flavin-based electron bifurcation, a new mechanism of biological energy coupling. *Chem. Rev.* **118**, 3862–3886 (2018).
68. Ljungdahl, L. G. The autotrophic pathway of acetate synthesis in acetogenic bacteria. *Annu. Rev. Microbiol.* **40**, 415–450 (1986).
69. Wood, H. G., Ragsdale, S. W. & Pezacka, E. The acetyl-CoA pathway of autotrophic growth. *FEMS Microbiol. Lett.* **39**, 345–362 (1986).
70. Ragsdale, S. W. & Pierce, E. Acetogenesis and the Wood–Ljungdahl pathway of CO2 fixation. *Biochim. Biophys. Acta Proteins Proteom.* **1784**, 1873–1898 (2008).
71. Chen, K. et al. Crystal structures of ferredoxin variants exhibiting large changes in [Fe-S] reduction potential. *Nat. Struct. Biol.* **9**, 188–192 (2002).
72. Appel, A. M. et al. Frontiers, opportunities, and challenges in biochemical and chemical catalysis of $CO_2$ fixation. *Chem. Rev.* **113**, 6621–6658 (2013).
73. Bierbaumer, S. et al. Enzymatic conversion of $CO_2$: from natural to artificial utilization. *Chem. Rev.* **123**, 5702–5754 (2023).
74. Lyu, Z., Shao, N., Akinyemi, T. & Whitman, W. B. Methanogenesis. *Curr. Biol.* **28**, R727–R732 (2018).
75. Kurth, J. M., Op den Camp, H. J. & Welte, C. U. Several ways one goal: methanogenesis from unconventional substrates. *Appl. Microbiol. Biotechnol.* **104**, 6839–6854 (2020).
76. Thauer, R. K., Kaster, A. K., Seedorf, H., Buckel, W. & Hedderich, R. Methanogenic archaea: Ecologically relevant differences in energy conservation. *Nat. Rev. Microbiol.* **6**, 579–591 (2008).
77. Zhu, X. & Tan, X. Metalloproteins/metalloenzymes for the synthesis of acetyl-CoA in the Wood-Ljungdahl pathway. *Sci. China B: Chem.* **52**, 2071–2082 (2009).
78. Greening, C. et al. Physiology, biochemistry, and applications of F420- and Fo-dependent redox reactions. *Microbiol. Mol. Biol. Rev.* **80**, 451–493 (2016).

