Engineering 16 (2022) 141–153

Contents lists available at ScienceDirect

Engineering

journal homepage: www.elsevier.com/locate/eng

Research
Green Chemical Engineering—Review

Microbial Electrosynthesis for Producing Medium Chain Fatty Acids

Na Chu [a], Wen Hao [b], Qinglian Wu [c], Qinjun Liang [a], Yong Jiang [a,*], Peng Liang [b], Zhiyong Jason Ren [d], Raymond Jianxiong Zeng [a]

[a] Fujian Provincial Key Laboratory of Soil Environmental Health and Regulation, College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou 350002, China
[b] State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China
[c] College of Architecture and Environment, Sichuan University, Chengdu 610065, China
[d] Department of Civil and Environmental Engineering & Andlinger Center for Energy and the Environment, Princeton University, Princeton, NJ 08544, USA

ARTICLE INFO

Article history:
Received 17 July 2020
Revised 25 January 2021
Accepted 3 March 2021
Available online 31 July 2021

Keywords:
Electro-fermentation
Microbial electrochemistry
Acetogen
Chain elongation
Electroactive microorganism

ABSTRACT

Microbial electrosynthesis (MES) employs microbial catalysts and electrochemistry to enhance CO_2 bioconversion to organics with concurrent waste biorefining capability. The aim of this review is to comprehensively discuss the current state of the art and prospects of medium chain fatty acids (MCFAs) production in MES from CO_2 and organic wastes. Fundamental mechanisms and development of MCFAs production via conventional fermentation are introduced as well. Studies on MCFAs production in MES are summarized, highlighting the strategy of multiple-electron donors (EDs). Challenges for MCFAs production in MES from CO_2 are presented, and the primary discussions included methanogenesis inhibition, adenosine triphosphate (ATP) limitations of acetogens, and production of limited EDs via solventogenesis. Possible applications of electrochemical approaches to promote the bioconversion of actual waste materials with MCFAs production are analyzed. Finally, future directions are explored, including multi-stage reactions, substrate supply, product extraction, and microbial pathways.

1. Introduction

Rapid urbanization and population growth have amplified problems of increased greenhouse gas emissions, significant generation of organic wastes, and energy crisis. Carbon dioxide (CO_2) is an important greenhouse gas, and there are significant economic and political incentives to develop technologies to capture and sequester CO_2 [1]. Global research and development (R&D) projects to improve CO_2 utilization have mainly focused on chemical production, mineralization, food processing, biological fixation, and energy storage [2]. Among these technologies, there is a growing interest in using CO_2 as a carbon source to produce high-valued chemicals. Many biosynthesis strategies cannot efficiently compete with chemosynthesis to produce chemicals due to lower productivity, yield, and titer [3]. However, biological routes provide higher specificity, lower energy costs under ambient conditions, and better avoid the poisoning effect of impurities [4]. Harnessing resources from microbial cell factories, CO_2 can be exploited as an alternative feedstock to sugar or biomass, and was described as the third generation biorefinery recently [4]. Higher energy efficiency of solar-electricity-products based on autotrophic electrosynthesis is expected from the direct bioconversion of CO_2 compared with the efficiency of solar-to-biomass-to-products [4]. In addition to CO_2, organic waste can also be used as sustainable feedstock for biochemical production [5]. Overall, there is strong interest in the development of technologies for resource recovery that are less environmentally harmful options for organic waste management [6,7]. The exploitation of renewable energy can help to decrease global anthropic CO_2 emissions, leading to an increased amount of renewable energy as a fraction of total electricity produced globally [8]. In the context of Power-to-*X* conversion technologies, renewable energy can be stored in fuels with a high volumetric or gravimetric storage capacity that are also compatible with existing fuel infrastructure [9].

Microbial electrosynthesis (MES), in a narrow sense, is a microbial electrochemical technology using microbes as electrode catalysts for chemical production from CO_2, and in a broad sense, includes the microbial electrochemical valorization of organic waste [10–12]. The development of MES together with the appropriate biological and electronic components has attracted an enormous amount of attention in recent years.

* Corresponding author.
E-mail address: jiangyongchange@163.com (Y. Jiang).

https://doi.org/10.1016/j.eng.2021.03.025

The application of MES for chemical production from CO_2, can use electrotrophic microbes for the generation of hydrocarbons from the sequestration of CO_2 and the storage of renewable energy [13–15]. Over the past decade, efforts to develop MES for chemical production from CO_2 has mainly centered on boosting the production rate and broadening the spectrum of chemicals that can be synthesized using this methodology [16]. For instance, production rate can be stimulated with adaptive laboratory evolution (ALE) of microbes, improvement of electrode materials, and the design of novel reactors with greater efficiency [17,18]. In addition, product spectrum can be expanded with the development of novel engineered biocatalyst to produce high value fine chemicals [19,20], or with the design of integrated process to produce bulk chemicals [21–23].

MES for chemical production from organic waste valorization should provide high selectivity by precisely controlling bioconversions with an increased operating range of redox conditions compared with that of traditional oxidation reduction potential (ORP)-based control [24]. In addition, the insertion of a solid electrode to serve as the terminal electron acceptor (EA) or electron donor (ED) can overcome the thermodynamic barrier of the fermentation process [25]. Compared with direct H_2 gas injection, reduction driven by an electrode should decrease energy consumption by stirring to overcome the gas–liquid mass transfer limitation [26]. MES for the valorization of organic wastes can be accomplished using fermentative microbes that are capable of extracellular electron transfer (EET) and co-fermentation with electroactive microbes. For example, the electro-fermentation of Gram-positive bacteria *Clostridium acetobutylicum* has been conducted in mediator-less reactors, and a shift in products was obtained using flavin-based EET [27]. With the development of enabling technologies, fine chemicals can be produced in MES [28]. However, the use of multiplex genome editing approaches and the assembly of too many different modules may overburden the chassis, causing imbalanced metabolism and insufficient efficiency [3]. Synthetic microbial consortia provide an alternative through labor division between two or more engineered strains that grow together [29]. However, the performance of synthetic microbial consortia in MES has not yet been demonstrated. The use of mixed cultures may decrease the capital and operating costs compared to single-organism cultures, or axenic systems, due to the use of unsterilized raw substrates [30]. It is thus attractive to create the mixed culture MES strategies for chemical production from practical organic waste valorization.

Medium chain fatty acids (MCFAs) are straight chain fatty acids with 6–12 carbon atoms (C6–C12) and one carboxylic group [31,32]. MCFAs have higher energy density than precursors such as short chain fatty acids (SCFAs, C2–C5), and can be used as antimicrobial agents, flavor additives, animal feed, and precursors of biofuels. Of these MCFAs, caproate (C6) has the lowest availability from traditional resources, but a high titer of C6 can be achieved by microbial production [33]. The market price of C1–C5 generally increases with carbon chain length (Fig. 1(a)), and C6 has an unrefined value of 1000 USD·t^{-1} and a refined value of 3800 USD·t^{-1} [8,17,34–36]. The global market value for carboxylates varies depending on the carbon chain (Fig. 1(b)), and it is estimated that by the end of 2023, the market value of MCFAs will reach ~8×10^9 USD [33]. The hydrophobicity of carboxylates is generally increased with the increase of carbon chain length. For instance, the solubilities of undissociated caproate and valerate are 10.82 and 49.7 g·L^{-1}, respectively, but other SCFAs are entirely miscible [37]. The stronger hydrophobicity of C6 leads to a nearly ten percent of energy consumption for extraction, compared with the energy required for C2 (Fig. 1(c)) [17].

There have been several excellent reviews on MES, describing the mechanisms of EET [38], configurations of bioreactors [39,40], strategies of energy efficiency improvement [41], discussions of energy consumption [17], developments of photosensitized materials [42], and applications of three-dimensional cathodes [43]. However, few review papers have focused on the use of MES for specific products. For example, the microbiome for methane or acetate production in MES has been discussed [44,45], the huge potential to merge the MES with existing biorefinery technologies was summarized [9,46,47], and prospects for improved butanol production using MES were discussed [48]. Conventional fermentation for MCFAs production can be potentially enhanced via microbial electrochemical regulation [31,32], and the production of MCFAs in MES should be economically profitable, especially compared to products such as methane and acetate [49,50]. The aim of this review is to comprehensively discuss the state of art and prospects for the use of MES to produce MCFAs. First, the fundamentals and recent advances of MCFAs production in conventional fermentation are introduced, and then recent works on MCFAs production in MES that optimized structure or parameters are summarized. Next, challenges for MCFAs

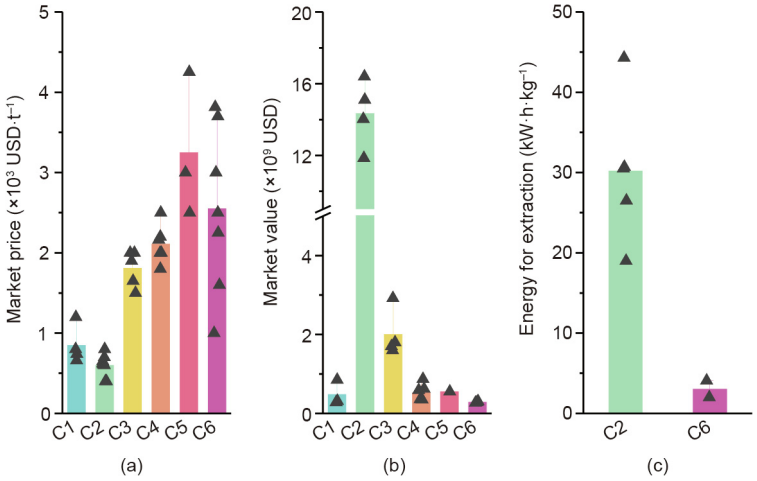

Fig. 1. (a) Market price of carboxylic acids. (b) Global market value of carboxylic acids projected to reach by 2026, and data are from different sources (https://www.marketsandmarkets.com/, https://www.marketwatch.com/, https://dataintelo.com/, http://www.gosreports.com/, and https://www.acumenresearchandconsulting.com/). (c) The energy consumption for extraction of carboxylic acids, and data are from an excellent review paper (Ref. [17]). Each data point is obtained from different sources, and bar height is the mean of these data. Data of market price are from Refs. [8,17,34–36].

production from CO_2 in MES are analyzed. Practical organic wastes can contain multiple-EDs, but in MES the electrode is used as a solid ED to promote bioconversion, so the use of multiple-EDs present both opportunity and challenge in both conventional fermentation and MES. Finally, future developments and perspectives for improved production in MES are outlined. Overall, this review should provide a common knowledge base for microbial electrochemical MCFAs production and may help guide future interdisciplinary work for increased application of this technology.

2. MCFAs production via conventional fermentation

In this section, recent advances in approaches to the production of sustainable MCFAs by conventional fermentation are introduced. Because practical organic wastes can contain multiple-EDs, the effects of multiple-EDs on the bioconversion are discussed.

2.1. Working principle and recent advances

Significant progress has been obtained in the field of microbial MCFAs production, with effective demonstration of industrialization by the company ChainCraft[†]. Microbes including *Clostridium kluyveri* (*C. kluyveri*), *Clostridium* sp. BS-1 (renamed *Caproiciproducens galactitolivorans*), *Megasphaera elsdenii*, and *Megasphaera hexanoica* are capable of MCFAs production, and the microbial chain elongation (CE) process based on the reverse β oxidation (RBO) pathway has been clarified [32,51,52]. Briefly, in the RBO pathway, acetyl coenzyme A (acetyl-CoA) generated from the oxidation of various EDs can interact in cyclic form with SCFAs that act as EAs, to extend the carbon chain length by two carbons for each cycle [32]. The effects of pH, yeast extract, and inorganic carbon on the model microbe *C. kluyveri* have been evaluated [53]. Microbial production of C8–C12 MCFAs with engineered microbes, for example, *Escherichia coli* (*E. coli*), is generally in the "milligram per liter" lower range, given that these chemicals are not endogenous to cells and it is difficult to control chain length [54]. In addition, MCFAs production in yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) was recently achieved by multidimensional manipulation of fatty acid synthases (FASs) [55]. Even-numbered, odd-numbered, and branched MCFAs can be produced by feeding of even-numbered, odd-numbered, and iso-forms of SCFAs, respectively [33]. Practical organic wastes have been used as feedstocks for MCFAs production, including food waste [56,57], acid whey waste [37,58], grass [59], activated sludge alkaline fermentation liquor [60], anaerobic fermentation liquid from sewage sludge [61], and Chinese Baijiu-making wastewater [62]. Granular sludge has been formed for MCFAs production [63,64]. Various bioreactors have been tested for MCFAs production, including upflow anaerobic filter (UAF), upflow anaerobic sludge blanket (UASB), anaerobic sequencing batch reactor (ASBR), continuous stirred tank reactor (CSTR), leach-bed reactor (LBR), and expanded granular sludge bed (EGSB) [65]. The long-term performance of continuous reactor systems to produce MCFAs for more than one year has also been evaluated [58,65,66].

