



A bacterial outlook on the caproate production from food waste

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Abstract As an essential strategy for transitioning towards a circular economy for a sustainable society, food waste (FW) can be efficiently used for the biological production of added-value compounds such as medium chain fatty acids (MCFAs). The microbial conversion of FW into MCFAs is an ecofriendly, sustainable and cost-effective approach that reduces the great pressure on land and water resources associated with traditional MCFAs production methods. Among the MCFAs, caproate holds high economic value and a large market size due to its widespread application in several industrial areas. The biological production of caproate from FW is a complex mechanism that requires a deep understanding of microbial dynamics, metabolic potentialities and functional stability for

process optimization and large-scale application. This review aims to outline the existing knowledge about bacterial component involved in the caproate production from FW. Innovative approaches to address current research gaps, ensuring a thorough and up-to-date understanding of the biological caproate production are herein identified and proposed.

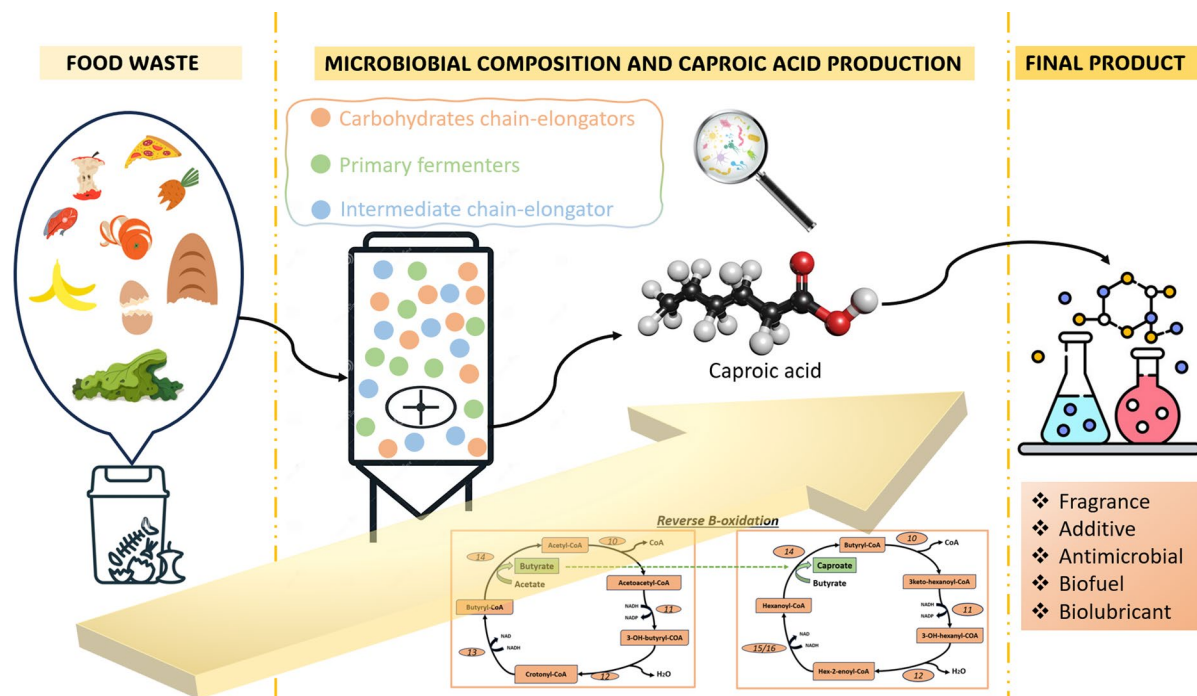
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Graphical Abstract



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1 Introduction

The growth of the world's population and the increasing levels of consumerism are driving a greater demand for non-renewable resources in the production of essential industrial goods (Atasoy et al. 2018). In 2022, it was estimated that approximately 82% of global energy demand was met using fossil fuels, such as oil, natural gas and coal (Energy Institute 2023). However, despite their essential importance in meeting the world's energy needs, these non-renewable fuels constitute the main sources of greenhouse gas emissions, responsible for global warming (Karakurt and Aydin 2023).

