

Converting Biowastes into Medium-chain Fatty Acid via Microbial Chain Elongation: A Mini Review

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ABSTRACT: The valorisation of organic biowastes into medium-chain fatty acids (MCFA) via microbial chain elongation has emerged as a promising strategy to recover carbon and energy while supporting a circular bioeconomy. MCFA production is primarily driven by the reversed β -oxidation (RBO) pathway, in which short-chain carboxylates are elongated using electron donors such as ethanol or lactate. Compared with conventional disposal routes or petrochemical synthesis, microbial chain elongation enables the sustainable conversion of diverse waste streams into high-value platform chemicals applicable to fuels, solvents, and specialty chemicals. This mini-review summarises recent advances in MCFA production from biowastes, with a particular focus on the biochemical fundamentals of the RBO pathway, key chain-elongating microorganisms, and the roles of different electron donors and carbon sources. We further discuss process engineering and ecological strategies for enhancing MCFA yields and steering product specificity, including feedstock pretreatment, electron donor supplementation, and regulation of operating parameters such as temperature, pH, and extraction. Finally, current challenges and future perspectives are highlighted, with emphasis on the apparent biological limitation to high-speed MCFA products.

Keywords: Caproate, Biowaste, Chain elongation, Reversed β -oxidation, Medium-chain fatty acids.

1. INTRODUCTION

The accelerating generation of organic waste worldwide, including food waste, agricultural residues and sewage sludge, poses a serious environmental and economic challenge [1]. Conventional treatment processes such as landfilling and incineration are increasingly recognised as unsustainable because they contribute to greenhouse gas emissions, degrade soil and water quality and squander the carbon and nutrient value embedded in biomass [2][3]. Addressing this problem requires technologies that both mitigate environmental harms and recover value from biowaste in order to support a circular bioeconomy. Currently, converting biowastes into high-value liquid chemicals such as medium-chain fatty acids (MCFA) via anaerobic fermentation has attracted great

attention for their role as primary precursors to produce jet fuels [4].

MCFA generally refers to the carboxylic acid (some articles use carboxylate) with 6-12 carbon atoms [5]. In anaerobic fermentation, the primary MCFA products include *n*-caproate (C6), heptanoate (C7) and caprylate (C8) [6]. Microbial production of MCFA is associated with a microbial metabolism process called chain elongation (CE) [7]. In this process, ethanol or lactic acid

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acting as electron donor drive the carbon chain of short-chain fatty acids (SCFAs) into medium chain by transferring two carbon atoms of ED in per reversed β -oxidation (RBO) cycle [8][9]. Compared to traditional chemical-based MCFA production, the microbial production provides a potential for lower production costs and a sustainable and environmentally friendly alternative for the circular economy.

Given current research progress in microbial chain elongation, this review aims to comprehensively summarize the MCFA production from various biowaste. To this end, this review discussed the mechanisms of microbial chain elongation process especially the main enzymes participating in RBO, analyzed the key microorganisms associated with chain elongation and summarized the commonly used electron donors and carbon sources in biowaste valorization. In addition, this review highlighted some strategies for enhancing microbial MCFA production. Finally, some perspectives were proposed to pave the path for practical application of microbial MCFA production from biowastes.

■ 2. FUNDAMENTALS OF MICROBIAL CHAIN ELONGATION

■ 2.1. Reversed β -oxidation Process

Biosynthesis of MCFA is fundamentally driven by the RBO cycle, a metabolic process dependent on the continuous availability of acetyl-CoA and reducing equivalents (Fig. 1) [10]. This process is initiated by the oxidation of electron donors (e.g., ethanol and lactic acid), which not only provide the carbon sources but also give the metabolic energy [11]. In terms of ethanol-based CE process, modeled extensively after *Clostridium kluyveri*, the metabolic flux is rigorously partitioned due to thermodynamic constraints. Research indicated that approximately one-sixth of the ethanol is oxidized to acetate to generate initial energy via substrate-level phosphorylation, while the remaining ethanol is converted into acetyl-CoA, which serves as the primer for the cyclic elongation process [4]. This cycle integrates acetyl-CoA with acyl-CoA intermediates (e.g., acetyl-CoA and butyryl-CoA), utilizing NADH and FADH₂ to elongate the carbon chain by two units per cycle, progressively forming *n*-butyrate and *n*-caproate [12].

