

Review

Advances in Electricity-Steering Organic Waste Bio-Valorization for Medium Chain Carboxylic Acids Production

Chao Liu , Yue Yin, Chuang Chen, Xuemeng Zhang, Jing Zhou, Qingran Zhang * and Yinguang Chen *

State Key Laboratory of Pollution Control and Resources Reuse, School of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Shanghai 200092, China

* Correspondence: qingran_zhang@tongji.edu.cn (Q.Z.); yinchentj@163.com (Y.C.)

Abstract: Medium chain carboxylic acids (MCCAs, e.g., caproic acid, caprylic acid, etc.) with 6–12 carbon atoms are valuable platform chemicals produced from organic waste via microbial chain elongation metabolism named as reversed β -oxidation and fatty acid-biosynthesis cyclical pathway. Recently, many articles reported that electricity could not only serve as the external electron donor and provide the reduction equivalent required for chain elongation but also regulate the microbiome structure and metabolic behaviors to promote MCCAs formation. Electricity-steering MCCAs bioproduction has become an appealing technique to valorize low-value organic waste, paving an alternative pathway for net-zero carbon emission energy systems and sustainable socio-economic development. However, the MCCAs' bioproduction from organic waste steered by electric field has not been comprehensively reviewed. From a systematical analysis of publicly available literature, we first covered the basic working principle, fermentation architecture, functional microflora, and metabolic pathway of MCCAs production driven by electricity. The strategies of substrate modulation, applied voltage/current regulation, electrode optimization, and microbial cooperation and stimulation for boosting electricity-driven MCCAs bioproduction are then scrutinized and extensively discussed. Ultimately, the pressing knowledge gaps and the potential path forward are proposed to provide pointers for consistently higher MCCAs yield and the transition from laboratory to market.

Keywords: electrofermentation; medium chain carboxylic acids; organic waste; valorization; bioproduction; anaerobic treatment; operation regulation; sustainable energy technologies



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1. Introduction

The transition to a climate-neutral future requires innovative avenues that guide net-zero carbon emission energy systems [1,2], and the substantial production of organic waste detrimentally influences the environment. The microbial valorization of organic waste feedstocks for manufacturing chemical products and bioenergy has been extensively considered as one of the key routes to confront these pressing obstacles [3–7]. Notably, anaerobic biotreatment of valorizing waste feedstocks is used widely due to its high energy recovery rate and low environmental impact [8–11]. In recent years, electrofermentation, a novel bioelectrochemical method based on electricity to regulate microbial fermentative metabolism with electrodes, has become an appealing, sustainable strategy to promote anaerobic fermentation [11–16]. Among them, the anaerobic production of medium chain carboxylic acids (MCCAs), such as caproic acid, using carbon chain elongation (CE) microbiota as biocatalyst, has garnered widespread interest in the past decade [17–20]. Actual organic waste has been applied as substrates for MCCAs formation, e.g., food waste [21], thin stillage [22], liquor wastewater [23,24], grass [25], acid whey waste [26], and alkaline fermentation liquor from waste activated sludge [27].

MCCAs, saturated monocarboxylic acids with 6–12 carbon atoms (C₆–C₁₂), are widely applied as “green” antibiotics, animal feed additive, anticorrosion agent, the precursor of lubricants, adhesives, surfactants, and jet fuel [11,28,29]. The considerable market value

of MCCAs was estimated at 8×10^9 USD by the end of 2023 [30]. Moreover, the relative ease of MCCAs recovery from fermentation broth due to their remarkable hydrophobicity would enable the downstream product extraction procedure to be more cost-effective and less energy-intensive compared to conventional bio-products such as short chain carboxylic acids (SCCAs) and ethanol [29,31]. Owing to the conspicuous benefits of waste-derived microbial MCCAs synthesis, considerable efforts to boost their productivities in an environmentally friendly and affordable manner have been dedicated to pursuing this route recently.

Since MCCAs are inhibitory and toxic to microorganisms [32], the extraction and separation of MCCAs play an important role. Due to the complex components of the reactors, the extraction and separation need to be highly selective [33]. Currently, in-line extraction strategies, such as liquid–liquid extraction (perstraction), electrodialysis, and anion exchange, show promising strength [34]. The perstraction system mainly includes two hollow fiber membranes and organic solvent (e.g., tri-*n*-octylphosphine oxide), which transfers MCCAs products from broth to an alkaline back-extraction agent. Then, the additional acid agent is added to obtain MCCAs (acid form) [35]. An electrodialysis system consisting of cathode, anode, cation exchange membranes, and anion exchange membranes, can self-produce H^+ in an anode chamber, thus replacing the additional acid [36]. Anion exchange is based on physical absorption and ion exchange of anion exchange resin, which has a higher affinity of MCCAs than that of SCCAs and thus can acquire a great selectivity for in-situ MCCAs recovery [34]. Although the MCCAs extracted from the fermentation broth by advanced separation method could remove impurities in organic waste as soon as possible, the safety issues, such as the transfer of residual trace antibiotics from broth to extracted solution, should be paid attention to when using these MCCAs in other processes.

Under the electrofermentation mode, the application of cathode potential can not only serve as the additional electron donor and provide the reduction equivalent required for carbon chain elongation during the MCCAs synthesis process [37,38], but also regulate microbial community structure and stimulate metabolic activities of microflora [39,40]. For example, a pioneering study demonstrated that MCCAs (i.e., caproate and caprylate) were generated by chain elongators when using the cathode electrode as the sole electron donor [37]. Moreover, Jiang et al. reported that the caproate selectivity was promoted by approximately 28% driven by electricity supply compared with the open circuit control, and the electrofermentation mode reshaped the microbial structure of biofilm and planktonic microbiota, thus redirecting the MCCAs fermentation pattern [39]. Moreover, the operating parameters such as initial substrate type and concentration [37,41,42], applied voltage/current [38,43], electrode material [38,44], and microbiome [45,46] are reported as the pivotal factors boosting MCCAs electrofermentation performance. Nevertheless, few articles systematically review the electricity-steering MCCAs production from organic waste and its enhanced strategies.

This paper aims to provide a critical and comprehensive state-of-the-art review of electricity-steering organic waste bio-valorization for MCCAs production. The fundamental working principle and fermentation architecture of MCCAs production driven by electricity are described first. A critical analysis of the functional microbes and metabolic pathways of electricity-driven MCCAs production from organic waste is conducted next. Then, the enhanced strategies for producing MCCAs from organic waste steered by electricity, including substrate modulation, applied voltage/current regulation, electrode optimization, and microbial cooperation and stimulation, are extensively discussed and summarized. Ultimately, the existing challenges and the potential path forward in the future are proposed, offering critical clues to further exploration for increasing the application of MCCAs electrofermentation in organic waste bio-valorization.

2. Working Principle of Electricity-Driven Medium Chain Carboxylic Acids Fermentation

Electrochemistry has been introduced into the fermentation system as a vital regulation method to promote the transformation of organic substrates into valuable platform

chemicals such as MCCAs (Figure 1a) [24,41,47–49]. The microbes attached to the electrode surface are excellent catalysts for electrode reaction due to low cost and high selectivity. In the meantime, an electric field can regulate the evolution of microbial community and provide reducing force. Better chain elongation (CE) performance was observed in electrofermentation systems than in conventional fermentation systems. *Clostridium kluyveri* and other functional microbes were revealed to respond positively to electricity [41,45,50]. The possibility of electric field as direct electron donor was investigated in pioneering research to lessen the addition of exogenous electron donors. A pioneering study found that medium chain carboxylic acids caproate and caprylate were generated from acetate in a bioelectrochemical system at -0.9 V vs. NHE (normal hydrogen electrode) cathode potential without an external mediator and electron donor addition [37]. Rase and coworkers also discerned that the external electric field could completely replace the external electron donor, showing the possibility of selectively regulating the product composition by external electric field [43].