The continuous upward trend in fossil fuel usage is projected to result in the rapid depletion of these valuable resources in the foreseeable future (Wu et al. 2019a). At the same time, the current level of consumerism results in very high waste production. The

European Member States generated in 2022 around 513 kg per capita of municipal waste (Eurostat 2023), of which about 34% was organic waste. Out of this organic waste, an estimated 60–70% specifically consists in food waste (FW) (Scherhauser et al. 2018; Eurostat 2023), having an impact on global warming equivalent to roughly 186 million tons of CO₂ per year (Scherhauser et al. 2018). Policies that enable waste reduction throughout the entire food supply chain are being implemented to address the significant economic and environmental impact. The waste reduction strategy can result in a decrease in greenhouse gas emissions of 800–1400 kg/ton (Bernstad Saraiva Schott and Andersson 2015). Additionally, technologies that allow for reuse, recycling, and valorization of food waste are being widely utilized (Braguglia et al. 2018; Molina-Peñate et al. 2022). The production of value-added bio-products, as well for example medium chain fatty acids (MCFAs), is receiving great attention in recent years due to its high potentialities as a sustainable and cost-effective approach for valorizing organic waste (Ren et al. 2022; Wu et al. 2022; Wang et al. 2023b; Tonanzi et al. 2024). Since

FW is rich in fermentable sugars with lower level of recalcitrant compounds in its structure, this carbon-rich biomass is a promising substrate for MCFAs production (Contreras-Dávila et al. 2020; Crognale et al. 2021; Wu et al. 2024a). MCFAs, consisting of fatty acids with carbon chain lengths between 6 and 12, exhibit unique physicochemical properties such as low melting points compared to long-chain fatty acids, efficient energy metabolism, and antimicrobial effects. Furthermore, they exhibit reduced dissolution properties allowing for an easy separation from liquid media, with economic advantages in minimizing separation expenses (Wu et al. 2024b). These characteristics give MCFAs a wide range of applications in various industries, including food, pharmaceuticals, and cosmetics (Reddy et al. 2018; Wu et al. 2019a; Chu et al. 2022). Microbial pathways, such as reverse beta-oxidation, have been identified as enabling carbon-chain elongation. Over the past years, chain elongation (CE) increased the technology readiness level from lab- to industrial scale, emerging as a key bioprocess for waste valorization in biorefineries (Strik et al. 2022). Biorefineries are integrated facilities that utilize various bio-based feedstocks to produce a range of valuable products, including MCFAs (Annamalai et al. 2020; Gianico et al. 2021; Perez-Zabaleta et al. 2021; Choi et al. 2023). These facilities employ a combination of biochemical and physicochemical processes to convert waste materials into high-value products, maximizing resource utilization and minimizing waste disposal costs, thus providing a sustainable and efficient approach to produce MCFAs, contributing to the development of a bio-based economy (McDowall et al. 2022).

Among the various MCFAs, caproate, a saturated fatty acid with six carbon atoms, has significant high economic interest (Debergh and Van Dael 2022) with a market size of approximately 25 thousand tons per year with a value of 2500 USD per ton (Zhang et al. 2021). It appears as a pale yellow oily liquid and is widely used as a fragrance, additive, and preservative in food, as well as a renewable precursor for diesel and aviation fuels (Cavalcante et al. 2017; Liu et al. 2022). Additionally, it serves as a biolubricant (Ghyssels et al. 2021). Caproate is typically derived from fossil fuels or obtained from animal fats and vegetable oils like coconut or palm oil with low yields and expensive processes (Ge et al. 2015). However, in the search of ecofriendly alternative, sustainable caproate

production methods utilizing organic feedstocks through biological processes as CE have recently been explored (Nzeteu et al. 2022). The microbial conversion of organic wastes into MCFAs offers several advantages over traditional methods, such as chemical synthesis or extraction from plant oils (Bao et al. 2019; Naresh Kumar et al. 2022), contributing to reduce the significant pressure on land and water resources associated with traditional MCFAs production methods. Thus, this approach holds great promise for addressing waste management challenges and promoting sustainable resource utilization in the future.

Like other microbial processes, CE performance depends on microbial activity. To optimize the production process, it is imperative to delve into the microbial metabolic pathways responsible for synthesizing MCFAs. The intricate biological organization for caproate production highlights the necessity of unveiling the pivotal role of specific bacterial consortia throughout the phases of anaerobic fermentation and chain elongation. A deep understanding of how microorganisms can react to variations in parameters such as pH, temperature, and organic loading rate (OLR) is of paramount importance. This comprehension is vital for maximizing process efficiency and guaranteeing optimal outcomes in terms of caproate yield.