A critical feature of the RBO pathway is its unique energy conservation mechanism [13]. While the most enzymes involved in CE are cytosolic, energy recovery is in the cell membrane. In *C. kluyveri*, this energy metabolic process relies on two key membrane-associated complexes, i.e.,

the ferredoxin:NAD oxidoreductase and ATP-synthase [14]. The thermodynamic driving force for CE process is generated during the reductive steps of the RBO cycle [15]. Specifically, the reduction of crotonyl-CoA to butyryl-CoA (catalyzed by butyryl-CoA dehydrogenase) and the analogous reduction of 5-hex-2-enoyl-CoA to hexanoyl-CoA (catalyzed by hexanoyl-CoA dehydrogenase) are energetically favorable. These exergonic reactions are coupled to the reduction of ferredoxin by NADH. The subsequent oxidation of reduced ferredoxin (Fd_{red}) and reduction of NAD⁺ drives the translocation of protons across the cell membrane, establishing a proton motive force that powers ATP synthesis [15,16]. Thus, the metabolic flow of hydrogen during chain elongation is intrinsically linked to cellular energy gain, allowing the bacteria to thrive even under thermodynamic pressure.

While ethanol provides a pathway for acetyl-CoA and NADH generation, lactic acid serves as an effective alternative electron donor. Lactic acid oxidation yields pyruvate, which is subsequently decarboxylated to acetyl-CoA and CO₂ [9]. However, lactic acid utilization is frequently complicated by the competing acrylate pathway, which directs carbon flux toward propionate production [9,17]. Although theoretical redox balancing suggests that propionate accumulation acts as an unavoidable electron sink, empirical evidence indicates that this competition is microbiome dependent [18]. For instance, certain open-culture systems exhibit negligible propionate formation even at varying lactate concentrations [19]. Furthermore, the Wood-Ljungdahl pathway may function as an auxiliary route, fixing CO₂ into acetyl-CoA precursors to supplement the RBO cycle.

The theoretical potential of the RBO cycle suggests indefinite chain elongation. However, accumulation of MCCs beyond C8 is rarely observed. Historically, this ceiling was attributed to the antimicrobial toxicity of longer-chain fatty acids, which disrupt cell membranes [10]. However, recent evidence challenges the notion that toxicity is the sole limiting factor. In systems employing efficient in-line extraction to maintain undissociated carboxylates below toxic thresholds, production still halts at C8 with no detectable longer-chain acid [20]. This strongly suggests that the limitation is enzymatic rather than purely toxicity-driven due to the steric specificity of the acyl-CoA dehydrogenases or transferases preventing the accommodation of carbon chains longer than C8.

■ 2.2. Key Microorganisms

To date, the capability for chain elongation via the RBO pathway has been identified across a diverse range of