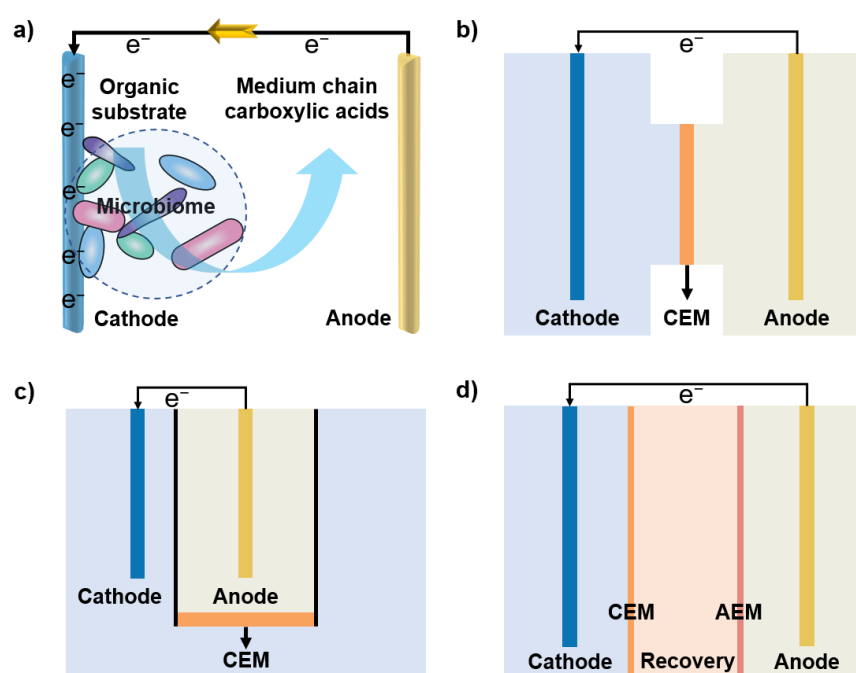


Figure 1. (a) Schematic diagram of electricity-steering MCCAs fermentation; (b) H-type configuration of the reactor; (c) bottle-type configuration of the reactor; (d) three-chamber configuration of the reactor; CEM: cation exchange membrane; AEM: anion exchange membrane.

The role of external electricity in MCCAs production mainly includes two aspects. Firstly, the external electric field can reshape the microbiome composition, biofilm growth, and microbial metabolic pathway [39,46]. It was reported that in an H-type double chamber reactor with a working volume of 250 mL in both anode and cathode compartments separated by a proton exchange membrane, the acclimated mixed culture was inoculated into granular activated carbon (GAC) fluidized cathode chamber [45], which obtained high MCCAs performance during the continuous operation. This cathode was made up of a piece of carbon felt ($4\text{ cm} \times 4\text{ cm} \times 0.3\text{ cm}$), and GAC (diameter 0.8–1.0 mm, specific surface area $900\text{ m}^2/\text{g}$) was loaded into the cathode chamber. On the other hand, electrodes can provide reducing power to microbial metabolic pathways. Hydrogen evolved on the cathode surface has been proven to promote electron transfer between microbes and the cathode or among microflora [23]. Moreover, ethanol was observed to be synthesized in situ on a cathode, and acetic acid can be elongated into butyric acid without an exogenous electron donor [43]. This study confirmed the possibility of replacing exogenous electron donors with electric current. Nevertheless, how microorganisms related to the chain

elongation process directly grab electrons from electrodes for MCCAs fermentation is still in its infancy, which would therefore be an attractive issue for further investigations.

Designing proper fermenter architectures that are effective at electricity-coupled medium chain carboxylates fermentation systems is crucial. The H-type geometry reactor has been widely used in many previous studies. In this configuration, anode and cathode are located in two cells, and a cation-exchange membrane (CEM) separates the two cells (Figure 1b). Thus, anode and cathode reactions would not be interfered with, keeping chain elongation reactions stable and efficient. The typically used anode electrode was titanium meshes coated with ruthenium and iridium, while carbon felt was usually used as a cathode electrode for microbial attachment and biofilm growth [44,50]. The H-type reactor was easy to assemble and stabilize, which was suitable for investigating novel electrode material adopted in MCCAs electrofermentation. However, the problems of low current density and surface/volume ratio still need to be settled [51].

The bottle-type geometry reactor is another typical configuration, consisting of one bottle and one tube [51]. The tube with a cation-exchange membrane divides the bottle into two chambers (Figure 1c). The anode is usually placed in the tube, while the bigger chamber is usually the cathode cell for chain elongation reaction. The structure of a single bottle is favorable for an even mixture, and the cation-exchange membrane ensures that electrode reactions are separated. Almost all MCCAs electrofermentation occurred in the cathode cell. The bottle reactor could flexibly adjust the anode cell and cathode, saving space and electrolyte solution utilization when large-scale engineering applications are implemented. Similar to the H-type configuration, the bottle-type reactor also confronted the challenges of high internal resistance and low current density [51]. In addition to reactor configuration, the flow direction of electrolyte solution inside the reactor would also affect MCCAs electrofermentation. A completely stirred tank reactor is the simplest and the most common, but substrate retention time and loading are limited by continuous flow [52]. In an up-flow reactor, microbial flocs had a better suspension condition, and the suspended conductive particles could form a fluidized cathode, which led to better electrochemical performance [50].

To sum up, an H-type reactor is usually used in the fundamental research stage at the laboratory scale, and a bottle-type reactor is more suitable for the pilot scale. Given the subsequent downstream separation and purification process, more advanced reactor configurations such as a three-chamber reactor (Figure 1d) [53,54], which has a third chamber beyond the anode and cathode chamber, i.e., separation chamber, seem more appealing. Future studies should focus on the reduction of internal resistance, the improvement of current density, and the long-term stable operation of the MCCAs electrofermentation reactor. Meanwhile, advanced methods of MCCAs in situ separation in bioreactors also need to be developed to improve the reactor efficacy by preventing MCCAs toxicity from chaining elongation, elevating the MCCAs concentration considerably, selecting the longer possible carbon chain, and building mildly acidic circumstance of the fermentation solution to also inhibit unwanted side reactions (e.g., acetoclastic methanation).

3. Functional Microbes and Metabolic Pathway of Electricity-Driven Medium Chain Carboxylic Acids Production from Organic Waste

3.1. Functional Microorganisms

Mixed microbial culture has been widely applied in electricity-steering MCCAs production, especially during the fermentation process of complex organic waste. Firstly, the cooperation of diverse microbial consortia benefits the biotransformation of complex organic feedstocks (e.g., liquor wastewater) to desired products by the total culture's metabolism [23,24], e.g., the synergy of microorganisms responsible for substrates hydrolysis, SCCAs production, and chain elongation process. That is, mixed culture avoids the limited metabolic potential of any given microbe containing single genetic makeup. Moreover, a mixed culture is more robust than a pure strain to fluctuating environment, thereby promising a more stable operation in the long-term fermentation process [55,56].

In addition, it is more realistic to set up and operate the mixed-culture fermentation system when treating actual organic waste. Furthermore, microbial heterogeneity is widely desired in mixed-culture mode under an electric field, which relied on dissimilarity in response to microorganisms' electrostimulation and growth rate [45]. For instance, *Clostridia*, *Oscillibacter*, and *Caproiciproducens* shifted from *Lactobacillus* to be the dominant bacteria on the cathode biofilm driven by electricity [50]. Changes in intracellular redox balance and promotion of interspecific electron transfer were reported to screen out specific MCCAs producers [23,39]. This simple culture system is often free of additional costs such as sterilization, specific nutrient additives, and rigorous process controls in pure or defined co-culture systems [57,58].

Mixed microbial cultures for MCCAs production can be acquired from various sources, such as acclimated anaerobic sludge, pit mud, and thin stillage. For MCCAs synthesis, a pretreating or acclimating operation is usually needed to enrich functional microorganisms and restrain the competing strains in mixed cultures. For instance, Wu et al. enriched MCCAs-producing microbiota by digesting the pretreated sludge by dosing 6.0 g/L anhydrous ethanol, 2.78 g/L sodium acetate, and some nutrients, with preheating seed sludge and adding 2.0 g/L sodium 2-bromoethanesulfonate to suppress the activities of methanogens [38]. Cheng and coworkers acclimated caproate-producing microbiome in a selective medium mainly containing electron acceptor acetate and electron donor ethanol, with the addition of 10 g/L of sodium 2-bromoethanesulfonate as the methanogenic inhibitor, and the temperature and pH were maintained at 35 °C and 6.5, respectively. It is noted that the initial inoculum in the selective medium was a mixture of activated sludge, garden soil, and anaerobic sludge [59].