2.2. Multiple-EDs in conventional fermentation

EDs are required for microbial production of MCFAs, and EDs can be from ethanol, methanol, lactate, H_2, and CO [31,32]. The characteristics of different EDs contribute significantly to the cost, the microbial community, and the carbon flux for MCFAs production. Ethanol has been proposed as the most ideal ED for microbial MCFAs production, considering that ethanol can be produced from various sources including industrial and agricultural waste while using as ED a high MCFAs productivity can be obtained [67]. However, the supply of additional ethanol has a negative environmental impact and an increased cost [68]. If ethanol is used as a source of ED, external CO_2 is required for protein synthesis of the corresponding MCFAs-forming bacteria, for example, *C. kluyveri* [69]. Cheap and readily available methanol has been used as the ED to produce *i*-butyrate and *n*-butyrate [66]. When lactate is used as ED, CO_2 is released, and this can decrease the carbon efficiency [70]. H_2 can be used as a gaseous energy carrier, but this is restricted by its low solubility. CO can provide a lower redox potential than that of H_2, and MCFAs can be produced with CO as the sole ED and carbon source [71]. *Clostridium ljungdahlii* (*C. ljungdahlii*) can produce different products, by shifting from acetate feeding with H_2/CO_2 to more reduced ethanol feeding with CO/CO_2 [72]. Also, CO can be used to directly upgrade SCFAs to generate MCFAs [73]. However, CO is inhibitory to various microbes to varying degrees, and is predicted to shape the microbial community due to its toxicity [74].

Substantial efforts have been made to evaluate the presence of multiple-EDs on microbial MCFAs production. There are two main lines of research: upgrading specific organic wastes that naturally contain multiple-EDs, or valuating the addition of external EDs to facilitate MCFAs production. Combining ethanol and lactate is expected to promote MCFAs production, as the CO_2 released from lactate compensates for the CO_2 requirement in ethanol-guided CE. Chinese Baijiu-making wastewater, which contains lactate, ethanol, and SCFAs, was used as substrate with an MCFAs selectivity (electron equivalents of MCFAs dividing by electrons from consumed substrates) of 80.34% ± 5.26% in serum bottles [62], and an MCFAs yield of 76.80% in long-term EGSB operation [65]. Concurrent use of methanol and ethanol in a CE reactor upgraded SCFAs into caproate and isobutyrate [75]. Addition of CO in ethanol fermentation increased the distribution of electrons to C3–C7 fatty acids and decreased the distribution to acetate and methane [76]. H_2 supply reduced the competing acrylate pathway of lactate-guided CE and improved MCFAs production [77].

Multiple-EDs can also potentially result in decreased MCFAs production, depending on the microbial community, the substrate composition, and operation parameters. For example, the supplementation of ethanol in a bioreactor fed with food waste decreased overall MCFAs production, but supplementation with H_2 resulted in improved performance [57]. Similarly, ethanol supplementation in a bioreactor fed with acid whey and operated with pH as low as 5.5 decreased the generation of MCFAs [78]. The reduced production of MCFAs with the addition of ethanol can be explained by the fact that the ethanol-guided caproate producer, for example, *C. kluyveri* is not a key microbe in these bioreactors, and lactate, not ethanol, mainly acts as the ED. *C. kluyveri* for ethanol-guided CE grows best at pH from 6.5 to 7.6, while Ruminococcaceae bacterium CPB6 for lactate-guided CE grows optimally at initial pH from 5.5 to 6 [79]. A recent study found that a pH value below 6 can stimulate MCFAs production from lactate over alternative pathways [80]. When feeding with a mixed substrate (such as lactose, lactate, acetate, and ethanol), bioaugmentation with *C. kluyveri* resulted in lower caproate production compared with the control reactor that was inoculated with only heat-treated anaerobic digester sludge. These results might be because *C. kluyveri* can repress the activity of microbes responsible for lactate utilization [81].

3. MCFAs production in MES

MCFAs production in MES should be more economically profitable than the production of methane or acetate as primary products. In addition, MCFAs production can provide new opportunities

[†] https://www.chaincraft.nl

for practical MES application by integration with other existing biorefinery technologies. In this section, studies on MCFAs production in MES are discussed.

3.1. CO_2 as the sole carbon source

In MES, acetate is generally the main product from CO_2 if methanogenesis is inhibited [17,38]. A few studies on MCFAs production in MES from CO_2 achieved a maximum C6 specificity that ranged from < 4% to 20%, with a lag phase up to 240 d (Table 1) [22,49,74,82–91]. The optimization of MES bioreactors and process parameters (e.g., pH and gas partial pressure) is critical for MCFAs generation using CO_2 as the sole carbon source. An MES bioreactor was constructed with forced-flow through of catholyte that allowed maintenance of a high current density (175 $A{\cdot}m^{-2}$); with a lag phase of 150 days, the reactor finally reached a maximum caproate concentration of 1.5 $g{\cdot}L^{-1}$, which is lower than that of butyrate (3.2 $g{\cdot}L^{-1}$) and acetate (8.6 $g{\cdot}L^{-1}$) [82]. A novel dual-biocathode MES was constructed to separately control the pH of a conventional biocathode (6.9) and a middle biocathode (4.9), with production of caproate (0.27 $g{\cdot}L^{-1}$), butyrate (1.49 $g{\cdot}L^{-1}$), and acetate (about 4.1 $g{\cdot}L^{-1}$) [22]. By operating the MES reactor at low pH and elevated H_2 partial pressure, a concentration of butyrate higher than that of acetate was obtained, with only a trace amount of caproate [84]. In these MES bioreactors, butyrate can be produced directly via the Wood–Ljungdahl pathway (WLP) coupled to acetyl-CoA reduction or via the RBO pathway-based CE process [92,93]. The detection of caproate in MES suggests the RBO pathway is active [84], but the relative contributions of these two pathways to butyrate production have not been determined. With the control of pH value to around 5 and after 462 days of operation, a maximum caproate concentration of 1.2 $g{\cdot}L^{-1}$ was obtained in MES, which was still lower than that of butyrate (3.1 $g{\cdot}L^{-1}$) and acetate (4.9 $g{\cdot}L^{-1}$) [49]. By regulating the CO_2 feeding rate in MES, a maximum caproate concentration of 3.1 $g{\cdot}L^{-1}$ was obtained after 200 days, again, still lower than the maximum concentrations of butyrate (9.3 $g{\cdot}L^{-1}$) and acetate (17.5 $g{\cdot}L^{-1}$) [83]. Considering the challenges of using CO_2 as the sole carbon source for MCFAs production, the use of SCFAs as an alternative to CO_2 has been applied in MES, with some of these studies discussed in section 4.3. In addition, MCFAs production in MES can also be stimulated with additional ED.

3.2. Multiple-EDs in MES

The strategy of multiple-EDs has been applied in MES for increased production of MCFAs (Fig. 2(a)). The concurrent use of electrode and ethanol as EDs in MES was achieved (Fig. 2(b)). Caproate was produced as the main product with a remarkably high selectivity of 80.28% ± 0.52% [90]. The maximum caproate concentration was (7.66 ± 1.38) $g{\cdot}L^{-1}$, much higher than that of butyrate ((1.22 ± 0.73) $g{\cdot}L^{-1}$) and acetate ((1.15 ± 0.77) $g{\cdot}L^{-1}$). According to the carbon balance calculation, 23.43% ± 0.69% of carbon in these liquid organic chemicals could originate from CO_2. The concurrent use of an electrode and CO as EDs in MES for MCFAs is not easy to implement, although a mixture of products including acetate, butyrate, propionate, isobutyrate, and isovalerate has been produced [94]. However, a recent study observed that caproate was produced with a specificity of 15.41% ± 1.48% in MES (Fig. 2(c)), feeding with CO:CO_2 (50:50) [74]. In contrast, in MES fed with pure CO_2, caproate was not detected [74].

The presence of multiple-EDs in MES might affect acetogenesis, solventogenesis, and the CE process [95]. The use of CO as the sole electron donor can sustain the autotrophic growth of acetogens via methyl branch after a water–gas shift reaction or directly via the carbonyl branch of WLP [96]. During growth on CO, the adenosine triphosphate (ATP) yields and growth rates are expected to be higher than those of H_2, as CO reduction only produces ferredoxin (Fd2) for ATP production, but bifurcation of electrons between reduced Fd2 and NADPH was observed when feeding with H_2/CO_2 [97]. The effects of a poised electrode on acetogenesis or solventogenesis have not been addressed. For example, it is not clear whether a poised electrode can directly feed acetogens for ferredoxin reduction to improve ATP yield [98]. More research is needed, but studies have suggested that a poised electrode might affect the CE process to some extent. As a global transcriptional regulator, Rex plays a key role in *C. kluyveri* to regulate metabolism in response to the cellular NADH/NAD^+ ratio [99], which is crucial for energy generation [100]. A poised electrode can drive the NADH pool to a more reduced state [3]. However, the caproate production of *C. kluyveri* in cathodic condition ((100.7 ± 8.2) $mmol{\cdot}L^{-1}$) was not statistically significant different from that obtained with open circuit control ((81.7 ± 12.0) $mmol{\cdot}L^{-1}$) [101]. Mixed culture electro-fermentation with a fresh cathode could increase the caproate specificity by about 28% from the bioconversion of acetate and ethanol, compared with the amount obtained by open circuit control [91]. However, the effect of an acclimated cathode on caproate production depended on the substrates (Fig. 2(d)) [91]. Microbial electrochemical regulation of MCFAs production from lactate should allow the recapture of released CO_2 from bioconversion, but this has not been shown experimentally.

4. Challenges for MCFAs production in MES from CO_2

MCFAs production from complex organic waste via conventional fermentation is a spontaneous process [100], consisting of

Table 1
Studies on MCFAs production in MES.

Carbon sources	Electron donors	Maximum MCFAs concentration ($g{\cdot}L^{-1}$)	Maximum C6 specificity[a] (%)	Lag phase of C6 (d)	Ref.
CO_2	Electrode	C6 (1.5)	20	164	[82]
CO_2	Electrode	C6 (3.1)	17	171	[83]
CO_2	Electrode	C6 (1.2)	16	240	[49]
CO_2	Electrode	C6 (0.27)	5	< 5	[22]
CO_2	Electrode	C6 (< 0.1)	< 4	NA	[84]
CO_2	Electrode	C6 (0.25), C7 (0.26)	14	1	[85]
Acetate, K_2CO_3	Electrode	C6 (0.739), C8 (0.036)	NA[b]	4	[86]
Acetate, CO_2	Electrode	C6 (0.06)	6	60	[87]
Acetate, butyrate	Electrode	C6 (0.02)	0.2	NA[b]	[88]
Acetate, butyrate	Electrode	C6 (0.3)	10	43	[89]
CO_2, ethanol	Electrode, ethanol	C6 (7.66), C7 (0.48)	84	1	[90]
Acetate, ethanol	Electrode, ethanol	C6 (6.6)	36.2	2	[91]
CO_2, CO	Electrode, CO	C6 (0.78)	15.4	44	[74]

[a] Electrons recovered in C6 compared with all identified liquid organic chemicals.
[b] Not available.