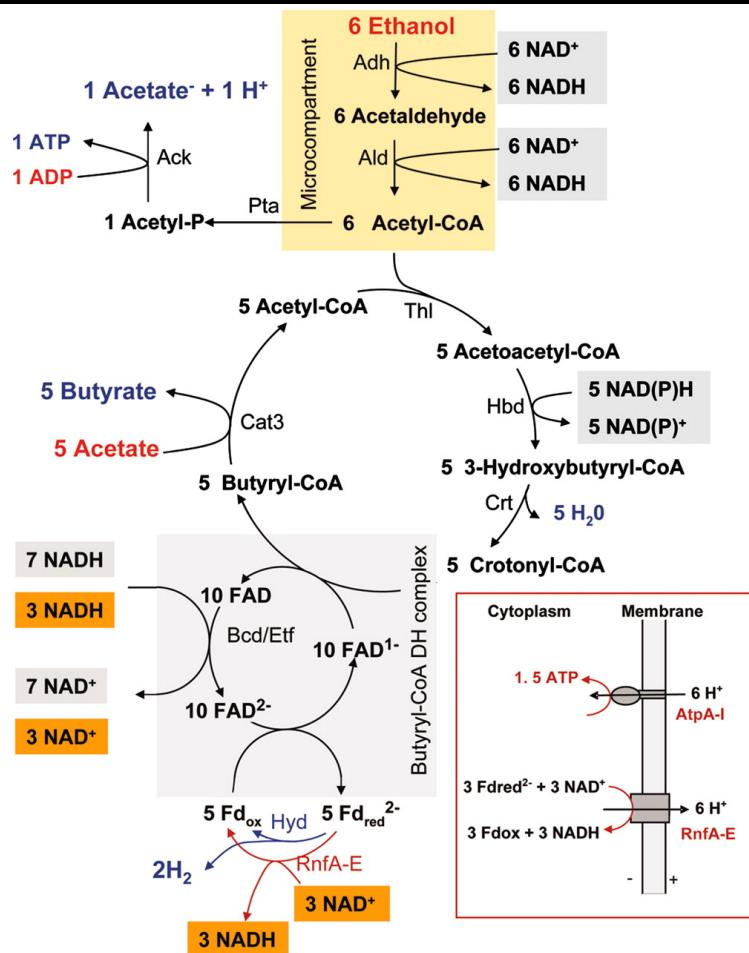


Figure 1: The metabolic process of RBO cycle [25]. Copyright © 2008 by The National Academy of Sciences of the USA.

phylogenetic groups. While all known chain elongators belong to the phylum *Firmicutes*, they exhibit distinct substrate preferences and metabolic strategies [6][21]. Here, we discuss the most significant genera: *Clostridium*, *Eubacterium*, *Megasphaera*, and some genera in *Ruminococcaceae* family [10,22].

Clostridium is the most well-documented genus capable of chain elongation, with *Clostridium kluyveri* as the model species for understanding the bioenergetics of this process [23]. *C. kluyveri*, initially isolated from canal mud, is a specialist that relies on ethanol as an electron donor to elongate acetate into C4 and C6 [24]. The metabolic superiority of *C. kluyveri* lies in its unique energy conservation system, which couples the exergonic reduction of crotonyl-CoA with the endergonic reduction of ferredoxin via electron bifurcation, subsequently driving ATP synthesis through the membrane-bound Rnf complex [25]. Furthermore, MCFA production in this genus is tightly regulated by the availability of inorganic carbon, which is essential not only for biomass synthesis but also for maintaining electron transport and cofactor regeneration [23].

Unlike the ethanol-specialist *C. kluyveri*, the genus *Eubacterium* demonstrates metabolic versatility regarding electron donors [26]. *Eubacterium limosum* is a notable species capable of utilizing one-carbon compounds, such as methanol, as an electron donor to elongate acetate into *n*-butyrate and *n*-caproate [27]. This trait makes it particularly relevant for syngas-based fermentation platforms. Another key species, *Eubacterium pyruvativorans*, originally isolated from the rumen, lacks the ability to utilize ethanol but can perform chain elongation using pyruvate, amino acids, and lactate [28]. Although members of *Eubacterium* (family *Eubacteriaceae*) have been consistently identified in chain-elongating microbiomes, genomic analyses suggest that not all members utilize the classical RBO pathway, implying the existence of alternative or distinct elongation mechanisms within this genus [29].

The genus *Megasphaera*, belonging to the class *Negativicutes*, represents a crucial group of lactate-driven chain elongators [30]. *Megasphaera elsdenii* is the best-characterized species in this group and is capable of converting lactate and sugars (e.g., glucose, fructose)

into even-chain carboxylates, including *n*-caproate [29,29,31]. A critical physiological feature of *M. elsdonii* is its substrate-dependent product profile; while it produces *n*-caproate from glucose, its elongation capability on lactate alone can be variable and condition-dependent. *Megasphaera* species are often found in competitive environments where they must contend with propionate producers (e.g., via the acrylate pathway) [31]. Recent phylogenetic analyses have expanded this group to include novel species such as *Megasphaera hexanoica*, further highlighting the importance of this genus in lactate-rich fermentation systems, such as those fed with acid whey or food waste [30,32].

The family *Ruminococcaceae* (taxonomically updated and often overlapping with *Oscillospiraceae*) has emerged as a functional core in mixed-culture chain elongation, particularly in systems fed with complex organic wastes or lactate [33,34]. Recent metagenomic and isolation efforts have identified *Caproiciproducens* spp. (e.g., *C. galactitolivorans*) and *Ruminococcaceae bacterium* CPB6 as prolific producers of *n*-caproate from lactate and carbohydrates [34,35]. Ecological studies by Wang *et al.* identified that *Caproiciproducens* and *Oscillospiraceae* exhibit high resilience to fluctuating operating conditions [20]. Notably, specific populations within this group show distinct substrate preferences; for instance, certain *Oscillospiraceae* lineages are positively correlated with high ethanol-to-lactate ratios and are instrumental in steering production toward longer chains like C8 [20]. This genus represents a primary target for ecological engineering strategies aimed at optimizing MCC specificity.