The electron donor, substrate source, and inoculum source can influence the microbial structure in the electricity-steering MCCAs fermentation system. The dominant microbes detected in MCCAs electrofermentation mixed microbial culture systems are hereafter discussed and summarized in Table 1. As seen in Table 1, the dominant microbes are different in MCCAs electrofermentation systems supported by different electron donors. For ethanol-supply electrofermentation systems, whether the ethanol was adsorbed or generated in situ by bioelectrochemical process, *Clostridium* spp. was widely detected [37,39,45,46,50,59]. For lactate-supply fermentation systems, previous studies revealed that lactate-utilizing chain elongation bacteria such as *Megasphaera elsdenii* and *Caproiciproducens* spp. have been widely examined [22–24]. Moreover, during the electron donors H₂ and ethanol generated in situ process, the presence of H₂ remarkably triggered the enrichment of *Oscillibacter*, which can catalyze acetate to produce ethanol and further use ethanol to elongate carbon chain for caproate generation [50,60].

In addition, substrate sources dramatically impact the distribution of dominant microbes. When short chain carboxylic acid such as acetic acid is used as electron acceptor, MCCAs-producing strains are more prevalent in the microbial community. In a system containing complex organic waste substrates [22,23], microorganisms bioconverting organic macromolecules to micromolecules dominated along with chain-elongating bacteria, for example, *Lactobacillus* spp. capable of transforming carbohydrates to produce lactic acid for the chain elongation process to become dominant. It was noted that electroactive microorganisms such as *Desulfovibrio* spp. and *Dysgonomonas* spp. could be enriched in the MCCAs electrofermentation system. For example, Jiang et al. found that *Dysgonomonas* genus occupied the highest proportion of 24.0% within the biofilm under −1.1 V conditions, which can utilize acetyl-CoA synthase for growth on acetate [39]. It was also reported that the electroactive microorganism *Desulfovibrio* that could accept electrons from the cathode electrode to catalyze the H₂ evolution reaction was enriched in the MCCAs production system with a relative population of 10.6–40.6% [45,62].

Table 1. Dominant microbes in MCCAs electrofermentation mixed microbial culture systems.

Feedstock	Electron Donor	Inoculum Source	Dominant Microbes	Maximum MCCAs Concentration/Productivity	Electrochemical Conditions	pH	Temperature (°C)	Reference
Acetic acid	Cathode; H ₂ and ethanol generated in situ	Microbes from a continuously operating anaerobic fixed film reactor for C4–C8 fatty acids production	<i>Clostridium kluyveri</i>	0.19 g/(L·d)	−0.9 V	6	30	[37]
Acetate, butyrate	Cathode; H ₂ and ethanol generated in situ	Acclimated microbes from CE reactors	<i>Clostridia</i> , <i>Oscillibacter</i> , and <i>Caproiciproducens</i>	7.8 ± 0.4 mM	−0.95 V	5	30	[50]
Acetic acid, butyric acid	Cathode; H ₂ and ethanol generated in situ	A mixed culture of sulfate reducers	<i>Curvibacter</i> , <i>Deltaproteobacteria</i> , <i>Desulfovibrionales</i> , <i>Desulfobacteraceae</i> , and <i>Syntrophobacteraceae</i>	0.02 g/L	160–210 Am ²	7.4	18–22	[61]
Acetate, ethanol	Cathode; H ₂ and ethanol generated in situ	Enriched caproate-producing microbial consortium	<i>Clostridium_sensu_stricto</i> , <i>Acetabacterium</i>	7454 mg COD/L	−0.8 V	6.0	22 ± 2	[59]
Acetate, ethanol	Cathode; ethanol; H ₂ and ethanol generated in situ	Enriched microbes from CE reactor	<i>Clostridium_sensu_stricto_12</i> , <i>Dysgonomonas</i> , and <i>Acetoanaerobium</i>	3.40 g/(L·d)	−1.1 V	7.2 (Initial pH)	30	[39]
Acetate, ethanol	Cathode; ethanol; H ₂ and ethanol generated in situ	Acclimated sludge from CE reactors	<i>Clostridia</i> , <i>Actinobacteria</i> , and <i>Negativicutes</i>	4.3 g/L	−0.7 V	7.0 (Initial pH)	36 ± 1	[46]
Acetate, ethanol	Cathode; ethanol; H ₂ and ethanol generated in situ	Acclimated CE mixed culture	<i>Clostridium_sensu_stricto_12</i> , <i>Desulfovibrio</i> , <i>Clostridium_sensu_stricto_13</i>	2.45 g/L	−0.7 V	/	/	[45]
Acetate, ethanol	Cathode; ethanol; H ₂ and ethanol generated in situ	Acclimated CE mixed culture + <i>Clostridium kluyveri</i>	<i>Clostridium_sensu_stricto_12</i> , <i>Desulfovibrio</i> , <i>Clostridium_sensu_stricto_13</i>	4.68 g/L	−0.7 V	/	/	[45]
Thin stillage	Cathode; ethanol; lactate; H ₂ and ethanol generated in situ	Thin stillage	<i>Megasphaera elsdenii</i> , <i>Lactobacillus</i> spp.	1.144 ± 0.275 g COD/(L·d)	100 mA	5.4–5.7	35	[22]
Liquor wastewater	Cathode; ethanol; lactate; H ₂ and ethanol generated in situ	Domesticated pit mud	<i>Rummeliibacillus</i> , <i>Clostridium_sensu_stricto_12</i> , and <i>Caproiciproducens</i>	4.04 g/L	−1.0 V	6.5 (Initial pH)	35 ± 2	[23]
Yellow water from a winery	Cathode; ethanol; lactate; H ₂ and ethanol generated in situ	Domesticated shallow pit mud	<i>Clostridium</i> and <i>Caproiciproducens</i>	2.14 g/L	−0.8 V	6.5	35	[24]

In addition, some vital bacteria presented in MCCAs electrofermentation system may derive from the inoculum, e.g., two well-known acetogenic bacteria *Acetabacterium* and *Acetobacter* favoring in forming acetate either through biotransformation of H_2 and CO_2 , or the incomplete oxidation of ethanol [46,59,63], which was able to provide key electron acceptor acetate for elongate carbon chain. Furthermore, a pioneering study by Sharma et al. indicated that a mixture of sulfate reducers consisting of *Curvibacter*, *Deltaproteobacteria*, *Desulfovibrionales*, *Desulfobacteraceae*, and *Syntrophobacteraceae* could bioelectrocatalyze reduction of acetic and butyric acids into alcohols, acetone, and caproate, controlled by direct electron transfer [61]. Moreover, Table 1 shows the demand for applied electricity, providing the vital driven force for MCCAs production and affecting the microbial community. Furthermore, the concentration of products obtained in MCCAs electrofermentation was not very high, which needs improving by effective and enhanced strategies in the future.

The open mixed bacterial culture system has been extensively used in electrofermentation due to its anti-stability. However, the open system has a wide range of microbial populations and different metabolic pathways, which makes it challenging to achieve the targeted accumulation of medium chain products. To overcome those problems, future studies are recommended to focus on the following aspects: (1) optimize the microflora structure, e.g., quickly build a mixed microbial system with chain elongation microbes as the dominant population; (2) enhance interspecies cooperation and use molecular biology to investigate the mechanisms of microbial cooperation, electron transfer, and community sensing between chain elongation microorganisms and other microbes, and tune microbial cooperation through stimulating factors or artificial inducers; (3) suppress side reactions and competition reactions such as anaerobic digestion, which consumed electron donors (e.g., ethanol) and/or electron acceptors such as SCCAs. Competitive reactions could be prevented via (1) using microorganisms with longer carbon chains as inoculum, (2) inhibiting the growth of competing microorganisms, (3) modulating operational parameters such as H_2 partial pressure, and (4) pretreating the substrate [11]. In addition to microbiome optimization, improving the competitive ability of chain elongators to acquire cathodic electrons is also beneficial for generating medium chain carboxylates.