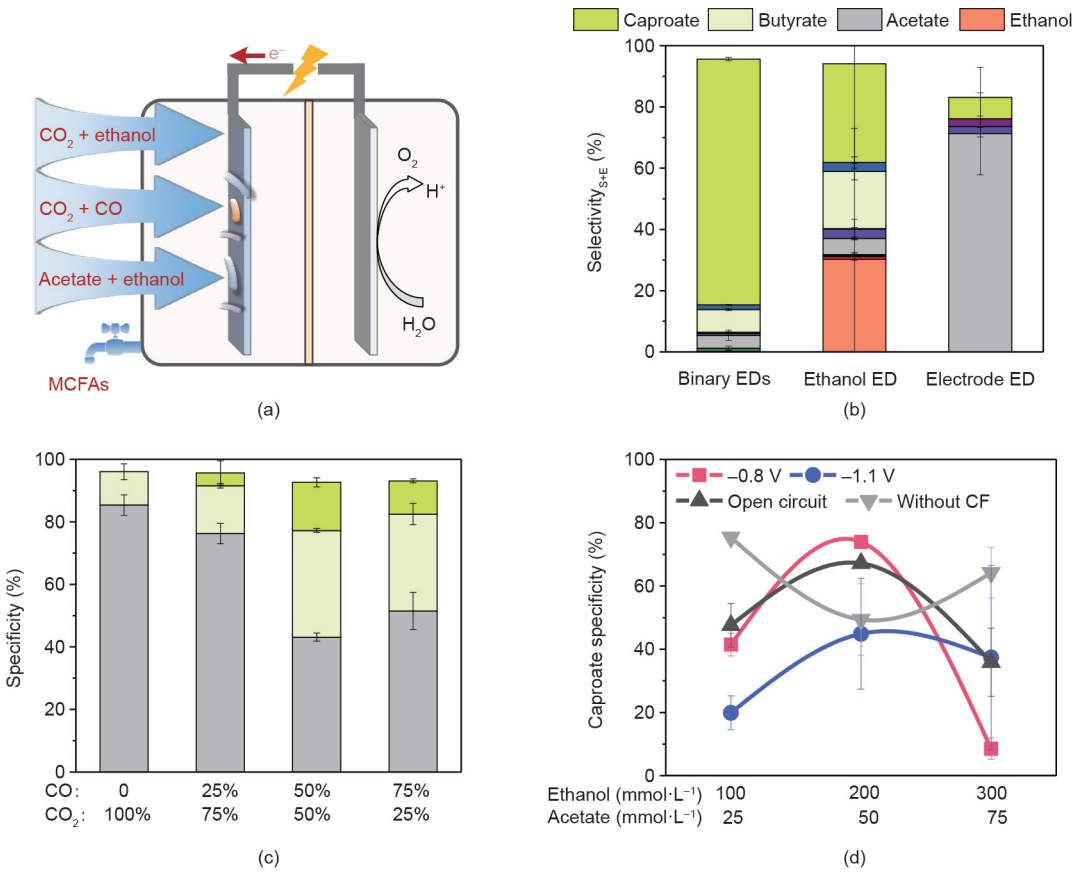

Fig. 2. (a) Strategy using multiple-EDs in MES for MCFAs production. (b) Concurrent use of electrode and ethanol as EDs in MES, with higher selectivity of caproate than that using ethanol or electrode alone. Reproduced from Ref. [90] with permission of Elsevier Ltd., © 2020. (c) Concurrent use of electrode and CO as EDs in MES; maximum caproate specificity achieved with 50% CO. Reproduced from Ref. [74] with permission of American Chemical Society, © 2020. (d) The effect of an additional acclimated cathode on mixed culture CE process under different substrate concentrations. Reproduced from Ref. [91] with permission of Elsevier Ltd., © 2020. CF: carbon felt; $Selectivity_{S+E}$: selectivity based on the substrate and electrode.

hydrolysis, primary fermentation, and CE (Fig. 3(a)). MCFAs production in MES with CO_2 as the sole carbon source is challenging, requiring processes of acetogenesis, solventogenesis, and CE (Fig. 3(b)) [22,49]. In this section, the challenges for MCFAs production in MES are comprehensively discussed.

4.1. Methanogenesis inhibition

In open culture fermentation, methanogens can consume substrates or intermediates (i.e., acetate and H_2) of the CE process to decrease overall yield of MCFAs [32,102]. Methanogens can thermodynamically affect the CE process, by consuming H_2 and CO_2 [32,103], as methane formation (ΔG = −130 kJ) is even thermodynamically more favorable than acetate formation (ΔG = −5 5 kJ). Methanogens are anaerobic prokaryotes, including aceticlastic methanogens (e.g., *Methanosaeta*), facultative aceticlastic methanogens (e.g., *Methanosarcina*), and hydrogenotrophic methanogens (e.g., *Methanobacterium*, *Methanoculleus*, and *Methanobrevibacter*) [104]. The current cost of CH_4 production via power-to-H_2-to-CH_4 is estimated at ~1.2 EUR·kg^{-1} CH_4 (assuming efficiency of H_2-to-CH_4 conversion of 90%), making this not an economic choice, given that the current wholesale price of natural gas is below 0.25 EUR·kg^{-1} [105].

Methanogenesis inhibition includes different approaches to reduce methanogenesis by suppressing the activity of methanogens. Methanogens can be manipulated with the addition of chemical inhibitors [106], redox mediators [107], or conductive materials, such as magnetite [108] and powder activated carbon [109]. 2-bromoethanesulfonate, is a frequently-used inhibitor but its addition increases overall cost [73], the effect is short-lived due to microbial degradation [110,111], and its addition may suppress branched MCFAs production [106]. An alternative strategy is to modify the operation parameters, as methanogens can be inhibited with low pH, low temperature, decreased sludge retention time (SRT), or decreased CO_2 and H_2 partial pressures [81]. It should be noted that these approaches for methanogenesis inhibition could potentially apply to MES as well as traditional fermentation.

4.2. ATP limitations of acetogen

Acetogens are defined as having a reductive acetyl-CoA pathway for CO_2 assimilation, with acetate formation relatively irrelevant [112]. Acetogens can work independently of other microbes. The model acetogen *Acetobacterium woodii* (*A. woodii*) is capable of hydrogen generation from fermentation and hydrogen consumption for CO_2 reduction in one cell, making the fermentation process energetically possible and independent from syntrophic components [113]. Acetogen is also of ecological importance. The WLP serves as more of an electron sink than a complete respiratory chain, so it can be potentially combined with the fermentation of various substrates to achieve redox balance [112].

WLP is the most suitable pathway for microbial CO_2 fixation under anaerobic condition [114], based on ATP consumption [4]. However, acetogen generally can only produce chemicals whose biosynthesis is coupled to ATP generation [115], due to its ATP limitation [98], and any diversion of acetyl-CoA flux away from acetate can further increase the ATP cost [98]. For instance, the

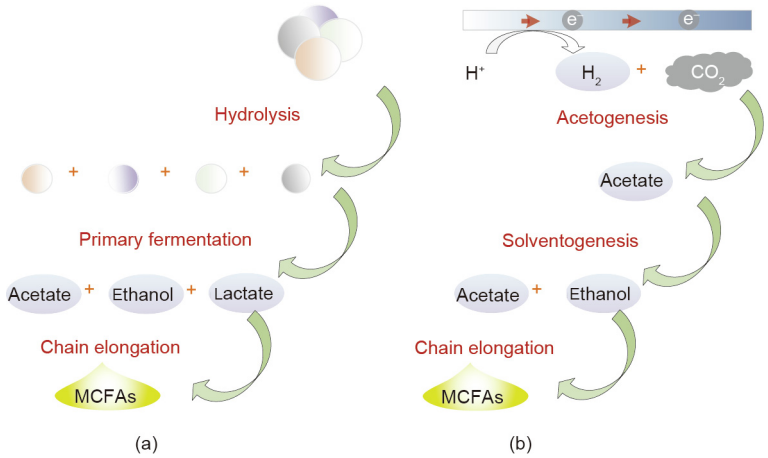

Fig. 3. MCFAs production via (a) conventional fermentation from complex organic waste and in (b) MES from CO_2.

yield of acetate can be 23.6 mol per 100 mol H_2, but the theoretical upper limit for butanol is only 0.2 mol [115]. Also, each mole of acetate produced supports the generation of only 0.25 to 0.63 mol ATP, leading to low overall yields and slow growth rates [116]. Given the energy limitations of this process, acetogen metabolism is controlled by thermodynamics through metabolite levels rather than at the energetically expensive posttranslational level [117].

With ATP limitation, the use of acetogen to produce chemicals with longer carbon chain, for example, MCFAs, is challenging, and products in MES reactors are mostly dominated by acetate. It is thus urgent to solve the problem of ATP limitation of acetogen in order to increase the volumetric production rate by increasing the biomass densities and expand the spectrum of chemicals by regulating the carbon flux. Strategies to address the ATP limitation of acetogen in conventional fermentation include the use of additional EAs for energy conservation and improving the yields of acetogens through mixotrophy (Fig. 4) [116,118–120].

As EA, nitrate is more energy favorable than CO_2 [116]. For example, nitrate addition improved the growth rate, biomass density, and ATP/adenosine diphosphate (ADP) ratio of *C. ljungdahlii* (Fig. 4(a)) [116]. However, *Moorella thermoacetica* (*M. thermoacetica*) cannot respire on nitrate while reducing CO_2, and *A. woodii* is unable to metabolize nitrate [116]. Negative effects of nitrate addition for energy conservation include the consumption of electrons, accumulation of ammonium, and the increase of pH. Nitrate addition can also lead to differences in growth and production rates, probably due to stochastic inhibition events [121].

WLP is active during autotrophic as well as heterotrophic growth [96], and many acetogens are capable of sourcing electrons from various EDs, including sugars, C1 compounds such as formate and CO, primary fermentation products such as alcohols and SCFAs, or methylated nitrogen compounds such as glycine and betaine, in addition to H_2. Mixotrophy of acetogens allow the concurrent utilization of both organic and inorganic substrates (Fig. 4(b)), by the decoupling of ATP production from CO_2 fixation [122]. Mixotrophy of microbes other than acetogens, such as cyanobacteria and microalgae, has also been reported [123,124]. For example, mixotrophic growth of *Chlorella sorokiniana* on SCFAs resulted in increased biomass productivity [125]. However, among the known CO_2 fixation pathways, only WLP is capable of stoichiometric conversion of six carbon sugars into three moles of acetate [126]. Mixotrophy is a trait of tested acetogens, including *A. Woodii*, *C. ljungdahlii*, *Clostridium autoethanogenum* (*C. autoethanogenum*), *Eubacterium limosum* (*E. limosum*), and *M. thermoacetica* [118]. Mixotrophy of acetogens can increase energy conservation by providing an alternative energy source for chemical production from CO_2 utilization [127]. Mixotrophy of acetogens can also improve the product yields from the fermentation of organic matters, as the released CO_2 can be fixed through WLP. In microbial fermentation, the conversion of pyruvate decarboxylation to acetyl-CoA with the release of CO_2 can cause at least 33% loss of the sugar-substrate carbon. The carbon efficiency can be further decreased during the production of more reduced chemicals [128], for example, with a theoretical yield of 51% for ethanol [118].

The types of organic substrates utilized can affect the mixotrophy of acetogens. For instance, fructose was found to be the only carbohydrate utilized for mixotrophy of 17 different acetogens [122]. Glucose is not expected to complete carbon fixation, given produced reducing equivalents approximately equal to the requirement of released CO_2 fixation, but some reducing equivalents will be consumed by biomass generation and maintenance [122]. Products with different degrees of reduction can affect the mixotrophy of acetogens. For more reduced products, for example, butanol, more reducing equivalents are directed towards product formation, so less CO_2 is fixed. H_2-enhanced mixotrophy is proposed to reduce overall CO_2 emissions while generating more reduced products from the fermentation of organic matters [118].