2.3. Electron Donors and Carbon Sources

The feasibility and efficiency of microbial chain elongation are intrinsically dictated by the availability of suitable electron donors [36]. These substrates play a dual role. On the one hand, they provide the carbon backbone (as acetyl-CoA) for chain extension and other hand, the reducing equivalents (NADH/NADPH) required to drive the thermodynamically constrained RBO cycles. While a variety of reduced compounds can theoretically fuel this process, their thermodynamic properties and metabolic pathways significantly influence the final product spectrum and the composition of the active microbiome [37,38].

2.3.1. Ethanol and other Alcohols

Ethanol represents the most established and thermodynamically favorable electron donor for chain

elongation and serves as the primary substrate for the model organism *Clostridium kluyveri* [23]. The oxidation of ethanol to acetyl-CoA generates two moles of NADH which provides abundant reducing power to drive the energy intensive reduction of crotonyl-CoA to butyryl-CoA via the Bcd-Etf complex [10]. Recent investigations have further highlighted the critical role of ethanol in driving deep chain elongation processes where research demonstrated that maintaining a high ethanol to lactate ratio creates a thermodynamic environment that favors the synthesis of *n*-caprylate over *n*-caproate while steering the core microbiome toward *Oscillospiraceae* dominance [20]. Beyond ethanol, other alcohols like methanol and propanol possess potential as electron donors yet their application in open culture systems remains limited [37,39]. Propanol utilization typically results in the formation of odd-chain carboxylates such as *n*-valerate or *n*-heptanoate while methanol is primarily utilized by specific specialists like *Eubacterium limosum* rather than the broader chain elongating community [39].

2.3.2. Lactic Acid

Lactic acid has emerged as another pivotal electron donor due to its prevalence in fermented organic waste streams like food waste and acid whey [17]. Unlike ethanol, the oxidation of lactate proceeds through a pyruvate intermediate with the release of carbon dioxide which fundamentally alters the thermodynamic landscape of the reaction [9]. While genera such as *Caproiciproducens* and *Megasphaera* can efficiently valorize lactate, this substrate introduces unique ecological challenges regarding product purity [40,41]. The primary issue is the competition from the acrylate pathway which diverts carbon flux toward propionate production and subsequently leads to the accumulation of odd-chain carboxylates [9,18]. Furthermore, recent findings suggest that lactate rich environments tend to select for *Caproiciproducens* dominated microbiomes that favor *n*-caproate accumulation whereas breaking the chain length barrier to achieve significant *n*-caprylate production often requires the supplemental thermodynamic driving force provided by ethanol [20].

2.3.3. Gas

Gaseous substrates including hydrogen, carbon monoxide, and carbon dioxide offer another avenue for chain elongation particularly within the context of syngas fermentation [42,43]. In most open culture reactor microbiomes these gases are not typically utilized directly by chain elongators but are instead converted by acetogens via the Wood-Ljungdahl pathway into

intermediate metabolites like ethanol or acetate which then feed the chain elongation process [44,45]. Although direct chain elongation from hydrogen and acetate is theoretically possible, thermodynamic modeling indicates that this pathway faces significantly higher energetic hurdles compared to ethanol-based elongation [46]. Consequently, hydrogen partial pressure is often manipulated in these systems as a thermodynamic control parameter to prevent the backward oxidation of ethanol or carboxylates rather than serving as the primary carbon source [4].

■ 2.3.4. Amino Acids

The utilization of amino acids as electron donors becomes particularly relevant when treating protein rich feedstocks such as slaughterhouse waste or manure [7]. Specialized organisms like *Eubacterium pyruvativorans* can employ Stickland reactions or deamination pathways to convert amino acids like alanine and glycine into pyruvate which subsequently enters the chain elongation cycle [28]. While this pathway is less ubiquitous than those driven by ethanol or lactate it holds significant ecological importance for the valorization of nitrogen rich waste streams.