3.2. Metabolic Pathway

It has been reported that the chain elongation process for MCCAs formation includes two pathways, i.e., reversed β -oxidation (RBO) pathway and fatty acid-biosynthesis (FAB) pathway. As seen in Figure 2, for RBO and FAB pathways, acetyl-CoA is a crucial intermediate usually derived from exogenous electron donors, including ethanol and lactate. Ethanol is widely deemed as the superior choice of electron donor, which is transferred to acetyl-CoA via only two steps [64]. Lactic acid, another typical electron donor used in electricity-steering MCCAs fermentation systems, must be converted into pyruvate before producing acetyl-CoA [65]. The key to promoting MCCAs production is providing enough acetyl-CoA via exogenous electron donors or other means. Compared to the RBO process, the FAB pathway is longer because acetyl-CoA is first transformed into malonyl-CoA, which is then converted into malonyl-ACP, and finally participates in the FAB cycle. Moreover, a previous study pointed out that malonyl-ACP synthesis needed one ATP per molecule, and more ATP was devoted to synthesizing ACP intermediates [66], indicating the reduced efficiency of the FAB process. This may be the reason for the wide observation of the RBO pathway in MCCAs-producing functional microbes such as *Clostridium* [67], *Megasphaera elsdenii* [68], and *Caproiciproducens* [69].

However, it is interesting that the FAB pathway is more dominant and critical during the chain elongation process in some mixed-culture systems [27,44,46], especially in MCCAs production driven by electricity. For example, Wang and colleagues used genome-centric metagenomics to reveal that the abundances of essential functional genes such as *accA*, *fabG*, *fadZ*, and *fasH* involved in the FAB pathway were much higher than those of the RBO pathway. This indicated the dominant role of FAB pathway in the microbial

electrosynthesis for the CE process that converted SCCAs with 1–5 carbon atoms and electron donor into MCCAs containing 6–12 carbon atoms [44]. Additionally, the FAB pathway was more active and easier to be enhanced than the RBO pathway accompanied by chain elongators enrichment and microbial redox activity improvement by quorum sensing signal N-octanoyl-L-homoserine lactone (C8-HSL), which promotes the electricity-driven MCCAs formation [46]. In detail, almost every bioconversion step in the FAB pathway had upregulated functional genes with C8-HSL-added. In contrast, in the RBO pathway, only one upregulated gene, K00074, for regulating the conversion of (C_{n+2}) - β -Hydroxyacyl-CoA to (C_{n+2}) -Enoyl-CoA was observed. Overall, exploring the critical factor and underlying mechanisms for determining the type of metabolic pathway during the chain elongation process is an intriguing issue.

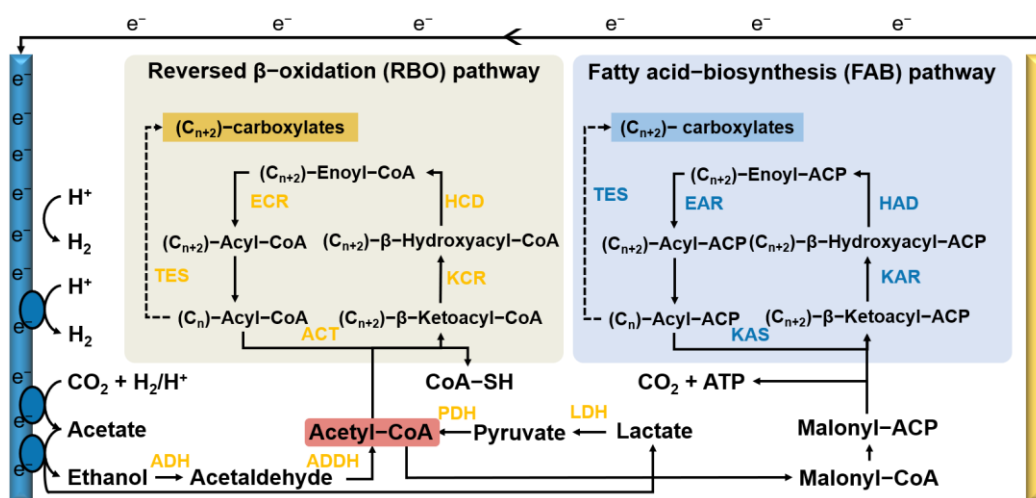


Figure 2. Schematic diagram of RBO and FAB pathways during chain elongation process in MCCAs electrofermentation. Abbreviation: CoA: coenzyme A; ACP: acyl carrier protein; LDH: lactic dehydrogenase; PDH: pyruvate dehydrogenase; ADH: alcohol dehydrogenase; ADDH: acetaldehyde dehydrogenase; ACT: Acetyl-CoA thiolase; KCR: ketoacyl-CoA reductase; HCD: hydroxyacyl-CoA dehydratase; ECR: enoyl-CoA reductase; TES: thioesterase; KAS: ketoacyl-ACP synthase; KAR: ketoacyl-ACP synthase; HAD: hydroxyacyl-ACP dehydratase; EAR: enoyl-ACP reductase [11,43,46].

Microbial metabolism is the cornerstone of electrofermentation. Both the low efficiency of electron transfer between electrogenic microorganisms and electrode interface and the weak ability of biofilm formation limited carbon chain elongation performance in MCCAs electrofermentation system. Firstly, future research is suggested to deeply analyze the electron transfer and biofilm formation mechanism including the variations of cytomembrane that could respond to environmental stimuli [70], based on the electrogenic microorganisms. Then, efforts are encouraged to optimize the electron transfer pathway of electrogenic microbes, improve the level of reducing power, and promote the formation of electroactive biofilm through synthetic biology techniques to design DNA machines, protein-based logic gates along with other components to enhance the electron transfer speed and efficiency. Moreover, the ability of carbon chain elongation microorganisms to obtain electrons directly from the cathode through genetic engineering techniques may significantly increase the selectivity of the products. Based on the microbial–electrode interaction, the role of methods such as cell modification by conductive materials and cell immobilization on the electrode surface in enhancing the electron transfer efficiency between microorganisms and electrodes is suggested to be investigated in depth, which will provide a theoretical basis for the development of new conductive nanomaterials and cell immobilization methods for enhancing MCCAs production.

4. Enhanced Strategies for Electricity-Steering Medium Chain Carboxylic Acids Generation from Organic Waste

The enhancement of medium chain carboxylic acids generation from organic waste is vital. In the last five years, considerable emerging studies have been focused on the electricity driven MCCAs bioproduction from many types of organic substrates (see Figure 3). To improve MCCAs formation, substrate modulation, applied voltage/current regulation, electrode optimization, and microbial cooperation and stimulation strategies have been adopted in previous scientific investigations, which are summarized in Table 2. Moreover, some illustrative details related to these strategies are provided in Figure 4.