For mixotrophy of acetogens, the CO_2 produced during glycolysis and the CO_2 uptake via WLP should be balanced to improve the product yields from the fermentation of organic matters. In addition, ATP production during glycolysis should be appropriate to the consumption for CO_2 uptake, cell growth, and chemical production. However, a critical concern for mixotrophy of acetogens is carbon catabolic repression (CCR), which is accompanied by the downregulation of genes for WLP in response to the presence of energetically favorable organic substrates. The occurrence of CCR varies between species of acetogens and culture conditions [129], and many species can use C1 gases and carbohydrates simultaneously [126]. However, CCR was observed when cofeeding *M. thermoacetica* with glucose and CO_2 [119]. Optimizing the culture conditions by feeding sugars with kinetically limiting concentrations could shift carbon substrate preferences towards CO_2 (Fig. 4(c)) [119]. Similarly, a recent study observed the co-uptake of xylose and CO by *C. autoethanogenum* for acetate production (Fig. 4(d)), under xylose-limited conditions [120]. The integration of MCFAs production with mixotrophy of acetogens was highlighted in an early review paper [126]. This proposal is based on a fact that under anaerobic condition, the fermentation of organic substrates is expected to generate efficient ATP for WLP, and products from WLP can serve as substrates for the RBO pathway. The concept of electrode-enhanced mixotrophy is proposed here,

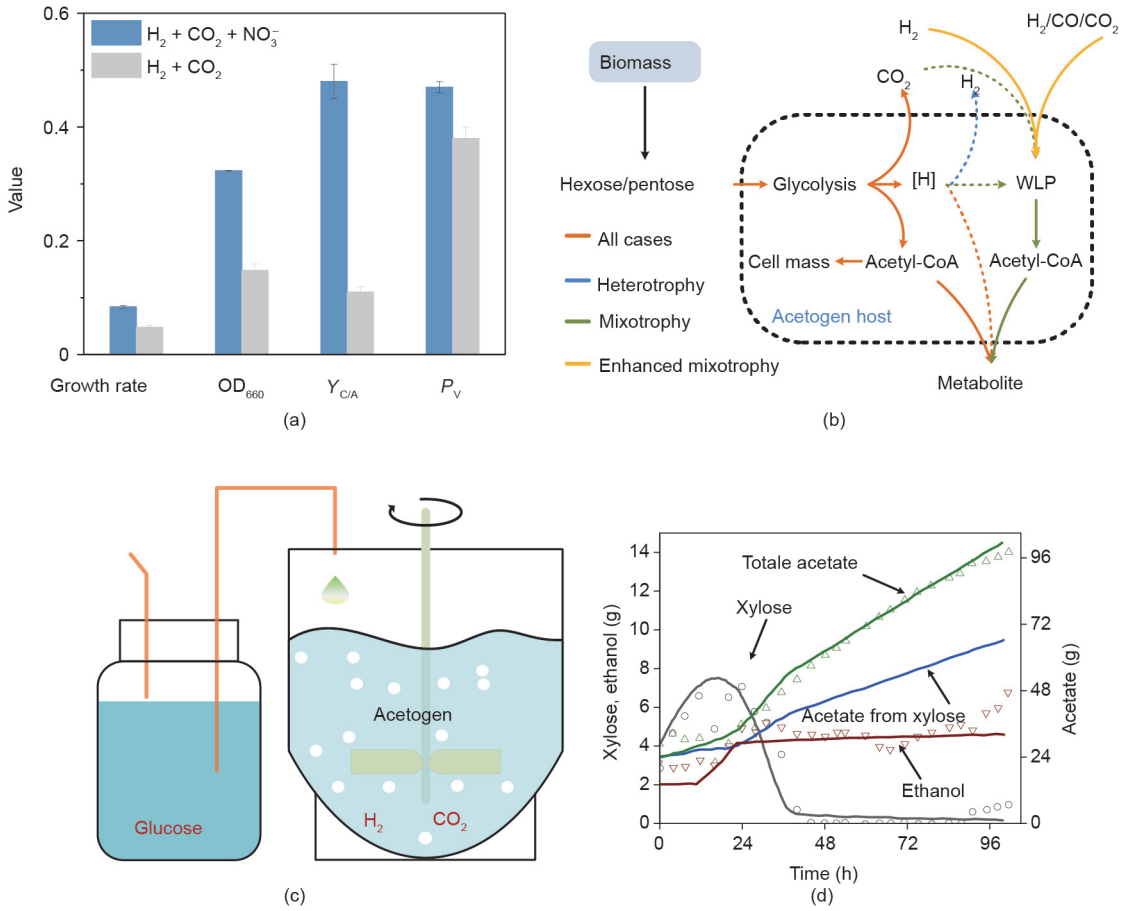

Fig. 4. Strategies to overcome the ATP limitations of acetogen. (a) Nitrate as additional EA provides reducing equivalents for ATP generation in *C. ljungdahlii* [116] (OD_{660}: optical density at 660 nm; $Y_{C/A}$: cellular yield per acetate; P_V: volumetric productivity; the unit of growth rate and P_V is h^{-1} and mmol $C{\cdot}h^{-1}{\cdot}L^{-1}$, respectively). (b) Different modes of fermentation including heterotrophy, mixotrophy, and H_2-enhanced mixotrophy. Reproduced from Ref. [118], with permission. (c) Feeding sugars at kinetically limiting concentrations to conquer CCR [119]. (d) Co-uptake of xylose and CO for acetate production under xylose-limited conditions. Reproduced from Ref. [120], with permission.

which might improve overall performance, in terms of CO_2 fixation and MCFAs production, although this has not been demonstrated experimentally (Fig. 5). In microbial electrochemical reactors, a solid electrode is used as the ED for extracellular or potential intracellular H_2 production [130]. However, the carbon flux of WLP may be essentially different in conventional fermentation using H_2 or in MES with an electrode as EDs, in terms of production rate and product spectrum [131,132].

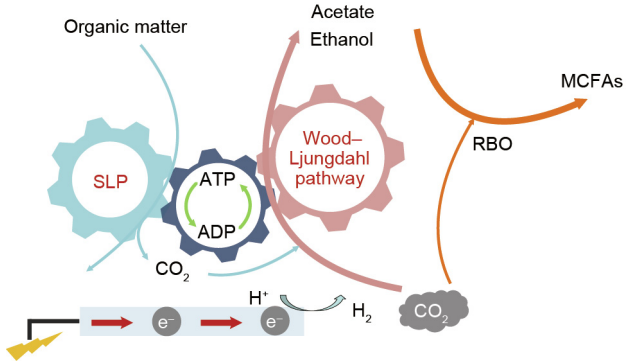

Fig. 5. Concept of electrode-enhanced mixotrophy for enhanced MCFAs production. SLP: substrate level phosphorylation. CO_2 released from SLP can be refixed by WLP. A proportion of CO_2 was used to supporting the growth of MCFAs-forming bacteria.

4.3. Limited EDs produced via solventogenesis for CE

Solventogenesis is a microbial process for the fermentative production of solvents (e.g., alcohols) [133]. Solventogenesis capacity has been tested with stains belonging to the genus *Clostridium*, including *Clostridium aceticum*, *C. ljungdahlii*, *C. carboxidivorans*, *C. ragsdalei*, and *C. autoethanogenum* [134]. The acetaldehyde ferredoxin oxidoreductase (AOR)–aldehyde/alcohol dehydrogenase (AdhE)-based acetate reduction to ethanol may play a more critical role for ethanol synthesis in *C. ljungdahlii*, compared with that of AdhE-based acetyl-CoA conversion [135]. Some acetogenic strains can grow well with a wider pH range from 5.4 to 9.8 [136]. However, mildly acidic pH (< 5) is critical for solventogenesis, in addition to the accumulation of carboxylate [133]. For example, decreased pH value increases the diffusion of undissociated acetic acid into cells, leading to increased uncoupling of the proton motive force (PMF), while a shift of products from acetate toward ethanol for energy conservation can achieve ATP homeostasis with low uncoupling of the PMF [137]. Longer-chain alcohols can be produced from syngas with multiple reactions. For example, a co-culture study found initial production of ethanol and acetate by *C. ljungdahlii*, followed by production of longer-chain carboxylates by *C. kluyveri*, and then production of longer-chain alcohols via the reduction of corresponding carboxylates by *C. ljungdahlii* [138].

Microbial electrochemical solventogenesis generally produces limited alcohols [38,88], limiting the follow-up CE process for

MCFAs production due to the lack of sufficient EDs [32]. Alcohols can be produced from CO_2 as the sole carbon source in MES. The use of a gas diffusion cathode can stimulate the solventogenesis process, although MCFAs were not produced [139]. Alcohols can also be produced by the reduction of SCFAs in MES, and optimized SCFAs loading and low-pH condition are essentially required to increase performance [140]. In a dual-chamber bioreactor and feeding with 6 $g{\cdot}L^{-1}$ acetate, microbial electrochemical CE was achieved with maximum concentrations of 0.74 $g{\cdot}L^{-1}$ for caproate, 0.26 $g{\cdot}L^{-1}$ for butyrate, 0.04 $g{\cdot}L^{-1}$ for caprylate, and 0.03 $g{\cdot}L^{-1}$ for ethanol [86]. In another dual-chamber electrochemical bioreactor fed with acetate and CO_2, butyrate was produced without the detection of alcohols, with production of propionate and caproate in only trace amounts [87]. A single chamber MES reactor was constructed for the reduction of both acetate and butyrate, and ethanol, methanol, and propanol were identified as the main products with a trace amount of butanol, with a reduction efficiency of about 50% for acetate, and 18%–40% for butyrate [141]. Thus, the maximum total concentration of alcohols was only (1.15 ± 0.07) grams of chemical oxygen demand per liter ($g\text{-}COD{\cdot}L^{-1}$) for substrate loading of 8.0 $g\text{-}COD{\cdot}L^{-1}$ [141]. Microbial electrochemical solventogenesis and thus the overall production of MCFAs can be affected by the presence of mediators [142], the types of SCFAs, and the process of microbial adaption [89]. Collectively, limited EDs are produced by solventogenesis in MES, with little or no production of MCFAs. The *in-situ* production and consumption of alcohols might lead to low accumulation. To achieve a higher caproate specificity via the CE process, a high substrate ratio of ethanol to acetate (e.g., 4:1) is required [143].

5. Electrochemical technologies enhanced MCFAs production

Most recent studies of MES for MCFAs production have used chemically defined artificial media. The bioconversion of practical organic matter and syngas for MCFAs production is a complex process, and electrochemical technologies can be potentially applied to enhance the production of MCFAs at different stages.

Tuning of the carbon-to-energy-to-cofactor ratios is crucial for the biorefinery of practical organic wastes using the reactor microbiome. The use of a single substrate naturally imposes stoichiometric constraints on available carbons, energy, and redox cofactors [119]. It is difficult to simultaneously satisfy all demands at the appropriate ratios using a single substrate, such as using glucose as the sole carbon source, which can lead to biosynthetic imbalance and suboptimal product yield [144]. Glucose can produce NADPH more directly; however, this is an energy deficient substrate. Acetate can support acetyl-CoA and ATP generation, but not NADPH generation [119]. The tuning of carbon-to-energy-to-cofactor ratios can help to alleviate protein burdens, since different substrates have different entry points to metabolic processes [119]. For example, limited quantities of glucose can be supplemented in xylose-based cultivation of *S. cerevisiae*, allowing for improved efficiency [145]. As EAs and EDs for MCFAs production, carboxylates and alcohols can be produced from organic wastes after pretreatment and primary fermentation process. However, MCFAs production from practical organic wastes might be restricted by an unbalanced ED/EA ratio. For instance, EAs are not sufficient after fermentation of sugarcane juice [146], while EDs are required with the fermentation of sludge [147]. The energy supply can be regulated with a poised electrode in MES reactors, and the available carbons and redox cofactors can be adjusted using co-substrates addition. For instance, co-fermentation and co-digestion have been proposed to increase H_2 or CH_4 recovery with increased nutrient balance, diluted inhibitors, and increased solid reduction [148,149].