The significant advances made in recent years for the production of MCFAs from organic waste and their potential biotechnological applications, including the optimization of fermentation processes and purification, have been critically discussed so far (Lee et al. 2019; Wu et al. 2019a; Wang et al. 2020a; Reddy et al. 2020; Stamatopoulou et al. 2020; Menon and Lyng 2021; Albizzati et al. 2021; Greses et al. 2022; Wang and Yin 2022; Tomás-Pejó et al. 2023). Nevertheless, to date the response mechanisms of different microorganisms to various influencing factors have not been clearly discussed, limiting the ability to design suitable systems and targeted regulation of caproate production. This review aims to analyze and discuss the existing literature, providing a comprehensive overview of the bacterial component involved in the caproate production from FW, with the focus on the diversity and metabolic pathways of pure and mixed cultures. Additionally, this review identifies and proposes innovative approaches to address current research gaps, ensuring a thorough and

up-to-date understanding of the biological caproate production.

2 Chain elongation pathway

The CE (Fig. 1) is a complex series of enzymatic reactions within metabolic pathways that ultimately lead to the synthesis of MCFAs from the short-chain fatty acids (SCFAs), typically volatile fatty acids, produced during primary or acidogenic fermentation process. These SCFAs act as electron acceptors for the formation of MCFAs (Andersen et al. 2015; Angenent et al. 2016). This pathway involves the cooperative actions

of different enzymes and proceeds through subsequent cycles of reverse β -oxidation reactions for CE (Wu et al. 2018). The main electron donors (EDs) used in this process are ethanol and lactate that play fundamental roles as starting substrates. They serve as sources of energy and provide necessary reducing equivalents, such as NADH, to power the process. Additionally, these compounds serve as direct precursors for the formation of acetyl-CoA, a key intermediate molecule involved in the sequence of reactions in the elongation process (Cavalcante et al. 2017). This process is essential for the biosynthesis of MCFAs and other compounds that require an extended carbon chain. Alternatively, saccharides can act as energy sources by undergoing an