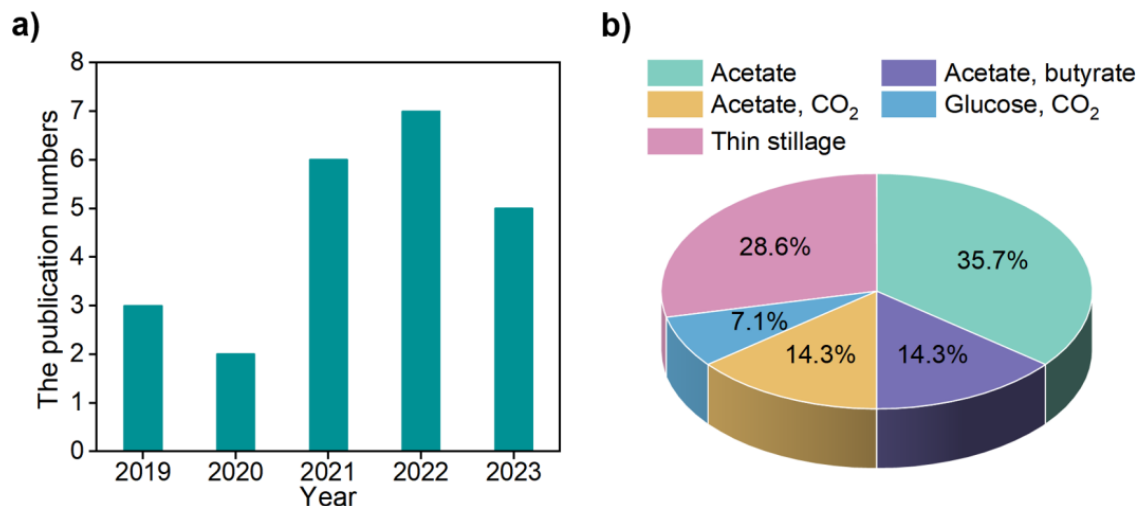


Figure 3. (a) The number of publications in the last five years related to MCCAs electrofermentation; (b) the raw materials used in MCCAs electrofermentation system.

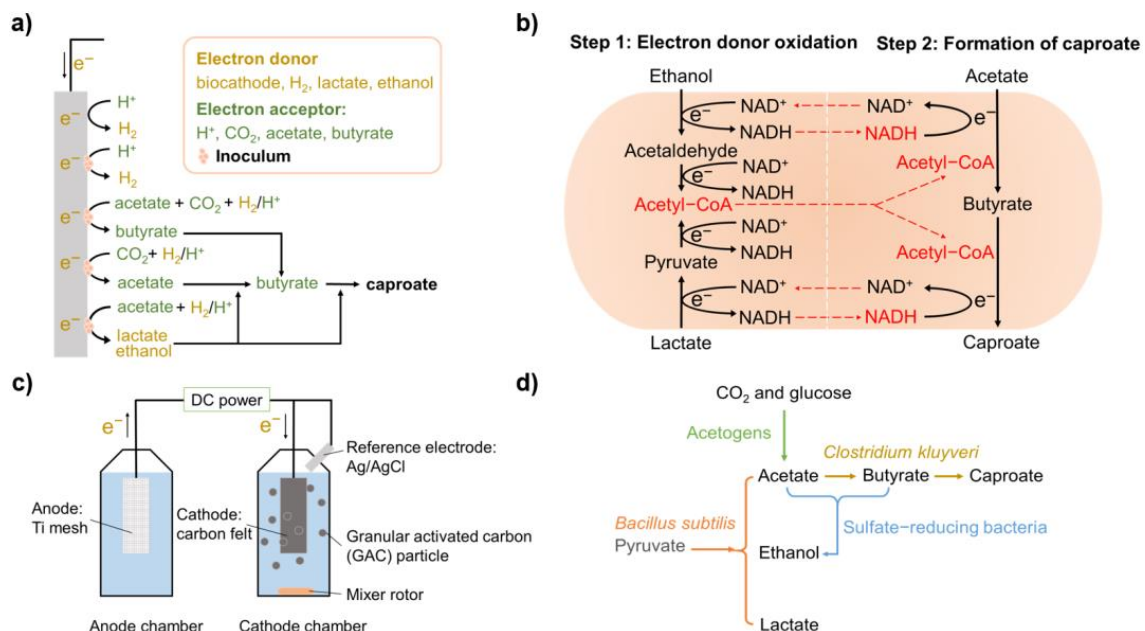


Figure 4. (a) All possible pathways occurring in MCCAs bioelectric production systems with acetate and CO₂ as substrates [43]; (b) reverse β oxidation mechanism and electron transfer direction [24]; (c) schematic diagram of fluidized cathode electric fermentation system [50]; (d) cooperation of microorganisms in bioaugmentation systems [44,45,61,71].

Table 2. Dominant microbes in MCCAs electrofermentation mixed microbial culture systems.

Substrate	Substrate Concentration	Electron Donor	Applied Voltage/Current	Voltage Supply Mode	Cathode Type	MCCAs Concentration or Productivity	Carbon/Electron Conversion or Recovery Rate (%)	MCFAs Selectivity (%)	References
Substrate modulation—composition regulation of the electron acceptor									
Acetic acid	100 mM acetic acid	Cathode; H ₂ and ethanol generated in situ	−0.9 V	Continuous	Graphite felt	739 mg/L caproate, 36 mg/L caprylate	Carbon recovery: 31%, electron recovery: 45%	26%	[37]
Acetic acid, n-butyrate	30 mM acetic acid, 30 mM n-butyrate	Cathode; H ₂ and ethanol generated in situ	29 mA	Continuous	Graphite felt	0.3 g/L caproate	/	83.4%	[41]
Acetate, CO ₂	10 mM acetic acid, 1 L/h CO ₂ gas	Cathode; H ₂ , ethanol, and lactate generated in situ	15 mA	Continuous	Graphite	0.07 ± 0.04 g/(L·d)	Electron recovery: 58.9 ± 9.8%	/	[43]
Substrate modulation—tuning the ratio of electron donor to electron acceptor									
Acetate, ethanol	25 mM acetate	Cathode; H ₂ and ethanol generated in situ	−0.8 V	Continuous	Carbon cloth coated with Pt/C catalyst	501 mg COD/L caproate	Carbon recovery: 51.4%	About 23%	[59]
	25 mM acetate, 37.5 mM ethanol					3016 mg COD/L caproate	Carbon recovery: about 92%	About 76%	
	25 mM acetate, 75 mM ethanol					7454 mg COD/L caproate	Carbon recovery: 98.6%	About 88%	
Substrate modulation—altering substrate concentration									
Thin stillage	0.14 gC/(L·d) acetate, 0.1 gC/(L·d) ethanol	Cathode; ethanol; lactate; H ₂ and ethanol generated in situ	100 mA	Continuous	Pre-enriched acetogens on the cathode carbon felt.	0.057 g COD/(L·d) caproate	Carbon conversion: 60 ± 2%	3%	[22]
	0.28 gC/(L·d) acetate, 0.1 gC/(L·d) ethanol	Cathode; ethanol; lactate; H ₂ and ethanol generated in situ	100 mA	Continuous		1.144 g COD/(L·d) caproate, 0.104 g COD/(L·d) heptanoate	Carbon conversion: 60 ± 11%	12%	
Glucose, CO ₂	0 g/L glucose, 4.2 g/L NaHCO ₃	Cathode; H ₂ and ethanol generated in situ	−1.0 V	Continuous		/	Electron recovery: 27.2%	0%	[44]
	0.1 g/L glucose, 4.2 g/L NaHCO ₃					/	Electron recovery: 23.5%	0%	
	0.2 g/L glucose, 4.2 g/L NaHCO ₃					0.37 ± 0.07 g/L caproate	Electron recovery: 23.1%	12.8 ± 1.5%	

Table 2. Cont.