Syngas generated from the thermal gasification, industrial emission, and electrolytic CO_2 reduction could be used to produce carboxylates and alcohols by syngas fermentation. The bioconversion of syngas could increase the CO_2 utilization potential, which is still relatively small compared to global CO_2 emissions [2]. The bioconversion of syngas from thermal gasification of non-alimentary feedstock can use biomass in its entirety, including lignin (10%–35% of lignocellulosic biomass), which is highly resistant to direct microbial degradation [96]. There are limited microbes that can be used to produce MCFAs and derivatives from syngas. For example, *C. carboxidivorans* and *E. limosum* can generate caproate [150], and *C. carboxidivorans* can form hexanol from syngas [151]. Bioconversion of CO could release CO_2, so additional EDs will be required for re-assimilation of these CO_2 [152]. For example, H_2 supply reduced carbon loss by releasing CO_2 and increasing the carbon flux towards ethanol for gas-fermentation with *C. autoethanogenum* [153]. However, hydrogenases and H_2 uptake may be inhibited by the presence of excess CO [117]. The co-fermentation of syngas with glucose also increased the conversion efficiencies [154]. A very recent study has suggested that a poised electrode as additional ED could increase the acetate production and biomass growth compared with traditional syngas fermentation, while the detail mechanism have not been fully clarified [155].

Electrochemical technologies can be potentially used to promote the bioconversion of wastes for MCFAs production at different process stages (Fig. 6). For example, electrochemical technologies can be used for ammonia and sulphur recovery from organic waste valorization with pretreatment, *in situ*, and side stream approaches [9,156]. The recovery of ammonia and sulphur as nutrients can increase economic benefits and help to increase MCFAs production by decreasing microbial toxicity and reducing unwanted flux of carbon or electrons [32]. Recovery of high-concentration charged carboxylates can be achieved with electrolytic extraction, while stripping of alcohols can be potentially obtained by electrocatalytic hydrogen evolution. In addition, the use of MES as a standalone technology can potentially use CO_2 to produce both acetate and ethanol with a tunable ratio. Microbial electrochemical solventogenesis can be used to adjust the ED/EA proportion before use as feedstock for CE process. For *in situ* enhancement, the microbial activity of primary fermentation, syngas fermentation, and CE process might be stimulated with electrochemical technologies by electrical stimulation, parameter regulation, and microbial synergistic interaction [157]. For instance, zerovalent iron, activated carbon, and biochar can enhance MCFAs production and selectivity by declining the ORP, decreasing the lag phase, altering the microbial community structure, and acting as an electron shuttle to improve electron transfer efficiency [158,159].

6. Research gaps and perspectives

Collectively, producing more valuable MCFAs will further promote the development of MES technologies with high economic return. Combining traditional bioconversion with MES could potentially improve the overall performance. In this section, the existing research gaps and future perspectives are identified.

6.1. Multi-stage reactions

MCFAs are produced via multi-stage reactions. The design of these multi-stage reactions depends on the types of wastes utilized, which may contain or can produce different EAs and EDs at different ratios. The involved microbes and optimized conditions for these reactions can differ, and functional division could be accomplished with the attachment of biofilm, or with use of a

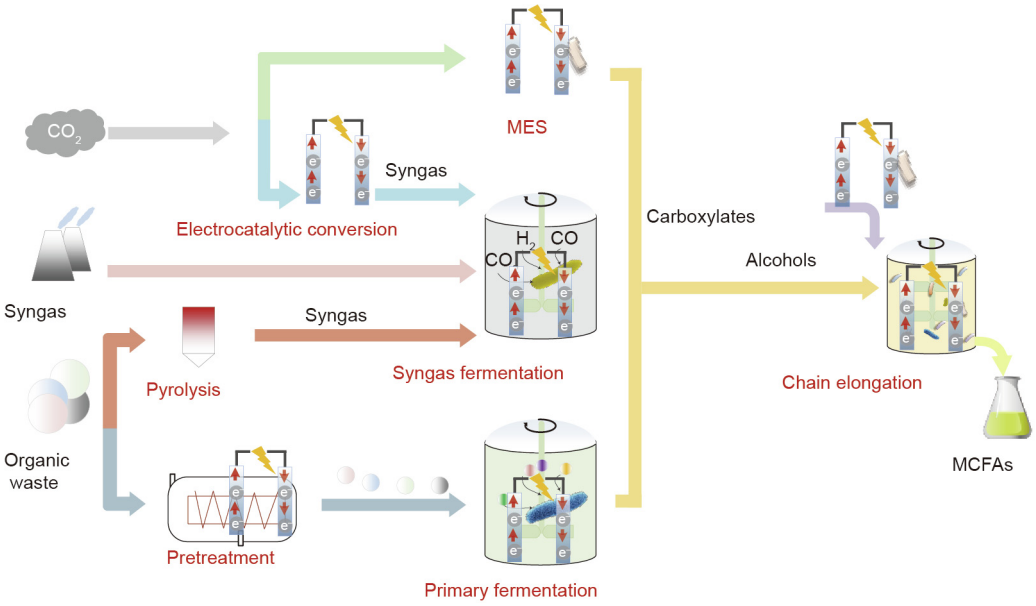

Fig. 6. Potential applications of electrochemical technologies to enhance bioconversion of wastes with MCFAs production.

second reactor. Also, the characteristics of the catholyte in MES, containing EAs and/or EDs, can further affect the CE process for MCFAs production. For example, mediators secreted by electrotrophic microbes could be used by MCFAs-forming microbes for redirected carbon and electron flux.

The production rate and consumption rate of intermediates that are used as EAs and/or EDs for MCFAs production should be compatible. Overall, the concentrations and types of intermediate products should be regulated for multi-stage reactions. For instance, the accumulation of acetate at a high concentration (> 10 $g \cdot L^{-1}$) can theoretically promote the efficiency of MES as a standalone technology for application in the food industry [17]. However, a high concentration of acetate might cause microbial toxicity for some multi-stage reactions [154]. Many microbes are incapable of direct electron transfer (DET), and bioconversion based on DET is constrained by very low current density [160]. The use of H_2 as a mediator raises safety issues due to its low solubility. To increase the potential of energy storage [161], and to balance charges between reduction and electromigration [162], formate might be a better intermediate product than acetate [163]. However, formate only carries two electrons per carbon, so the re-release of the initially reduced CO_2 for use as an intermediate for bioconversion would be restrictive [14].

6.2. Substrates supply and products extraction

The supply of substrates and the extraction of products are both important considerations in the design of MES reactors. The sparging of gaseous substrate with low solubility can increase mass transfer, but this will potentially reduce the accumulation of alcohols with non-trivial vapor pressure to thus restrict MCFAs production. Extraction of products is important for MCFAs production, as the inhibitory action is generally increased with the increase of carbon chain. Also, the toxicity of MCFAs can be enhanced by low pH and the presence of ethanol [55]. It is important to increase the robustness under toxic environment with novel reactors [147]. In addition, extraction and separation units may require a more compact structure, while maintaining the capability to selectively extract and perform phase separation of MCFAs [164,165].

6.3. Electrochemical effects on microbes and pathways

The involved microbes and pathways for MCFAs production in MES reactors can be regulated with the exchange of mass and electrons. The integration of electrochemistry into bioreactors could affect mass transfer and thus the overall bioconversion process. Gradients of substrates and products can form from the bulk liquid towards the surface of the electrode. The creation of an electrical field can affect the mass transfer of charged ions, for example, SCFAs and MCFAs in anionic form. The insertion of a solid electrode with forced EET could reduce the growth yields of some specific microbes, including *C. autoethanogenum* [166] and *C. pasteurianum* [167]. Also, the insertion of an electrode could affect microbial gene expression, as the expression profile of cathodic biofilm can be remarkably different from that with H_2 as ED [168]. There can be significant variation in the reactor microbiome, as microbial communities attached on electrodes can be distinct from those in the mixed liquor [156]. For instance, biofilm growth on the electrode allows the retention of slow-growing microbes. In addition, the insertion of a conductive electrode with a large surface area can stimulate electron exchange between microbes, that is, interspecies electron transfer (IET), to create mutualistic or nonmutualistic interactions [169]. It is necessary to determine changes in metabolic pathways under dynamic and complex conditions in an MES reactor for MCFAs production. Approaches for rational microbiome engineering have been proposed to manipulate microbial ecosystems, by integrating basic scientific discovery with engineering [170]. Metagenomic analysis is helpful to determine the key genes and their regulation, and this approach should be performed together with other investigative strategies such as microscopy and 13C-labeling analyses [168].

7. Conclusions

Substantial efforts have been made to produce MCFAs via conventional fermentation or via microbial electrochemical regulation of carbon flux in MES. However, MCFAs production from CO_2 in MES is limited by methanogenesis inhibition, ATP limitations of acetogens, and limited production of EDs. Opportunity and challenge are presented both in conventional fermentation and MES

with the application of multiple-EDs. Electrochemical approaches can be developed to promote the bioconversion of waste for MCFAs production at different process stages. Further studies are required on the design of multi-stage reactions, substrate supply, product extraction, and electrochemical effects on microbes and pathways.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (51908131), Special Fund of State Key Joint Laboratory of Environment Simulation and Pollution Control (19K05ESPCT), the Chinese Academy of Sciences (CAS) Key Laboratory of Environmental and Applied Microbiology & Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences (KLCAS-2019-1), and the Fujian Provincial Natural Science Foundation of China (2020J01563).

Compliance with ethics guidelines

Na Chu, Wen Hao, Qinglian Wu, Qinjun Liang, Yong Jiang, Peng Liang, Zhiyong Jason Ren, and Raymond Jianxiong Zeng declare that they have no conflict of interest or financial conflicts to disclose.