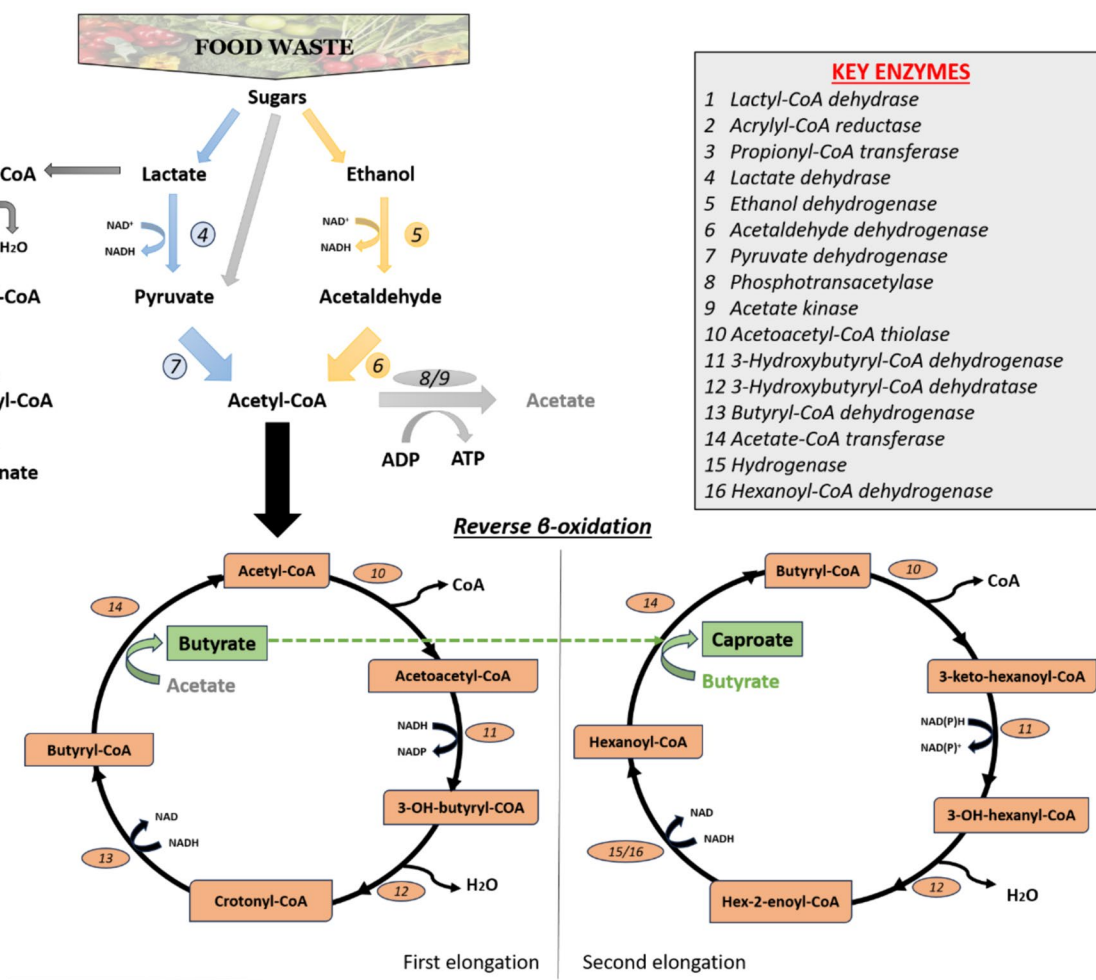
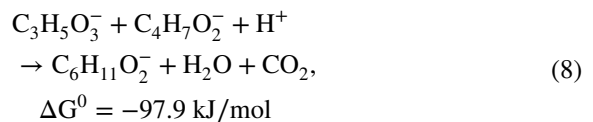
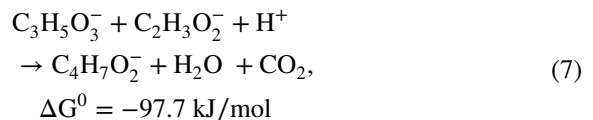
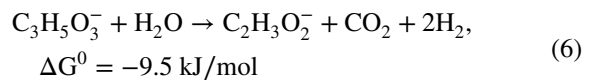
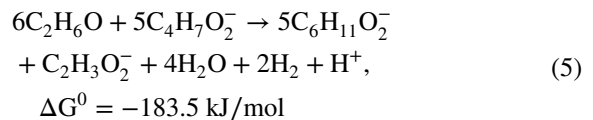
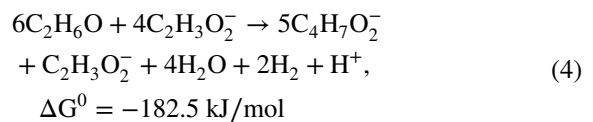
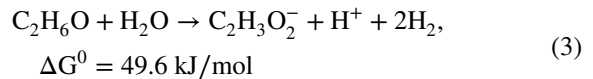
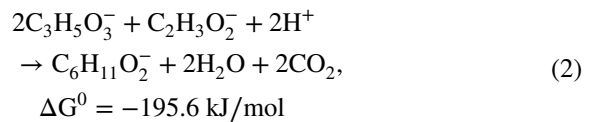
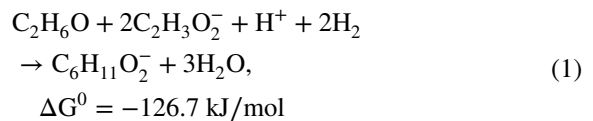


Fig. 1 Graphic representation of the chain elongation process according to Wu et al. (2019a). The intermediates of reverse beta-oxidation highlighted in orange delineate the pathway

from condensation to the end products highlighted in green (medium-chain fatty acids). Image created by using PowerPoint 2016

initial oxidation process, resulting in the formation of pyruvate and subsequent production of acetyl-CoA. The production of caproate through CE process using ethanol or lactate as EDs, is thermodynamically feasible under standard conditions (1 atm, 25 °C, and 1 M; Table S1) (Eqs. (1), (2)) (Coma et al. 2016; Zhu et al. 2017).