Substrate	Substrate Concentration	Electron Donor	Applied Voltage/Current	Voltage Supply Mode	Cathode Type	MCCAs Concentration or Productivity	Carbon/Electron Conversion or Recovery Rate (%)	MCFAs Selectivity (%)	References
Tuning applied voltage/current			0			Not detected	/	/	
Acetate	8.34 g/L acetate	Cathode; H ₂ and ethanol generated in situ	−0.6 V	Continuous	Carbon felts	0.12 g/(L·d) caproate	Electron recovery: 84%	/	[38]
			−1.2 V			Not detected	/	/	
			−1.8 V			Not detected	Electron recovery: 22%	/	
			−2.5 V			Not detected	Electron recovery: 20%	/	
			−0.8 V			2.4 g/L caproate approximately	/	About 41%	
Ethanol, CO ₂ , acetate	100 mM ethanol, 25 mM acetate, 4.2 g/L NaHCO ₃ 200 mM ethanol, 50 mM acetate, 4.2 g/L NaHCO ₃ 100 mM ethanol, 25 mM acetate, 4.2 g/L NaHCO ₃ 200 mM ethanol, 50 mM acetate, 4.2 g/L NaHCO ₃	Cathode; ethanol; H ₂ and ethanol generated in situ	−0.8 V	Continuous	Acclimated cathode	7.3 g/L caproate approximately	/	About 73%	[39]
			−1.1 V			1.3 g/L caproate approximately	/	About 20%	
			−1.1 V			4.2 g/L caproate approximately	/	About 45%	
			5 mA			0	Electron recovery: 73.7 ± 12.6%	/	
Acetate, CO ₂	10 mM acetic acid, 1 L/h CO ₂ gas	Cathode; H ₂ , ethanol and lactate generated in situ	15 mA	Continuous	Fresh cathode: graphite	0.07 ± 0.04 g/(L·d)	Electron recovery: 58.9 ± 9.8%	/	[43]
						1.47 g/L caproate	/	/	
Chinese liquor wastewater	11.90 ± 0.14 g/L ethanol, 13.21 ± 0.11 g/L lactate, 8 g/L acetate, 0.08 ± 0.03 g/L propionate, 0.74 ± 0.13 g/L butyrate, 0.98 ± 0.04 g/L caproate	Cathode; ethanol; lactate; H ₂ and ethanol generated in situ	−0.8 V	Electrical stimulation in first half (0–6 d)	/	2.14 g/L caproate	/	/	[24]
				Electrical stimulation in first half (6–12 d)		1.93 g/L caproate	/	/	
				Continuous electrical stimulation (0–12 d)		0.55 g/L caproate	/	/	
				Without electricity					

Table 2. Cont.

Substrate	Substrate Concentration	Electron Donor	Applied Voltage/Current	Voltage Supply Mode	Cathode Type	MCCAs Concentration or Productivity	Carbon/Electron Conversion or Recovery Rate (%)	MCFAs Selectivity (%)	References
Electrode acclimation and cathode material optimization									
Ethanol, CO ₂ , acetate	100 mM ethanol, 50 mM acetate, 4.2 g/L NaHCO ₃	Cathode; ethanol; H ₂ and ethanol generated in situ	−0.8 V	Continuous	Fresh cathode: carbon felt	2.0 g/L caproate approximately	Electron recovery: 101.28% ± 7.88%	36.16 ± 1.67%	[39]
	100 mM ethanol, 50 mM acetate, 4.2 g/L NaHCO ₃		−1.1 V	Continuous		1.8 g/L caproate approximately	Electron recovery: 93.45% ± 0.11%	36.22 ± 1.89%	
	100 mM ethanol, 25 mM acetate, 4.2 g/L NaHCO ₃		−0.8 V	Continuous	Acclimated cathode	2.4 g/L caproate approximately	/	About 41%	
	200 mM ethanol, 50 mM acetate, 4.2 g/L NaHCO ₃		−0.8 V	Continuous		7.3 g/L caproate approximately	/	About 74%	
	300 mM ethanol, 75 mM acetate, 4.2 g/L NaHCO ₃		−1.1 V	Continuous		6.0 g/L caproate approximately	/	About 38%	
Acetate, butyrate	5.0 g/L acetate, 50 mmol/L butyrate	Cathode; H ₂ and ethanol generated in situ	−0.95 V	Continuous	Fresh fluidized cathode: carbon felt	3.4 ± 0.2 mM caproate	Carbon recovery: about 39%; electron recovery: about 35%	/	[50]
					Fresh fluidized cathode: carbon felt with 8% filling ratio granular activated carbon	7.8 ± 0.4 mM caproate	Carbon recovery: 69.3 ± 0.5%; electron recovery: 61.1% ± 1.9%	/	
Glucose, CO ₂	0.2 g/L glucose, 4.2 g/L NaHCO ₃	Cathode; H ₂ and ethanol generated in situ	−1.0 V	Continuous	Fresh cathode without acetogens	Not detected	Electron recovery: 70.3%	/	[44]
					Pre-enrichment of acetogens on the cathode carbon felt	0.37 ± 0.07 g/L caproate	Electron recovery: 23.1%	12.8 ± 1.5%	

Table 2. Cont.

Substrate	Substrate Concentration	Electron Donor	Applied Voltage/Current	Voltage Supply Mode	Cathode Type	MCCAs Concentration or Productivity	Carbon/Electron Conversion or Recovery Rate (%)	MCFAs Selectivity (%)	References
Microbial cooperation and stimulation									
Ethanol, acetate	6 g/L ethanol, 2.0 g/L sodium acetate	Cathode; ethanol; H ₂ and ethanol generated in situ	−0.7 V	Continuous	Acclimated cathode carbon felts	2.45 g/L caproate 4.68 g/L caproate (bioaugmentation by <i>C. kluyveri</i>) 3.77 g/L caproate (with quorum sensing signals)	Carbon conversion: 83.37% Carbon conversion: 92.07%	/	[45]
Ethanol, acetate	6 g/L ethanol, 2.73 g/L sodium acetate	Cathode; ethanol; H ₂ and ethanol generated in situ	−0.7 V	Continuous	Acclimated cathode carbon felts	10 µM C6-HSL 4.30 g/L caproate (10 µM C8-HSL) 3.96 g/L caproate (10 µM 3OC10-HSL)	/	/	
Glucose, CO ₂	0.2 g/L glucose, 4.2 g/L NaHCO ₃	Cathode; ethanol and H ₂ generate in situ	−1.0 V	Continuous	Fresh cathode without acetogens pre-enrichment	Not detected	Electron recovery: 70.3%	/	[44]
					Pre-enriched acetogens on the cathode carbon felt	0.37 ± 0.07 g/L caproate	Electron recovery: 23.1%	12.8 ± 1.5%	

4.1. Substrate Modulation

The regulation of the composition and concentration of substrate can promote the carbon chain elongation process in electricity-steering MCCAs generation. The composition of the electron acceptor influenced the synthesis of MCCAs when no external electron donor was added (Figure 4a). When only acetate was used as the electron acceptor for MCCAs synthesis, the carbon chain elongation reaction in the cathode chamber produced MCCAs, including caproate and caprylate, without adding electron donors such as ethanol and hydrogen. Still, the MCCAs selectivity was only 26%, and the efficiencies of carbon recovery and electron recovery were both less than 50% [37]. The main reason was that H_2 diffused through the membrane to the anode, and other electron acceptors at the cathode, such as sulfate, scrambled electrons [37]. When acetate and n-butyrate were used together as carbon sources and electron acceptors, MCCAs selectivity could reach 83.4% due to the thermodynamic advantage of n-butyrate over acetate for synthesizing caproate [41]. Moreover, the electron recovery efficiency in MCCAs electrofermentation system could be improved by 58.9% when using inorganic carbon CO_2 and organic acetic acid together as substrates [43]. In this process, CO_2 and H_2 are metabolized by autotrophic acetic acid-producing strains to biosynthesize acetic acid as a substrate for carbon chain elongation. Some microorganisms could use acetate and CO_2 directly to achieve carbon chain elongation.

Furthermore, the ratio of electron donor to electron acceptor is an important parameter affecting carbon chain elongation (Table 2). Cheng et al. found that caproate yield and selectivity were promoted as the ratio of electron donor to electron acceptor increased [59], but electron recovery efficiency decreased with the diffusion of H_2 . The modulation of substrate concentrations could boost the synthesis of MCCAs (Table 2). When using thin stillage as substrate, the elevation of the organic load and current resulted in an increase of the formation and proportion of caproate in the product spectrum and induced heptanoate generation. This was because the electrofermentation process could produce more H_2 and lactate as electron donors for fermentation when increasing substrate concentration and applying current [22]. In the domesticated cathode systems, the efficiency of electrofermentation depended on the substrate concentration. As the substrate concentration increased, the selectivity of caproate tended to increase first and then decrease due to excessive ethanol and caproate toxicity to microbes [39]. Furthermore, the substrate concentration affected not only the concentration and selectivity of MCCAs, but also the composition of the microbial community. This is because different caproate-synthesizing microorganisms have different tolerance to substrate concentrations. *Caproiciproducens* could endure high ethanol and lactic acid concentrations, but *Rummeliibacillus* had a limited metabolic behavior in environments with high ethanol concentrations, and *Clostridium sensu stricto* 12 was the major caproate producing microorganism at 40% substrate concentration when using liquor waste water as feedstock [23].