References

[1] De Luna P, Hahn C, Higgins D, Jaffer SA, Jaramillo TF, Sargent EH. What would it take for renewably powered electrosynthesis to displace petrochemical processes? Science 2019;364(6438):eaav3506.
[2] Zhang Z, Pan SY, Li H, Cai J, Olabi AG, Anthony EJ, et al. Recent advances in carbon dioxide utilization. Renew Sustain Energy Rev 2020;125:109799.
[3] Chen Z, Zhou J, Wang Y, Wang Y. Nano on micro: tuning microbial metabolisms by nano-based artificial mediators to enhance and expand production of biochemicals. Curr Opin Biotechnol 2020;64:161–8.
[4] Liu Z, Wang K, Chen Y, Tan T, Nielsen J. Third-generation biorefineries as the means to produce fuels and chemicals from CO_2. Nat Catal 2020;3(3):274–88.
[5] Kaza SY, Lisa C, Bhada-Tata P, Van Woerden F. What a waste 2.0: a global snapshot of solid waste management to 2050 [Internet]. Washington, DC: The World Bank Group; 2018 Sep 20 [cited 2020 Jul 15]. Available from: https://www.worldbank.org/en/news/infographic/2018/09/20/what-a-waste-20-a-global-snapshot-of-solid-waste-management-to-2050.
[6] Tan W, Xi B, Zhao X, Dang Q. Emerging views on the overall process treatment of municipal domestic waste for the sustainable use of landfills in China. Engineering 2020;6(7):733–5.
[7] Qu JH, Wang HC, Wang KJ, Yu G, Ke B, Yu HQ, et al. Municipal wastewater treatment in China: development history and future perspectives. Front Environ Sci Eng 2019;13(6):88.
[8] Grim RG, Huang Z, Guarnieri MT, Ferrell JR, Tao L, Schaidle JA. Transforming the carbon economy: challenges and opportunities in the convergence of low-cost electricity and reductive CO_2 utilization. Energy Environ Sci 2020;13(2):472–94.
[9] Chu N, Liang Q, Jiang Y, Zeng RJ. Microbial electrochemical platform for the production of renewable fuels and chemicals. Biosens Bioelectron 2020;150:111922.
[10] Rabaey K, Rozendal RA. Microbial electrosynthesis—revisiting the electrical route for microbial production. Nat Rev Microbiol 2010;8(10):706–16.
[11] Nevin KP, Woodard TL, Franks AE, Summers ZM, Lovley DR. Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. mBio 2010;1:e00103-10-e-10.
[12] Jiang Y, Su M, Zhang Y, Zhan G, Tao Y, Li D. Bioelectrochemical systems for simultaneously production of methane and acetate from carbon dioxide at relatively high rate. Int J Hydrogen Energy 2013;38(8):3497–502.
[13] Barbosa SG, Peixoto L, Alves JI, Alves MM. Bioelectrochemical systems (BESs) towards conversion of carbon monoxide/syngas: a mini-review. Renew Sustain Energy Rev 2021;135:110358.
[14] Salimijazi F, Kim J, Schmitz AM, Grenville R, Bocarsly A, Barstow B. Constraints on the efficiency of engineered electromicrobial production. Joule 2020;4(10):2101–30.
[15] Chen H, Simoska O, Lim K, Grattieri M, Yuan M, Dong F, et al. Fundamentals, applications, and future directions of bioelectrocatalysis. Chem Rev 2020;120(23):12903–93.
[16] Lee SY, Oh YK, Lee S, Fitriana HN, Moon M, Kim MS, et al. Recent developments and key barriers to microbial CO_2 electrobiorefinery. Bioresour Technol 2021;320:124350.
[17] Bian B, Bajracharya S, Xu J, Pant D, Saikaly PE. Microbial electrosynthesis from CO_2: challenges, opportunities and perspectives in the context of circular bioeconomy. Bioresour Technol 2020;302:122863.
[18] Jiang Y, Chu N, Zhang W, Ma J, Zhang F, Liang P, et al. Zinc: a promising material for electrocatalyst-assisted microbial electrosynthesis of carboxylic acids from carbon dioxide. Water Res 2019;159:87–94.
[19] Krieg T, Sydow A, Faust S, Huth I, Holtmann D. CO_2 to terpenes: autotrophic and electroautotrophic alpha-humulene production with *Cupriavidus necator*. Angew Chem Int Ed Engl 2018;57(7):1879–82.
[20] Mayr JC, Grosch JH, Hartmann L, Rosa LFM, Spiess AC, Harnisch F. Resting Escherichia coli as chassis for microbial electrosynthesis: production of chiral alcohols. ChemSusChem 2019;12(8):1631–4.
[21] Chatzipanagiotou KR, Jourdin L, Buisman CJN, Strik DPBTB, Bitter JH. CO_2 conversion by combining a copper electrocatalyst and wild-type microorganisms. ChemCatChem 2020;12(15):3900–12.
[22] Vassilev I, Kracke F, Freguia S, Keller J, Krömer JO, Ledezma P, et al. Microbial electrosynthesis system with dual biocathode arrangement for simultaneous acetogenesis, solventogenesis and carbon chain elongation. Chem Commun 2019;55(30):4351–4.
[23] Jiang Y, Zeng RJ. Expanding the product spectrum of value added chemicals in microbial electrosynthesis through integrated process design—a review. Bioresour Technol 2018;269:503–12.
[24] Ter Heijne A. Bioelectrochemistry for flexible control of biological processes. Environ Sci Ecotechnol 2020;1:100011.
[25] Jiang Y, Lu L, Wang H, Shen R, Ge Z, Hou D, et al. Electrochemical control of redox potential arrests methanogenesis and regulates products in mixed culture electro-fermentation. ACS Sustain Chem Eng 2018;6(7):8650–8.
[26] Salehizadeh H, Yan N, Farnood R. Recent advances in microbial CO_2 fixation and conversion to value-added products. Chem Eng J 2020;390:124584.
[27] Engel M, Bayer H, Holtmann D, Tippkotter N, Ulber R. Flavin secretion of *Clostridium acetobutylicum* in a bioelectrochemical system—is an iron limitation involved? Bioelectrochemistry 2019;129:242–50.
[28] Mayr JC, Rosa LFM, Klinger N, Grosch JH, Harnisch F, Spiess AC. Response-surface-optimized and scaled-up microbial electrosynthesis of chiral alcohols. ChemSusChem 2020;13(7):1808–16.
[29] Jiang Y, Dong W, Xin F, Jiang M. Designing synthetic microbial consortia for biofuel production. Trends Biotechnol 2020;38(8):828–31.
[30] Cavalcante WA, Leitão RC, Gehring TA, Angenent LT, Santaella ST. Anaerobic fermentation for *n*-caproic acid production: a review. Process Biochem 2017;54:106–19.
[31] Venkateswar Reddy M, Kumar G, Mohanakrishna G, Shobana S, Al-Raoush RI. Review on the production of medium and small chain fatty acids through waste valorization and CO_2 fixation. Bioresour Technol 2020;309:123400.
[32] Wu Q, Bao X, Guo W, Wang B, Li Y, Luo H, et al. Medium chain carboxylic acids production from waste biomass: current advances and perspectives. Biotechnol Adv 2019;37(5):599–615.
[33] De Leeuw KD, Buisman CJN, Strik DPBTB. Branched medium chain fatty acids: iso-caproate formation from iso-butyrate broadens the product spectrum for microbial chain elongation. Environ Sci Technol 2019;53(13):7704–13.
[34] Zhou M, Yan B, Wong JWC, Zhang Y. Enhanced volatile fatty acids production from anaerobic fermentation of food waste: a mini-review focusing on acidogenic metabolic pathways. Bioresour Technol 2018;248:68–78.
[35] Jouny M, Luc W, Jiao F. General techno–economic analysis of CO_2 electrolysis systems. Ind Eng Chem Res 2018;57(6):2165–77.
[36] Andersen SJ, Candry P, Basadre T, Khor WC, Roume H, Hernandez-Sanabria E, et al. Electrolytic extraction drives volatile fatty acid chain elongation through lactic acid and replaces chemical pH control in thin stillage fermentation. Biotechnol Biofuels 2015;8(1):221.
[37] Xu J, Hao J, Guzman JJL, Spirito CM, Harroff LA, Angenent LT. Temperature-phased conversion of acid whey waste into medium-chain carboxylic acids via lactic acid: no external e-donor. Joule 2018;2(2):280–95.
[38] Chen H, Dong F, Minteer SD. The progress and outlook of bioelectrocatalysis for the production of chemicals, fuels and materials. Nat Catal 2020;3(3):225–44.
[39] Noori MT, Vu MT, Ali RB, Min B. Recent advances in cathode materials and configurations for upgrading methane in bioelectrochemical systems integrated with anaerobic digestion. Chem Eng J 2020;392:123689.
[40] Dessì P, Rovira-Alsina L, Sánchez C, Dinesh GK, Tong W, Chatterjee P, et al. Microbial electrosynthesis: towards sustainable biorefineries for production of green chemicals from CO_2 emissions. Biotechnol Adv 2021;46:107675.
[41] Prévoteau A, Carvajal-Arroyo JM, Ganigué R, Rabaey K. Microbial electrosynthesis from CO_2: forever a promise? Curr Opin Biotechnol 2020;62:48–57.
[42] Sahoo PC, Pant D, Kumar M, Puri SK, Ramakumar SSV. Material–microbe interfaces for solar-driven CO_2 bioelectrosynthesis. Trends Biotechnol 2020;38(11):1245–61.
[43] Flexer V, Jourdin L. Purposely designed hierarchical porous electrodes for high rate microbial electrosynthesis of acetate from carbon dioxide. Acc Chem Res 2020;53(2):311–21.
[44] LaBelle EV, Marshall CW, May HD. Microbiome for the electrosynthesis of chemicals from carbon dioxide. Acc Chem Res 2020;53(1):62–71.
[45] Zakaria BS, Dhar BR. Progress towards catalyzing electro-methanogenesis in anaerobic digestion process: fundamentals, process optimization, design and scale-up considerations. Bioresour Technol 2019;289:121738.
[46] Sun M, Zhai LF, Mu Y, Yu HQ. Bioelectrochemical element conversion reactions towards generation of energy and value-added chemicals. Pror Energy Combust Sci 2020;77:100814.
[47] Lin R, Deng C, Zhang W, Hollmann F, Murphy JD. Production of bio-alkanes from biomass and CO_2. Trends Biotechnol 2021;39(4):370–80.