■ 2.3.5. Carbohydrate

Carbohydrates represent the most abundant fraction in organic waste streams but are rarely utilized directly by chain elongators in mixed cultures due to intense competition from fast growing acidogens [47]. The bioconversion of carbohydrates in these systems typically relies on trophic cascades where synergistic bacteria such as lactic acid bacteria first ferment sugars into lactate or ethanol which are then available for chain elongation [48]. Although certain species like *Megasphaera elsdenii* possess the metabolic capacity to channel glucose directly into chain elongation their product specificity is often unstable and highly dependent on substrate conditions [29,49]. Therefore, a common engineering strategy involves the deliberate pre-fermentation of carbohydrates into lactate or ethanol to ensure a more controllable and selective chain elongation process.

■ 3. STRATEGIES FOR ENHANCED MCFA PRODUCTION

■ 3.1. Pretreatment of Biowastes

The efficient conversion of complex organic residues requires overcoming the rate-limiting step of hydrolysis to make biodegradable substrates accessible for the chain

elongating microbiome [50]. As chain elongators typically cannot directly utilize complex polymers like lignocellulose or particulate proteins, a pretreatment or pre-fermentation step is often indispensable to unlock the carbon potential of biowastes [51,52]. Biological pretreatment strategies such as two-stage anaerobic digestion are widely implemented where the first stage is dedicated to hydrolysis and acidogenesis [53][54]. This spatial separation allows for the maximization of short-chain carboxylate and lactate production in the first stage which subsequently serve as electron acceptors and donors in the second chain elongation stage. Furthermore, thermal or chemical pretreatment methods can be employed to solubilize organic matter and increase the bioavailability of substrates although these must be carefully optimized to avoid the generation of inhibitory byproducts that could severely impact the sensitive bioenergetics of the chain elongation machinery [55][5].

■ 3.2. Additives

In many waste valorization scenarios, the intrinsic carbon-to-nitrogen ratio or the electron donor capacity of the feedstock is insufficient to support extensive chain elongation [56]. The strategy of supplementing exogenous additives, particularly electron donors, has proven effective in boosting MCFA titers [8]. The addition of ethanol or lactate to feedstock deficient in reducing equivalents can thermodynamically drive the RBO cycle toward longer carbon chains. For instance, supplementing dilute ethanol streams to acetate-rich residues creates a favorable thermodynamic condition for *n*-caproate and *n*-caprylate synthesis [43,57,58]. Beyond substrates, the addition of conductive materials such as biochar or activated carbon has been explored to facilitate direct interspecies electron transfer although the specific mechanisms in chain elongation remain under investigation [59–63]. Crucially, the use of methanogenic inhibitors like 2-bromoethanesulfonate is a common additive strategy in lab-scale systems to suppress competitive methane production, yet recent studies advocate for ecological steering via operating parameters rather than chemical inhibitors to maintain economic viability and process sustainability [21].

■ 3.3. Regulating Operating Parameters to Enhance MCFA Production

Precise control over bioreactor operating conditions acts as a powerful ecological selection pressure to shape the microbiome and steer metabolic fluxes. The ratio of electron donor to electron acceptor is a deterministic

factor for product specificity [64–66]. Recent breakthroughs have demonstrated that manipulating the ethanol-to-lactate ratio can actively steer the system toward specific chain lengths [20]. Specifically, a high ethanol load relative to lactate provides the necessary thermodynamic driving force to extend carbon chains beyond *n*-caproate. Research indicates that increasing the ethanol-to-lactate ratio to approximately 3 to 1 significantly shifts the metabolic output toward *n*-caprylate production while simultaneously enriching for *Oscillospiraceae* populations within the microbiome [21]. However, contrasting perspectives still exist. For instance, Arhin *et al.* reported that a lactate-to-ethanol molar ratio of about 1.5 was optimal, facilitating a self-buffering pH environment (stabilizing at ~5.6) that minimized carbon flux dispersion toward odd-chain carboxylates and solventogenesis [67]. This condition yielded high concentrations and selectivity of *n*-caproate and *n*-caprylate. Another study found that in the co-fermentation of lactose with lactate and ethanol, the presence and concentration of a fermentable sugar (lactose) could override the simple ethanol/lactate ratio dynamic [68]. In their study, a high initial lactate load coupled with lactose fermentation created a low-pH environment that inhibited propionate producers and subsequently favoured lactate-driven chain elongation to caproate, achieving high selectivity (65%). The presence of complex wastes or additional fermentable carbohydrates can shift microbial communities and metabolic networks, altering the apparent optimal ratio observed in synthetic media studies. Therefore, follow-up studies should further investigate the effects of co-electron donor in microbial chain elongation process.