Moreover, a fermentative hydrogen production system can produce caproic and heptanoic acid with higher concentrations than that of electro-fermentation systems. This may be because the fermentative hydrogen production system not only supplemented a mass of costly electron donors but also used some enhanced methods, e.g., increasing reaction time, and complementing iron, nitrogen, and phosphorus [72,73]. Although the MCCAs yield in the electrofermentation system was tentatively lower, the sustainable production mode of this system, such as free of vast external electron donors' addition, makes it an appealing and alternative fermentation strategy.

4.2. Tuning Applied Voltage/Current

The modulation of the applied voltage is vital to optimizing the synthesis of MCCAs in bio-electrofermentation systems. It was reported that a voltage of -0.6 V to -1.2 V is a suitable interval for performing carbon chain elongation [38]. In this range, the substrate acetate is first converted to ethanol in the presence of a biocathode, followed by the formation of butyrate and caproate. Most of the electron donor comes from ethanol

generated in situ, with 10% coming directly from the electrode (including H₂-mediated electron transfer from the electrode to the biofilm) [38]. Raes et al. also found that the increased current boosted the yield of n-butyrate by 1.5 times and that only at higher currents was caproate of synthesis (Table 2) [43]. More interestingly, the pathway of electron transfer can be regulated by adjusting the voltage. For example, *Bacillus subtilis* metabolizes pyruvate to form the highest amount of ethanol and acetic acid at -0.8 V and lactate at -0.6 V, respectively. These metabolic products are all carbon chain extension substrates [71]. Therefore, in systems containing *Bacillus subtilis*, the carbon chain elongation process can be indirectly regulated by manipulating the voltage.

In addition, exceeding the optimum voltage adversely affected the concentration and selectivity of caproate synthesis. Jiang and colleagues found that the maximum selectivity of caproate synthesis at a voltage of -0.8 V was about 74%, which was significantly higher than that of caproate synthesis at -1.1 V (about 40%) due to the reason that excessive hydrogen partial pressure at higher voltage inhibited the synthesis of MCCAs by restraining microbial activity [39]. When the voltage exceeds 1.8 V, microorganisms lost their ability to transfer electrons to and from the electrode, and the electrons were consumed mainly by acetate to produce H₂ (diffused into the headspace rather than dissolved in water), CO₂, and other carbon-containing substances, and microorganisms metabolized acetate for their energy supply rather than ethanol and MCCAs synthesis [38]. The voltage regulation could produce enough amount of H₂ to mediate electron transfer without exogenous electron mediators addition [74], prevent oxidation of SCCAs, and provide electrons for fixing CO₂ to form acetic acid in the presence of acetic acid-producing bacteria [43]. Moreover, it is well-known that H₂ and CO₂ can also be converted to acetate by homoacetogens without electrodes, so voltage tuning could couple different microbial processes not needing the permanent addition of electricity for producing needed intermediaries for MCCAs production. The suitable voltage created the appropriate environment (including pH and redox potential) for the growth of carbon-chain-elongating microorganisms so that the biocathode has a more efficient catalytic effect. Therefore, manipulating voltage is a crucial tool to augment the synthesis of MCCAs.

Notably, the intermittent voltage supply was more favorable to promoting carbon chain elongation than the continuous voltage supply mode (Table 2). This is because the reverse β oxidation of MCCAs synthesis is divided into two steps (Figure 4b). The first step is the conversion of lactate and ethanol to acetyl-CoA. In the second step, acetyl-CoA is converted back to butyrate and caproate. Biocathode, as an exogenous electron donor, could promote the reduction reaction in the second part and suppress the oxidation reaction of the electron donor in the first step. Hence, the synthesis of caproic acid was more favorably promoted by applying voltage only in the second step [24]. Compared to the control fermentation system without applied electricity, the continuously powered bioelectric fermentation triggered faster substrate consumption and a higher caproate yield of 250.9%. Nonetheless applied voltage only to the second step of reverse β oxidation was more effective, which increased the synthesis of hexanoic acid by 288.5% [24]. From the microflora point of view, an open circuit in the first stage was more favorable for the enrichment of carbon chain-lengthening functional microorganisms, especially *Clostridium* 12, while in the second stage, electrical stimulation not only favored the enrichment of carbon chain-lengthening functional microorganisms but also reduced the abundance of microorganisms with negative effects on carbon chain lengthening [24].

4.3. Electrode Acclimation and Cathode Material Optimization

In the bioelectric fermentation system for producing MCCAs, electrodes could be fresh or acclimated. A fresh electrode means that the electrode is free of microorganisms at the beginning of the experiment, and the microbes are directly inoculated in the cathode chamber. In contrast, an acclimated cathode is one in which the microorganisms are pre-attached to a biofilm on the cathode before the fermentation [39]. Although most experiments used fresh cathodes because the experimental operation process is more straightforward [43,50],

adopting an acclimated cathode could enhance the caproate yield under appropriate substrate concentration (see Table 2). Jiang et al. compared the effects of a fresh carbon felt cathode (stage I) and an acclimated cathode with the formation of biofilm (stage II) on the chain elongation process, and the microbes in stage II came from stage I [39]. The microbial analysis showed that microbes in the cathode biofilm were different from planktonic microorganisms in the acclimated cathode system, and the relative abundance of planktonic chain elongation functional microorganism *Clostridium_sensu_stricto_12* was higher than that of *Clostridium_sensu_stricto_12* in the biofilm in the acclimated cathode system, and the relative abundance of the acetate-producing bacteria *Acetobacterium* on the biofilm increased when applying electricity. Compared with the planktonic condition, biofilm formation on the acclimated electrode improved resistance to ethanol and caproate [39].

The optimization of the cathode's texture and morphology could also promote fermentation efficiency. In previous investigations, the cathode material was mainly carbon felt, but the small specific surface area of carbon felt impeded the reaction efficiency. For this situation, Ma et al. used granular activated carbon (GAC) to construct a fluidic electrode (see Figure 4c) [50]. When the cathode was used as the sole electron donor, the current, caproate yield, carbon recovery, and electron recovery efficiency were increased by 3.1, 2.1, 1.8, and 1.6 times, respectively, at an 8% filling rate of GAC compared to the control group without GAC filling, and the relative abundance of functional microbiota for chain elongation on the cathodic biofilm was increased. The reason was that GAC has a porous structure with a large specific surface area, which could supply many contact sites for microbes and improve the contact area between feedstocks and microbes. However, when the filling amount of GAC was too high, it led to elevated internal resistance, hindering electron transfer and reducing the production of MCCAs [50].

4.4. Microbial Cooperation and Stimulation

In mixed microbial systems for the synthesis of MCCAs, the cooperation of microorganisms can reinforce the tolerance of microorganisms to unfavorable environments and expand the range of fermentation substrates, promoting the carbon chain elongation efficiency. The external addition of microorganisms to achieve biofortification is an alternative method to construct powerful MCCAs-producing microbial consortia (Figure 4d). One recent bioaugmentation study was the addition of *Clostridium kluyveri*, a carbon chain elongator, directly improved the elongation efficiency, which increased caproic acid production by 41.62% compared to the control group (Table 2) [45]. This is because the electric field could promote the colonization of *C. kluyveri* at the cathode. The electrons and H₂ produced in situ at the cathode boost the fixation of CO₂ for acetic acid production by the acetic acid-producing bacterium *Acetobacterium woodii*, so *A. woodii* and *C. kluyveri* had a synergistic effect of increasing the production of caproate in the electrofermentation system [45].