[48] Liu X, Yu X. Enhancement of butanol production: from biocatalysis to bioelectrocatalysis. ACS Energy Lett 2020;5(3):867–78.
[49] Vassilev I, Hernandez PA, Batlle-Vilanova P, Freguia S, Krömer JO, Keller J, et al. Microbial electrosynthesis of isobutyric, butyric, caproic acids, and corresponding alcohols from carbon dioxide. ACS Sustain Chem Eng 2018;6(7):8485–93.
[50] Jiang Y, May HD, Lu L, Liang P, Huang X, Ren ZJ. Carbon dioxide and organic waste valorization by microbial electrosynthesis and electro-fermentation. Water Res 2019;149:42–55.
[51] Spirito CM, Richter H, Rabaey K, Stams AJ, Angenent LT. Chain elongation in anaerobic reactor microbiomes to recover resources from waste. Curr Opin Biotechnol 2014;27:115–22.
[52] Kim H, Jeon BS, Sang BI. An efficient new process for the selective production of odd-chain carboxylic acids by simple carbon elongation using *Megasphaera hexanoica*. Sci Rep 2019;9(1):11999.
[53] San-Valero P, Abubackar HN, Veiga MC, Kennes C. Effect of pH, yeast extract and inorganic carbon on chain elongation for hexanoic acid production. Bioresour Technol 2020;300:122659.
[54] Sarria S, Kruyer NS, Peralta-Yahya P. Microbial synthesis of medium-chain chemicals from renewables. Nat Biotechnol 2017;35(12):1158–66.
[55] Zhu Z, Hu Y, Teixeira PG, Pereira R, Chen Y, Siewers V, et al. Multidimensional engineering of Saccharomyces cerevisiae for efficient synthesis of medium-chain fatty acids. Nat Catal 2020;3(1):64–74.
[56] Contreras-Dávila CA, Carrion VJ, Vonk VR, Buisman CNJ, Strik D. Consecutive lactate formation and chain elongation to reduce exogenous chemicals input in repeated-batch food waste fermentation. Water Res 2020;169:115215.
[57] Nzeteu CO, Trego AC, Abram F, O'Flaherty V. Reproducible, high-yielding, biological caproate production from food waste using a single-phase anaerobic reactor system. Biotechnol Biofuels 2018;11(1):108.
[58] Duber A, Jaroszynski L, Zagrodnik R, Chwialkowska J, Juzwa W, Ciesielski S, et al. Exploiting the real wastewater potential for resource recovery—*n*-caproate production from acid whey. Green Chem 2018;20(16):3790–803.
[59] Khor WC, Andersen S, Vervaeren H, Rabaey K. Electricity-assisted production of caproic acid from grass. Biotechnol Biofuels 2017;10(1):180.
[60] Wu SL, Sun J, Chen X, Wei W, Song L, Dai X, et al. Unveiling the mechanisms of medium-chain fatty acid production from waste activated sludge alkaline fermentation liquor through physiological, thermodynamic and metagenomic investigations. Water Res 2020;169:115218.
[61] Wang Q, Zhang P, Bao S, Liang J, Wu Y, Chen N, et al. Chain elongation performances with anaerobic fermentation liquid from sewage sludge with high total solid as electron acceptor. Bioresour Technol 2020;306:123188.
[62] Wu Q, Guo W, Bao X, Meng X, Yin R, Du J, et al. Upgrading liquor-making wastewater into medium chain fatty acid: insights into co-electron donors, key microflora, and energy harvest. Water Res 2018;145:650–9.
[63] Roghair M, Strik DPBTB, Steinbusch KJJ, Weusthuis RA, Bruins ME, Buisman CJN. Granular sludge formation and characterization in a chain elongation process. Process Biochem 2016;51(10):1594–8.
[64] Carvajal-Arroyo JM, Candry P, Andersen SJ, Props R, Seviour T, Ganigué R, et al. Granular fermentation enables high rate caproic acid production from solid-free thin stillage. Green Chem 2019;21(6):1330–9.
[65] Wu Q, Feng X, Guo W, Bao X, Ren N. Long-term medium chain carboxylic acids production from liquor-making wastewater: parameters optimization and toxicity mitigation. Chem Eng J 2020;388:124218.
[66] De Leeuw KD, de Smit SM, van Oossanen S, Moerland MJ, Buisman CJN, Strik DPBTB. Methanol-based chain elongation with acetate to *n*-butyrate and isobutyrate at varying selectivities dependent on pH. ACS Sustain Chem Eng 2020;8(22):8184–94.
[67] Wu SL, Wei W, Sun J, Xu Q, Dai X, Ni BJ. Medium-chain fatty acids and long-chain alcohols production from waste activated sludge via two-stage anaerobic fermentation. Water Res 2020;186:116381.
[68] Moscoviz R, Trably E, Bernet N, Carrère H. The environmental biorefinery: state-of-the-art on the production of hydrogen and value-added biomolecules in mixed-culture fermentation. Green Chem 2018;20(14):3159–79.
[69] Seedorf H, Fricke WF, Veith B, Brüggemann H, Liesegang H, Strittmatter A, et al. The genome of *Clostridium kluyveri*, a strict anaerobe with unique metabolic features. Proc Natl Acad Sci USA 2008;105(6):2128–33.
[70] Kucek LA, Nguyen M, Angenent LT. Conversion of l-lactate into *n*-caproate by a continuously fed reactor microbiome. Water Res 2016;93:163–71.
[71] He P, Han W, Shao L, Lu F. One-step production of C6–C8 carboxylates by mixed culture solely grown on CO. Biotechnol Biofuels 2018;11(1):4.
[72] Zhu HF, Liu ZY, Zhou X, Yi JH, Lun ZM, Wang SN, et al. Energy conservation and carbon flux distribution during fermentation of CO or H_2/CO_2 by *Clostridium ljungdahlii*. Front Microbiol 2020;11:416.
[73] Liu C, Luo G, Liu H, Yang Z, Angelidaki I, O-Thong S, et al. CO as electron donor for efficient medium chain carboxylate production by chain elongation: microbial and thermodynamic insights. Chem Eng J 2020;390:124577.
[74] Chu N, Liang Q, Zhang W, Ge Z, Hao W, Jiang Y, et al. Waste C1 gases as alternatives to pure CO_2 improved the microbial electrosynthesis of C4 and C6 carboxylates. ACS Sustain Chem Eng 2020;8(23):8773–82.
[75] Chen WS, Huang S, Plugge CM, Buisman CJN, Strik DPBTB. Concurrent use of methanol and ethanol for chain-elongating short chain fatty acids into caproate and isobutyrate. J Environ Manage 2020;258:110008.
[76] Esquivel-Elizondo S, Miceli 3rd J, Torres CI, Krajmalnik-Brown R. Impact of carbon monoxide partial pressures on methanogenesis and medium chain fatty acids production during ethanol fermentation. Biotechnol Bioeng 2018;115(2):341–50.
[77] Wu Q, Guo W, You S, Bao X, Luo H, Wang H, et al. Concentrating lactate–carbon flow on medium chain carboxylic acids production by hydrogen supply. Bioresour Technol 2019;291:121573.
[78] Duber A, Zagrodnik R, Chwialkowska J, Juzwa W, Oleskowicz-Popiel P. Evaluation of the feed composition for an effective medium chain carboxylic acid production in an open culture fermentation. Sci Total Environ 2020;728:138814.
[79] Zhu X, Zhou Y, Wang Y, Wu T, Li X, Li D, et al. Production of high-concentration *n*-caproic acid from lactate through fermentation using a newly isolated Ruminococcaceae bacterium CPB6. Biotechnol Biofuels 2017;10(1):102.
[80] Candry P, Radić L, Favere J, Carvajal-Arroyo JM, Rabaey K, Ganigué R. Mildly acidic pH selects for chain elongation to caproic acid over alternative pathways during lactic acid fermentation. Water Res 2020;186:116396.
[81] Zagrodnik R, Duber A, Łężyk M, Oleskowicz-Popiel P. Enrichment versus bioaugmentation—microbiological production of caproate from mixed carbon sources by mixed bacterial culture and *Clostridium kluyveri*. Environ Sci Technol 2020;54(9):5864–73.
[82] Jourdin L, Raes SMT, Buisman CJN, Strik DPBTB. Critical biofilm growth throughout unmodified carbon felts allows continuous bioelectrochemical chain elongation from CO_2 up to caproate at high current density. Front Energy Res 2018;6:7.
[83] Jourdin L, Winkelhorst M, Rawls B, Buisman CJN, Strik DPBTB. 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 2019;7:100284.
[84] Batlle-Vilanova P, Ganigue R, Ramio-Pujol S, Baneras L, Jimenez G, Hidalgo M, et al. Microbial electrosynthesis of butyrate from carbon dioxide: production and extraction. Bioelectrochemistry 2017;117:57–64.
[85] Jabeen G, Farooq R. Bio-electrochemical synthesis of commodity chemicals by autotrophic acetogens utilizing CO_2 for environmental remediation. J Biosci 2016;41(3):367–80.
[86] Van Eerten-Jansen MCAA, Ter Heijne A, Grootscholten TIM, Steinbusch KJJ, Sleutels THJA, Hamelers HVM, et al. Bioelectrochemical production of caproate and caprylate from acetate by mixed cultures. ACS Sustain Chem Eng 2013;1(5):513–8.
[87] Raes SMT, Jourdin L, Buisman CJN, Strik DPBTB. Continuous long-term bioelectrochemical chain elongation to butyrate. ChemElectroChem 2017;4(2):386–95.
[88] Sharma M, Aryal N, Sarma PM, Vanbroekhoven K, Lal B, Benetton XD, et al. Bioelectrocatalyzed reduction of acetic and butyric acids via direct electron transfer using a mixed culture of sulfate-reducers drives electrosynthesis of alcohols and acetone. Chem Commun 2013;49(58):6495.
[89] Raes SMT, Jourdin L, Buisman CJN, Strik DPBTB. Bioelectrochemical chain elongation of short-chain fatty acids creates steering opportunities for selective formation of *n*-butyrate, *n*-valerate or *n*-caproate. ChemistrySelect 2020;5(29):9127–33.
[90] Jiang Y, Chu N, Qian DK, Jianxiong ZR. Microbial electrochemical stimulation of caproate production from ethanol and carbon dioxide. Bioresour Technol 2020;295:122266.
[91] Jiang Y, Chu N, Zhang W, Zhang L, Jianxiong ZR. Electro-fermentation regulates mixed culture chain elongation with fresh and acclimated cathode. Energy Convers Manag 2020;204:112285.
[92] Ganigué R, Puig S, Batlle-Vilanova P, Balaguer MD, Colprim J. Microbial electrosynthesis of butyrate from carbon dioxide. Chem Commun 2015;51(15):3235–8.
[93] Pepè Sciarria T, Batlle-Vilanova P, Colombo B, Scaglia B, Balaguer MD, Colprim J, et al. Bio-electrorecycling of carbon dioxide into bioplastics. Green Chem 2018;20(17):4058–66.
[94] Eun Song Y, Kim C, Li S, Baek J, Seol E, Park C, et al. Supply of proton enhances CO electrosynthesis for acetate and volatile fatty acid productions. Bioresour Technol 2021;320:124245.
[95] Izadi P, Fontmorin JM, Virdis B, Head IM, Yu EH. The effect of the polarised cathode, formate and ethanol on chain elongation of acetate in microbial electrosynthesis. Appl Energy 2021;283:116310.
[96] Latif H, Zeidan AA, Nielsen AT, Zengler K. Trash to treasure: production of biofuels and commodity chemicals via syngas fermenting microorganisms. Curr Opin Biotechnol 2014;27:79–87.
[97] Bertsch J, Müller V. Bioenergetic constraints for conversion of syngas to biofuels in acetogenic bacteria. Biotechnol Biofuels 2015;8(1):210.
[98] Emerson DF, Stephanopoulos G. Limitations in converting waste gases to fuels and chemicals. Curr Opin Biotechnol 2019;59:39–45.
[99] Hu L, Huang H, Yuan H, Tao F, Xie H, Wang S. Rex in *Clostridium kluyveri* is a global redox-sensing transcriptional regulator. J Biotechnol 2016;233:17–25.
[100] Angenent LT, Richter H, Buckel W, Spirito CM, Steinbusch KJJ, Plugge CM, et al. Chain elongation with reactor microbiomes: open-culture biotechnology to produce biochemicals. Environ Sci Technol 2016;50(6):2796–810.
[101] Koch C, Kuchenbuch A, Kracke F, Bernhardt PV, Kromer J, Harnisch F. Predicting and experimental evaluating bio-electrochemical synthesis—a case study with *Clostridium kluyveri*. Bioelectrochemistry 2017;118:114–22.
[102] Gao T, Zhang H, Xu X, Teng J. Integrating microbial electrolysis cell based on electrochemical carbon dioxide reduction into anaerobic osmosis membrane reactor for biogas upgrading. Water Res 2021;190:116679.
[103] Jiang Y, Su M, Li D. Removal of sulfide and production of methane from carbon dioxide in microbial fuel cells–microbial electrolysis cell (MFCs–MEC) coupled system. Appl Biochem Biotechnol 2014;172(5):2720–31.