79. Houtkooper, R. H., Cantó, C., Wanders, R. J. & Auwerx, J. The secret life of $NAD^+$: an old metabolite controlling new metabolic signaling pathways. *Endocr. Rev.* **31**, 194–223 (2010).
80. Katsyv, A. & Müller, V. Overcoming energetic barriers in acetogenic C1 conversion. *Front. Bioeng. Biotechnol.* **8**, 621166 (2020).
81. Kracke, F., Vassilev, I. & Krömer, J. O. Microbial electron transport and energy conservation—the foundation for optimizing bioelectrochemical systems. *Front. Microbiol.* **6**, 575 (2015).
82. González-Cabaleiro, R., Lema, J. M., Rodríguez, J. & Kleerebezem, R. Linking thermodynamics and kinetics to assess pathway reversibility in anaerobic bioprocesses. *Energy Environ. Sci.* **6**, 3780–3789 (2013).
83. Angenent, L. T. et al. Chain elongation with reactor microbiomes: Open-culture biotechnology to produce biochemicals. *Environ. Sci. Technol.* **50**, 2796–2810 (2016).
84. Vassilev, I. et al. Microbial electrosynthesis system with dual biocathode arrangement for simultaneous acetogenesis, solventogenesis and carbon chain elongation. *Chem. Commun.* **55**, 4351–4354 (2019).
85. Lundie, L. L. & Drake, H. L. Development of a minimally defined medium for the acetogen *Clostridium thermoaceticum*. *J. Bacteriol.* **159**, 700–703 (1984).
86. Mohanakrishna, G., Vanbroekhoven, K. & Pant, D. Impact of dissolved carbon dioxide concentration on the process parameters during its conversion to acetate through microbial electrosynthesis. *React. Chem. Eng.* **3**, 371–378 (2018).
87. Roy, M., Yadav, R., Chiranjeevi, P. & Patil, S. A. Direct utilization of industrial carbon dioxide with low impurities for acetate production via microbial electrosynthesis. *Bioresour. Technol.* **320**, 124289 (2021).
88. Raes, S. M. T., Jourdin, L., Buisman, C. J. N. & Strik, D. P. Continuous long-term bioelectrochemical chain elongation to butyrate. *ChemElectroChem* **4**, 386–395 (2017).
89. Hille, R. Molybdenum and tungsten in biology. *Trends Biochem. Sci.* **27**, 360–367 (2002).
90. Zegers, M. A. J., Roy, M. & Jourdin, L. Identifying key drivers of product formation in microbial electrosynthesis with a mixed linear regression analysis. *Cell Rep. Phys. Sci.* **6**, 102934 (2025).
91. Ammam, F., Tremblay, P. L., Lizak, D. M. & Zhang, T. Effect of tungstate on acetate and ethanol production by the electrosynthetic bacterium *Sporomusa ovata*. *Biotechnol. Biofuels* **9**, 163 (2016).
92. Schulz, S., Molitor, B. & Angenent, L. T. Acetate augmentation boosts the ethanol production rate and specificity by *Clostridium ljungdahlii* during gas fermentation with pure carbon monoxide. *Bioresour. Technol.* **369**, 128387 (2023).
93. Lee, M. et al. Microbial cell viability-driven operational strategy for enhanced acetate production in syngas fermentation. *J. Environ. Chem. Eng.* **13**, 116531 (2025).
94. Lee, J. et al. Domestication of the novel alcohologenic acetogen *Clostridium* sp. AWRP: From isolation to characterization for syngas fermentation. *Biotechnol. Biofuels* **12**, 228 (2019).
95. Shen, S. et al. Effect of temperature and surfactant on biomass growth and higher-alcohol production during syngas fermentation by *Clostridium carboxidivorans* P7. *Bioresour. Bioprocess.* **7**, 56 (2020).
96. Shen, S. et al. Enhanced alcohol titre and ratio in carbon monoxide-rich off-gas fermentation of *Clostridium carboxidivorans* through combination of trace metals optimization with variable-temperature cultivation. *Bioresour. Technol.* **239**, 236–243 (2017).
97. Allaart, M. T., Korkontzelos, C., Sousa, D. Z. & Kleerebezem, R. A novel experimental method to determine substrate uptake kinetics of gaseous substrates applied to the carbon monoxide-fermenting *Clostridium autoethanogenum*. *Biotechnol. Bioeng.* **121**, 1325–1335 (2024).
98. Valgepea, K. et al. Maintenance of ATP homeostasis triggers metabolic shifts in gas-fermenting acetogens. *Cell Syst.* **4**, 505–515.e5 (2017).
99. Heffernan, J. K. et al. Enhancing $CO_2$-Valorization Using Clostridium autoethanogenum for Sustainable Fuel and Chemicals Production. *Front. Bioeng. Biotechnol.* **8**, 204 (2020).