Other promising bioaugmentation channels were conducted by introducing satellite microbes of chain elongation strains, such as *Bacillus subtilis*, sulfate-reducing bacteria, and acetogens, which can provide electron donors and/or acceptors for the MCCAs-producing process. This was because *Bacillus subtilis* could metabolize pyruvate to produce ethanol, acetate, and lactate [71], sulfate-reducing bacteria have been shown to consume acetate and butyrate to produce alcohols and acetone by direct electron transfer [61], and acetogens can use CO₂ and glucose to produce acetate [44]. Thus, a wider variety of substances can be converted into substrates for carbon chain elongation, promoting MCCAs synthesis and improving carbon chain elongation applications.

In addition, quorum sensing signals could stimulate microbial interactions and cooperation, promoting biofilm formation and carbon chain elongation. N-acyl homoserine lactones are a typical signaling molecule that could mediate population sensing among gram-negative bacteria, which regulated biofilm formation [75]. In MCCAs electrofermentation systems with acetate and ethanol as substrates, the addition of acyl-homoserine lactones such as N-hexanoyl-L-homoserine lactone (C6-HSL), N-octanoyl-L-homoserine lactone (C8-HSL), and N-(3-oxodecanoyl)-L-homoserine lactone (3OC10-HSL) all increased

microbial attachment to the electrode and the proportion of live cells in the biofilm, improved redox activity in the electroactive biofilm, and significantly boosted the caproate production (Table 2) [46]. Microbial community analysis showed that acyl homoserine lactones (AHLs) significantly enhanced the relative activity of *Negativicutes* (chain elongation Gram-negative bacteria). The most apparent effect of the addition of C8-HSL on the caproate synthesis was 61.48% higher than that of the control group. Metatranscriptomic analysis showed that C8-HSL increased CoA-transferase activity and promoted carbon chain elongation metabolism, especially the fatty acid biosynthesis pathway [46].

Collectively, the yield, selectivity, and electron recovery efficiency of MCCAs in bioelectric fermentation systems can be improved by regulating the concentration and composition of the substrate, the level and supply mode of voltage, the material and morphology of electrode, and the cooperation between microbial species. Nevertheless, there is still a lot of space for improvement, such as seeking more competitive cathode materials than carbon felt used at present. Although some pioneering studies improved the specific surface area by adding granular activated carbon, scarce attention has been devoted to the modification of cathode material, such as the combination of nanomaterials with biological enzymes. In addition, the effects of acclimated and fresh cathodes on MCCAs synthesis under the same substrate conditions need investigation, which can provide the data basis for biofilm formation. Moreover, the electron transfer between electrode and microorganism is mainly reported to be mediated by H_2 rather than direct electron transfer. However, the solubility of H_2 in water is low and predominantly diffuses to the headspace, thus causing electron loss. In addition, the present studies argued that the interaction between microorganisms in the bioaugmentation system was mostly substance transfer, but did not notice electron transfer, which is a well-known vital interaction mode in the microbiome. The bio-electric fermentation systems are feasible and stable in long-term operations, which shows great potential for industry application [76]. However, the relative abundance shift of different microbes during long term operation is seldom reported. Therefore, the underlying process of bioaugmentation by key strains, such as electroactive microorganisms promoting carbon chain elongation, and the influence of possible microbial population shift when long term operation need further to be deciphered.

Furthermore, the electrofermentation synthesis of MCCAs is mostly a lab-scale study, and numerous factors must be considered to move from the lab to practical applications. For example, it needs to be determined whether the system is based on biofilms or planktonic cells, as this factor affects the reactor design. If the system is based on biofilm, the biocompatibility of the electrodes and the microbial enrichment capacity need to be improved. In the case of planktonic-based systems, there is a need to justify the electron transfer medium to boost electron transfer and to maintain suitable biomass residence time and appropriate reaction conditions. Moreover, ports are needed in the reactor design to allow for the housing of multiple electrodes, which can expand these electrodes' radius of influence. The scale-up MCCAs electrofermentation systems also require the design of simple and robust reactors and facilitate real-time feedstock adjustment and operation time according to microbial growth. Overall, the optimization of electrode materials, reactor structure, volume, electrode-specific surface area, and electrode chamber volume ratio should also be considered to improve the synthesis yield, rate, and selectivity of the target products in large-scale MCCAs production.

5. Future Perspectives

With the development of large-scale MCCAs production, the substrate supply needs to be more stable, more economical, and widely available. Except for the reported substrates used in electricity-driven MCCAs production systems, the widely present biomass and organic waste such as grass, waste activated sludge, and food waste could be good feedstock sources. Appropriate pretreatment is required for structurally complex substrates, such as residual sewage sludge or food waste. Although numerous pretreatment tools have been reported for promoting the production of chain elongation precursors and caproic

acid [77–79], the combination of pretreatment tools and electrofermentation is still less well investigated. In addition to applying pretreated fermentation broth for multiphase electrofermentation, the combination of pretreatment and electrofermentation systems has opened a new research frontier. For example, it is interesting to explore whether electric field stimulation under alkaline sludge fermentation conditions would further improve the hydrolysis of difficult-to-degrade organic matters, such as proteins, and whether it would influence the enrichment of chain elongation microbes.

The purpose of pretreatment is to hydrolyze complex organic matter into smaller intermediate molecules, such as glucose, amino acids, and fatty acids. The differential structure and configuration significantly impact their biotransformations. For instance, the short chain carboxylic acids production from the widely present D (dextrorotatory)-amino acid was dramatically lower than that from corresponding L-enantiomers, i.e., L (levorotatory)-amino acid [80]. It could be speculated that the metabolic pathways and metabolic rates of microbes using different structural and configurational substrates in a mixed-culture electrofermentation system vary widely. Nevertheless, as for these vital intermediate products, not only their structural and conformational change but the interaction effects among these organic substances have not been investigated in MCCAs electrofermentation so far. Therefore, the expansion of feedstock sources, the investigation of substrate–substrate and substrate–microbe interactions, and the methods of enhancing substrate transformation could help construct a metabolic network for MCCAs electrofermentation and achieve targeted product accumulation through substrate regulation.

Expanding the product spectrum of carbon chain elongation, such as producing branched MCCAs and medium chain bio-alkanes, is vital to improving the value and charm of the carboxylate fermentation platform. It was reported that branched MCCAs have a higher viscosity, lower boiling point, and higher oxidative stability [30]. The addition of branched MCCAs to the substrate of Kolbe electrolysis can produce more fuel, such as octane [81]. The accumulation of MCCAs is known to be deleterious to CE biocatalysts by inhibiting microbial growth and metabolism, necessitating further effort in product separation. A recent discovery reported the transformation of MCCAs to value-added bio-alkanes with higher energy density by integrating photo/bio/electrochemical catalysis [82]. Considering the diverse use, valorization opportunities, and high economic value of renewable bio-alkanes [82], this novel innovative circular cascading system offers a promising pathway for simultaneous product separation and valorization of MCCAs electrofermentation whilst achieving carbon circularity [1]. Nonetheless, progressing to the future application of this system needs further efforts to synthesize longer-chain bio-alkanes, increase product yield and selectivity, and reduce the economic cost.

Based on all the above information, although encouraging results have been acquired so far, there are several research gaps and existing challenges ahead before electricity-driven MCCAs bioproduction with stable high-yield performance and realistic. Future potential directions are recommended in the following aspects: (1) expanding feedstock sources and promoting substrate utilization, (2) widening product spectrum and improving selectivity, (3) deepening the inward mechanism of microbe–electricity interaction and shaping a more efficient microbiome, and (4) optimizing operation process and transitioning from laboratory to scale-up industrial level.

6. Conclusions

The present review article systematically analyzed and summarized the microbial production of MCCAs from organic waste steered by electricity. First, the fundamental working principle, fermentation architecture, functional microflora, and metabolic pathway of electricity-driven MCCAs production were comprehensively covered. We then scrutinized and extensively discussed the strategies of substrate modulation, applied voltage/current regulation, electrode acclimation, and cathode material optimization, and microbial cooperation and stimulation for boosting electricity-driven MCCAs synthesis. Last but not least, the pressing challenges and the potential avenue are proposed, providing

clues for developing MCCAs electrofermentation as an effective technology for organic waste valorization.

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