[104] Yang Z, Wang W, Liu C, Zhang R, Liu G. Mitigation of ammonia inhibition through bioaugmentation with different microorganisms during anaerobic digestion: selection of strains and reactor performance evaluation. Water Res 2019;155:214–24.
[105] De Vrieze J, Verbeeck K, Pikaar I, Boere J, Van Wijk A, Rabaey K, et al. The hydrogen gas bio-based economy and the production of renewable building block chemicals, food and energy. N Biotechnol 2020;55:12–8.
[106] Huang S, Kleerebezem R, Rabaey K, Ganigué R. Open microbiome dominated by *Clostridium* and *Eubacterium* converts methanol into *i*-butyrate and *n*-butyrate. Appl Microbiol Biotechnol 2020;104(11):5119–31.
[107] Yang HY, Wang YX, He CS, Qin Y, Li WQ, Li WH, et al. Redox mediator-modified biocathode enables highly efficient microbial electro-synthesis of methane from carbon dioxide. Appl Energy 2020;274:115292.
[108] Viggi CC, Colantoni S, Falzetti F, Bacaloni A, Montecchio D, Aulenta F. Conductive magnetite nanoparticles enhance the microbial electrosynthesis of acetate from CO_2 while diverting electrons away from methanogenesis. Fuel Cells 2020;20(1):98–106.
[109] Jiang Y, Liang Q, Chu N, Hao W, Zhang L, Zhan G, et al. A slurry electrode integrated with membrane electrolysis for high-performance acetate production in microbial electrosynthesis. Sci Total Environ 2020;741:140198.
[110] Rago L, Guerrero J, Baeza JA, Guisasola A. 2-Bromoethanesulfonate degradation in bioelectrochemical systems. Bioelectrochemistry 2015;105:44–9.
[111] Salvador AF, Cavaleiro AJ, Paulo AMS, Silva SA, Guedes AP, Pereira MA, et al. Inhibition studies with 2-bromoethanesulfonate reveal a novel syntrophic relationship in anaerobic oleate degradation. Appl Environ Microbiol 2019;85:e01733–1818.
[112] Schuchmann K, Muller V. Energetics and application of heterotrophy in acetogenic bacteria. Appl Environ Microbiol 2016;82(14):4056–69.
[113] Wiechmann A, Ciurus S, Oswald F, Seiler VN, Müller V. It does not always take two to tango: "Syntrophy" via hydrogen cycling in one bacterial cell. ISME J 2020;14(6):1561–70.
[114] Cotton CA, Edlich-Muth C, Bar-Even A. Reinforcing carbon fixation: CO_2 reduction replacing and supporting carboxylation. Curr Opin Biotechnol 2018;49:49–56.
[115] Fast AG, Papoutsakis ET. Stoichiometric and energetic analyses of non-photosynthetic CO_2-fixation pathways to support synthetic biology strategies for production of fuels and chemicals. Curr Opin Chem Eng 2012;1(4):380–95.
[116] Emerson DF, Woolston BM, Liu N, Donnelly M, Currie DH, Stephanopoulos G. Enhancing hydrogen-dependent growth of and carbon dioxide fixation by *Clostridium ljungdahlii* through nitrate supplementation. Biotechnol Bioeng 2019;116(2):294–306.
[117] Mahamkali V, Valgepea K, de Souza Pinto Lemgruber R, Plan M, Tappel R, Köpke M, et al. Redox controls metabolic robustness in the gas-fermenting acetogen *Clostridium autoethanogenum*. Proc Natl Acad Sci USA 2020;117(23):13168–75.
[118] Jones SW, Fast AG, Carlson ED, Wiedel CA, Au J, Antoniewicz MR, et al. CO_2 fixation by anaerobic non-photosynthetic mixotrophy for improved carbon conversion. Nat Commun 2016;7(1):12800.
[119] Park JO, Liu N, Holinski KM, Emerson DF, Qiao K, Woolston BM, et al. Synergistic substrate cofeeding stimulates reductive metabolism. Nat Metab 2019;1(6):643–51.
[120] Mann M, Munch G, Regestein L, Rehmann L. Cultivation strategies of *Clostridium autoethanogenum* on xylose and carbon monoxide combination. ACS Sustain Chem Eng 2020;8(7):2632–9.
[121] Klask CM, Kliem-Kuster N, Molitor B, Angenent LT. Nitrate feed improves growth and ethanol production of *Clostridium ljungdahlii* with CO_2 and H_2, but results in stochastic inhibition events. Front Microbiol 2020;11:724.
[122] Maru BT, Munasinghe PC, Gilary H, Jones SW, Tracy BP. Fixation of CO_2 and CO on a diverse range of carbohydrates using anaerobic, non-photosynthetic mixotrophy. FEMS Microbiol Lett 2018;365(8):365.
[123] Subashchandrabose SR, Ramakrishnan B, Megharaj M, Venkateswarlu K, Naidu R. Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation. Environ Int 2013;51:59–72.
[124] Kanno M, Carroll AL, Atsumi S. Global metabolic rewiring for improved CO_2 fixation and chemical production in cyanobacteria. Nat Commun 2017;8(1):14724.
[125] Lacroux J, Trably E, Bernet N, Steyer JP, van Lis R. Mixotrophic growth of microalgae on volatile fatty acids is determined by their undissociated form. Algal Res 2020;47:101870.
[126] Fast AG, Schmidt ED, Jones SW, Tracy BP. Acetogenic mixotrophy: novel options for yield improvement in biofuels and biochemicals production. Curr Opin Biotechnol 2015;33:60–72.
[127] Hu GP, Li Y, Ye C, Liu LM, Chen XL. Engineering microorganisms for enhanced CO_2 sequestration. Trends Biotechnol 2019;37(5):532–47.
[128] Charubin K, Papoutsakis ET. Direct cell-to-cell exchange of matter in a synthetic *Clostridium* syntrophy enables CO_2 fixation, superior metabolite yields, and an expanded metabolic space. Metab Eng 2019;52:9–19.
[129] Schreiber F, Ackermann M. Environmental drivers of metabolic heterogeneity in clonal microbial populations. Curr Opin Biotechnol 2020;62:202–11.
[130] Izadi P, Fontmorin JM, Godain A, Yu EH, Head IM. Parameters influencing the development of highly conductive and efficient biofilm during microbial electrosynthesis: the importance of applied potential and inorganic carbon source. npj Bio Microbio 2020;6:40.
[131] Zeppilli M, Chouchane H, Scardigno L, Mahjoubi M, Gacitua M, Askri R, et al. Bioelectrochemical vs hydrogenophilic approach for CO_2 reduction into methane and acetate. Chem Eng J 2020;396:125243.
[132] Mayer F, Enzmann F, Lopez AM, Holtmann D. Performance of different methanogenic species for the microbial electrosynthesis of methane from carbon dioxide. Bioresour Technol 2019;289:121706.
[133] Chakraborty S, Rene ER, Lens PNL, Veiga MC, Kennes C. Enrichment of a solventogenic anaerobic sludge converting carbon monoxide and syngas into acids and alcohols. Bioresour Technol 2019;272:130–6.
[134] Arslan K, Bayar B, Nalakath Abubackar H, Veiga MC, Kennes C. Solventogenesis in *Clostridium aceticum* producing high concentrations of ethanol from syngas. Bioresour Technol 2019;292:121941.
[135] Zhang L, Zhao R, Jia D, Jiang W, Gu Y. Engineering *Clostridium ljungdahlii* as the gas-fermenting cell factory for the production of biofuels and biochemicals. Curr Opin Chem Biol 2020;59:54–61.
[136] Li C, Zhu X, Angelidaki I. Carbon monoxide conversion and syngas biomethanation mediated by different microbial consortia. Bioresour Technol 2020;314:123739.
[137] Valgepea K, de Souza Pinto Lemgruber R, Meaghan K, Palfreyman RW, Abdalla T, Heijstra BD, et al. Maintenance of ATP homeostasis triggers metabolic shifts in gas-fermenting acetogens. Cell Syst 2017;4(5):505–15.
[138] Richter H, Molitor B, Diender M, Sousa DZ, Angenent LT. A narrow pH range supports butanol, hexanol, and octanol production from syngas in a continuous co-culture of *Clostridium ljungdahlii* and *Clostridium kluyveri* with in-line product extraction. Front Microbiol 2016;7:1773.
[139] Srikanth S, Singh D, Vanbroekhoven K, Pant D, Kumar M, Puri SK, et al. Electro-biocatalytic conversion of carbon dioxide to alcohols using gas diffusion electrode. Bioresour Technol 2018;265:45–51.
[140] Gavilanes J, Reddy CN, Min B. Microbial electrosynthesis of bioalcohols through reduction of high concentrations of volatile fatty acids. Energy Fuels 2019;33(5):4264–71.
[141] Gavilanes J, Noori MT, Min B. Enhancing bio-alcohol production from volatile fatty acids by suppressing methanogenic activity in single chamber microbial electrosynthesis cells (SCMECs). Bioresour Technol Rep 2019;7:100292.
[142] Kondaveeti S, Min B. Bioelectrochemical reduction of volatile fatty acids in anaerobic digestion effluent for the production of biofuels. Water Res 2015;87:137–44.
[143] Spirito CM, Marzilli AM, Angenent LT. Higher substrate ratios of ethanol to acetate steered chain elongation toward *n*-caprylate in a bioreactor with product extraction. Environ Sci Technol 2018;52(22):13438–47.
[144] Liu N, Santala S, Stephanopoulos G. Mixed carbon substrates: a necessary nuisance or a missed opportunity? Curr Opin Biotechnol 2020;62:15–21.
[145] Uranukul B, Woolston BM, Fink GR, Stephanopoulos G. Biosynthesis of monoethylene glycol in *Saccharomyces cerevisiae* utilizing native glycolytic enzymes. Metab Eng 2019;51:20–31.
[146] Gehring T, Cavalcante W, Colares A, Angenent LT, Santaella S, Lübken M, et al. Optimal pH set point for simultaneous production and pertraction of *n*-caproic acid: an experimental and simulation study. J Chem Technol Biotechnol 2020;95(12):3105–16.
[147] Wu Q, Feng X, Chen Y, Liu M, Bao X. Continuous medium chain carboxylic acids production from excess sludge by granular chain-elongation process. J Hazard Mater 2021;402:123471.
[148] Ma Y, Liu Y. Turning food waste to energy and resources towards a great environmental and economic sustainability: an innovative integrated biological approach. Biotechnol Adv 2019;37(7):107414.
[149] Yang G, Hu Y, Wang J. Biohydrogen production from co-fermentation of fallen leaves and sewage sludge. Bioresour Technol 2019;285:121342.
[150] Fernández-Naveira Á, Veiga MC, Kennes C. Selective anaerobic fermentation of syngas into either C2–C6 organic acids or ethanol and higher alcohols. Bioresour Technol 2019;280:387–95.
[151] Baleeiro FCF, Kleinsteuber S, Neumann A, Sträuber H. Syngas-aided anaerobic fermentation for medium-chain carboxylate and alcohol production: the case for microbial communities. Appl Microbiol Biotechnol 2019;103(21–22):8689–709.
[152] Christodoulou X, Velasquez-Orta SB. Microbial electrosynthesis and anaerobic fermentation: an economic evaluation for acetic acid production from CO_2 and CO. Environ Sci Technol 2016;50(20):11234–42.
[153] Valgepea K, de Souza Pinto Lemgruber R, Abdalla T, Binos S, Takemori N, Takemori A, et al. H_2 drives metabolic rearrangements in gas-fermenting *Clostridium autoethanogenum*. Biotechnol Biofuels 2018;11(1):55.
[154] Liu C, Wang W, O-Thong S, Yang Z, Zhang S, Liu G, et al. Microbial insights of enhanced anaerobic conversion of syngas into volatile fatty acids by co-fermentation with carbohydrate-rich synthetic wastewater. Biotechnol Biofuels 2020;13(1):53.
[155] Roy M, Yadav R, Chiranjeevi P, Patil SA. Direct utilization of industrial carbon dioxide with low impurities for acetate production via microbial electrosynthesis. Bioresour Technol 2021;320:124289.
[156] De Vrieze J, Arends JBA, Verbeeck K, Gildemyn S, Rabaey K. Interfacing anaerobic digestion with (bio)electrochemical systems: potentials and challenges. Water Res 2018;146:244–55.
[157] So JY, Lee J, Ahn Y, Kang D, Jung W, Bae WG. The synergistic effect of biomimetic electrical stimulation and extracellular-matrix-mimetic nanopattern for upregulating cell activities. Biosens Bioelectron 2020;167:112470.
[158] Wang Y, Wei W, Wu SL, Ni BJ. Zerovalent iron effectively enhances medium-chain fatty acids production from waste activated sludge through improving

sludge biodegradability and electron transfer efficiency. Environ Sci Technol 2020;54(17):10904–15.
[159] Ghysels S, Buffel S, Rabaey K, Ronsse F, Ganigué R. Biochar and activated carbon enhance ethanol conversion and selectivity to caproic acid by *Clostridium kluyveri*. Bioresour Technol 2021;319:124236.
[160] Claassens NJ, Cotton CAR, Kopljar D, Bar-Even A. Making quantitative sense of electromicrobial production. Nat Catal 2019;2(5):437–47.
[161] Schwarz FM, Müller V. Whole-cell biocatalysis for hydrogen storage and syngas conversion to formate using a thermophilic acetogen. Biotechnol Biofuels 2020;13(1):32.
[162] Verbeeck K, Gildemyn S, Rabaey K. Membrane electrolysis assisted gas fermentation for enhanced acetic acid production. Front Energy Res 2018;6:88.
[163] Schuchmann K, Müller V. Direct and reversible hydrogenation of CO_2 to formate by a bacterial carbon dioxide reductase. Science 2013;342 (6164):1382–5.
[164] Xu J, Guzman JJL, Angenent LT. Direct medium-chain carboxylic acid oil separation from a bioreactor by an electrodialysis/phase separation cell. Environ Sci Technol 2021;55(1):634–44.
[165] Carvajal-Arroyo JM, Andersen SJ, Ganigué R, Rozendal RA, Angenent LT, Rabaey K. Production and extraction of medium chain carboxylic acids at a semi-pilot scale. Chem Eng J 2021;416:127886.
[166] Kracke F, Virdis B, Bernhardt PV, Rabaey K, Krömer JO. Redox dependent metabolic shift in *Clostridium autoethanogenum* by extracellular electron supply. Biotechnol Biofuels 2016;9(1):249.
[167] Choi O, Kim T, Woo HM, Um Y. Electricity-driven metabolic shift through direct electron uptake by electroactive heterotroph *Clostridium pasteurianum*. Sci Rep 2014;4(1):6961.
[168] Reiner JE, Geiger K, Hackbarth M, Fink M, Lapp CJ, Jung T, et al. From an extremophilic community to an electroautotrophic production strain: identifying a novel Knallgas bacterium as cathodic biofilm biocatalyst. ISME J 2020;14(5):1125–40.
[169] Moscoviz R, Quemener ED, Trably E, Bernet N, Hamelin J. Novel outlook in microbial ecology: nonmutualistic interspecies electron transfer. Trends Microbiol 2020;28(4):245–53.
[170] Lawson CE, Harcombe WR, Hatzenpichler R, Lindemann SR, Löffler FE, O'Malley MA, et al. Common principles and best practices for engineering microbiomes. Nat Rev Microbiol 2019;17(12):725–41.