100. Lee, M. et al. Proposition of cost-effective industrial grade medium for synthesis gas fermentation through evaluation of economic feasibility. *Bioresour. Technol. Rep.* **19**, 101208 (2022).
101. Rittmann, B. E. & McCarty, P. L. *Environmental Biotechnology: Principles and Applications* 39-41 (McGraw-Hill, 2001).
102. Sathasivan, A. & Ohgaki, S. Application of new bacterial regrowth potential method for water distribution system–a clear evidence of phosphorus limitation. *Water Res.* **33**, 137–144 (1999).
103. Duboc, P., Schill, N., Menoud, L., Van Gulik, W. & Von Stockar, U. Measurements of sulfur, phosphorus and other ions in microbial biomass: Influence on correct determination of elemental composition and degree of reduction. *J. Biotechnol.* **43**, 145–158 (1995).
104. Kim, J., Park, C. W., Lee, K. W. & Lee, T. S. Adsorption of ethylenediaminetetraacetic acid on a gel-type ion-exchange resin for purification of liquid waste containing Cs ions. *Polymers* **11**, 297 (2019).
105. Wei, W., Xu, J., Chen, W., Mi, L. & Zhang, J. A review of sodium chloride-based electrolytes and materials for electrochemical energy technology. *J. Mater. Chem. A* **10**, 2637–2671 (2022).
106. Batlle-Vilanova, P. et al. Biogas upgrading, CO2 valorisation and economic revaluation of bioelectrochemical systems through anodic chlorine production in the framework of wastewater treatment plants. *Sci. Total Environ.* **690**, 352–360 (2019).
107. Lei, Y., Hidayat, I., Saakes, M., van der Weijden, R. & Buisman, C. J. N. Fate of calcium, magnesium and inorganic carbon in electrochemical phosphorus recovery from domestic wastewater. *Chem. Eng. J.* **362**, 453–459 (2019).
108. Wang, Y., Liu, Y., Wiley, D., Zhao, S. & Tang, Z. Recent advances in electrocatalytic chloride oxidation for chlorine gas production. *J. Mater. Chem. A* **9**, 18974–18993 (2021).
109. Zheng, W. et al. Discovering the importance of ClO• in a coupled electrochemical system for the simultaneous removal of carbon and nitrogen from secondary coking wastewater effluent. *Environ. Sci. Technol.* **54**, 9015–9024 (2020).
110. Zhou, X. et al. Reducing chloride ion permeation during seawater electrolysis using double-polyamide thin-film composite membranes. *Environ. Sci. Technol.* **58**, 391–399 (2024).
111. Bremer, E. & Krämer, R. Responses of microorganisms to osmotic stress. *Annu. Rev. Microbiol.* **73**, 313–334 (2019).
112. Khosravi, M. et al. Sustainable seawater electrolysis: evaluating environmental impacts and technological development opportunities. *Sustain. Energy Fuels* **9**, 4238–4261 (2025).
113. Outaleb, H., Kouzbour, S., Audonnet, F., Vial, C. & Gourich, B. Electrocatalytic nitrate reduction for brackish groundwater treatment: From engineering aspects to implementation. *Appl. Sci.* **14**, 8986 (2024).
114. Radchenko, M. V. et al. Potassium/proton antiport system of Escherichia coli. *J. Biol. Chem.* **281**, 19822–19829 (2006).
115. Yu, L., Duan, J., Du, X., Huang, Y. & Hou, B. Accelerated anaerobic corrosion of electroactive sulfate-reducing bacteria by electrochemical impedance spectroscopy and chronoamperometry. *Electrochem. Commun.* **26**, 101–104 (2013).
116. Agostino, V. & Rosenbaum, M. A. Sulfate-reducing electroautotrophs and their applications in bioelectrochemical systems. *Front. Energy Res.* **6**, 55 (2018).

## Acknowledgements
This project received funding from the project "C1to6: CO2 into hexanoic acid using microbial electrosynthesis" (NWO OTP 19771).

## Author contributions
D. van der Lee, M. Lee: conceptualization, data analysis, writing, and reviewing. M. Gabriëls: major contribution to metabolic pathway analysis and reviewing. R. Kleerebezem: conceptualization and reviewing. L. Jourdin: funding acquisition, conceptualization, and reviewing.

## Competing interests
The authors declare no competing interests.

## Additional information
**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1038/s41467-026-69414-z.

**Correspondence** and requests for materials should be addressed to Mungyu Lee or Ludovic Jourdin.

**Peer review information** *Nature Communications* thanks Sanne de Smit, Deepak Pant and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.