Operating temperature exerts a profound influence on both the kinetics of enzymatic reactions and the thermodynamics of competitive pathways. While mesophilic conditions are generally preferred, distinct temperature optima exist for different product spectra [69]. For instance, Lim *et al.* observed that a temperature of 35°C supported the highest overall carboxylate yield (23.5 g/L), outperforming both 25°C and 45°C [70]. Notably, however, the production of C6 at 35°C was lower than that achieved at 45°C, suggesting a potential trade-off between total acid yield and the selectivity for longer-chain products [71].

The regulation of pH is critical not only for microbial activity but also for product toxicity management. Chain elongation typically operates best at near-neutral or slightly acidic pH values between 5.0–7.0 which balances the growth requirements of the microbiome with the

efficiency of downstream extraction [6,72,73]. To prevent product inhibition caused by the accumulation of toxic undissociated fatty acids, implementing in-line product extraction is essential [66,74]. Pertraction or membrane-based separation systems allow for the continuous removal of MCFAs thereby maintaining their concentration in the bioreactor below toxic thresholds and enabling high-rate production [75].

■ 4. PERSPECTIVES AND CHALLENGES

Despite significant advances in understanding and engineering the chain elongation process, several critical bottlenecks remain that hinder its widespread industrial implementation.

A persistent scientific conundrum is the apparent limitation of biological chain elongation to eight carbon atoms. While thermodynamic calculations suggest that elongation to C8 or longer chains is feasible, experimental observation of these products is rare. Historically, this was attributed to the high toxicity of longer-chain carboxylates which disrupt cell membranes [72]. However recent evidence from systems with highly efficient in-line extraction, where toxicity is effectively negated, still failed to produce longer-chain acid [20]. This suggests that the limitation may be fundamentally enzymatic, potentially involving steric hindrances in the specific acyl-CoA dehydrogenases or transferases [76]. Future research must combine structural biology with omics approaches to unravel these enzymatic constraints and explore the potential of genetic engineering or bioprospecting for novel chain elongators capable of surpassing the C8 limit.

Maintaining a stable functional microbiome in the face of fluctuating waste streams remains a challenge. The dynamic nature of competition microbiome interactions means that some pathways like propionate formation or methanogenesis can rapidly destabilize the process. While strategies such as temperature control have shown promise in suppressing competitors, long-term stability often requires sophisticated ecological engineering. Future perspectives lie in the development of synthetic microbiomes or robust consortia that can be bio-augmented into reactors to ensure resilience. Additionally, elucidating the role of dormant microbiome members and their activation triggers will be crucial for designing dynamic feeding strategies that can adaptively steer production toward high-value products like *n*-caprylate.

The separation and purification of MCFAs from complex fermentation broths account for a significant portion of

total process costs. Although in-line extraction solves toxicity issues, the subsequent recovery of pure chemicals requires further innovation in membrane materials and solvent chemistry. Integrating the carboxylate platform with existing anaerobic digestion infrastructure represents a viable transition strategy but requires rigorous techno-economic and life cycle assessments to validate the environmental benefits over conventional biogas production. Future efforts must focus on bridging the gap between fundamental microbiological insights and practical process engineering to realize the full potential of the circular carboxylate economy.

5. CONCLUSION

Microbial chain elongation for converting organic biowastes into MCFA represents a promising pathway for carbon and energy recovery in a circular bioeconomy. This review has synthesized recent advances in the field, leading to several key conclusions. First, the RBO pathway serves as the core metabolic process for MCFA production, where its energy conservation mechanisms and thermodynamic driving forces fundamentally govern the efficiency and limits of carbon-chain elongation. Second, functional microorganisms (such as *Clostridium*, *Caproiciproducens*, and *Megasphaera*) constitute the ecological functional member that drives this process. Their substrate preferences and metabolic strategies directly shape the final product spectrum. Third, the type and ratio of electron donors (e.g., ethanol and lactate) act as critical levers for steering product selectivity and microbiome composition. Meanwhile, precise control of process parameters (e.g., pH and temperature) is essential for achieving high production rates and selectivity.

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