When using ethanol as the electron donor (ED), the first step of the CE process begins with the oxidation of ethanol into acetaldehyde by the enzyme *ethanol dehydrogenase*, which is subsequently converted into acetyl-CoA through the action of *acetaldehyde dehydrogenase*. Approximately 1/6 of the resulting acetyl-CoA is transformed into acetate through substrate-level phosphorylation (*phosphotransacetylase* and *acetate kinase*) (Eq. (3)), while the remaining 5/6 enters the cyclic reverse β -oxidation pathway (Angenent et al. 2016) for chain elongation. In fact, in each cycle acetyl-CoA undergoes a two-carbon addition by coupling to another CoA derivative (Wu et al. 2019a). The first cycle involves the elongation of acetate into n-butyrate (Eq. (4)), achieved by coupling acetyl-CoA to another acetyl-CoA through *acetoacetyl-CoA thiolase*. This sequence of enzymatic reactions continues until the production of the critical intermediate, butyryl-CoA due to the action of *butyryl-CoA dehydrogenase*. The released acetyl-CoA reacts with other acetyl-CoA derived from ethanol oxidation to reinitiate the elongation of acetate. Simultaneously, the acetyl-CoA couples with butyryl-CoA to initiate the second cycle of n-butyrate elongation into caproate (Eq. (5)), with various intermediates. If lactate is used as the ED, it is oxidized to pyruvate via *lactate dehydrogenase*, then subsequently converted to acetyl-CoA through the action of *pyruvate dehydrogenase*. A portion of the resulting acetyl-CoA is converted into acetate (Eq. (6)). The acetyl-CoA generated from lactate oxidation enters the cyclic reverse β -oxidation to extend acetate into n-butyrate (Eq. (7)), and then into caproate (Eq. (8)) like the ethanol pathway. The formation of propionate can occur via the acrylate pathway in contrast to the CE pathway (Kucek et al. 2016). This competing pathway generally occurs when a high content of lactate is available inducing the formation of lactyl-CoA, key intermediate for the propionate production (Prabhu et al. 2012; Sträuber et al. 2016).



3 Microorganisms involved in CE

In the biological CE process different microorganisms can exhibit different metabolic capabilities in response to abiotic factors, such as pH, temperature and applied OLR. Equally important, a variety of biotic factors can influence the interactions within the microbial communities involved in the synthesis

of MCFAs. These factors include competition for resources, metabolic cooperation, cellular communication, and the production of secondary metabolites. For example, some microorganisms might compete for the same substrate, while others might synergistically cooperate to exploit different metabolic pathways, thus optimizing the overall efficiency of MCFAs production. Furthermore, cellular communication through chemical signals such as quorum sensing can regulate the growth and behavior of microbial populations, further influencing the dynamics of communities involved in anaerobic fermentation. The production of secondary metabolites, such as antibiotics or signaling molecules, may also play a role in modulating the composition and function of the microbial community (Ho et al. 2016). These interactions, ranging from competition for resources to metabolic cooperation and cellular communication, deeply influence the efficiency and stability of bioprocesses (Cabrol and Malhautier 2011). Recognizing the intricate interplay of these factors has profound implications for real-world applications, particularly in bioprocess optimization. Indeed, in the context of industrial fermentation for MCFAs production, understanding and exploiting these biotic interactions can have a significant impact on process stability, yield and cost-effectiveness. Overall, rather than the persistence of a constant microbial community, a given arrangement among populations seems more important to sustain high functional performance and stability of the bioprocess (Cabrol et al. 2017). By elucidating the mechanisms underlying microbial community dynamics, researchers and practitioners can implement strategies to improve process efficiency, minimize resource consumption, and mitigate environmental impacts.

Generally, *Archaea* are not actively involved in the CE process; as described by De Groof et al., (2020), their relative abundance in CE systems from FW is generally low, commonly below 10%. Various strategies, as for example the use of methane inhibitor 2-bromoethanesulfonate and a pH < 6, can be used for inhibiting archaeal selection in the CE systems (Steinbusch et al. 2011; Vasudevan et al. 2014). In any case, the identified archaeal species were more oriented towards methanogenesis rather than CE, thus suggesting a distinct separation and a competition between different microbial functions.

As well as *Archaea*, also members of domain *Eukaryota* are not commonly involved in CE process. In some cases, different species of Fungi as *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, and *Pichia stipites* were used for ethanol production from acid whey (Crognale et al. 2021). However, reports on their capability to ferment FW are not available to date. In contrast, the sole observation of yeasts and filamentous fungi in CE process from FW was related to their presence in the feed due to their known use in cheese production and their pathogenic activity against fruits and vegetables (Crognale et al. 2021).

Instead, various bacterial taxa have been identified and investigated for their capacity to synthesize MCFAs and will be deeply described in the subsequent sections of this review.

3.1 Bacterial strains in pure cultures

The metabolic potential of different bacterial strains has been tested in pure culture using synthetic growth media without the addition of any real and complex substrate such as for example FW. Overall, these strains mostly belong to the genera *Caproiciproducens*, *Clostridium*, *Megasphaera*, *Pseudoramibacter*, and to the families *Eubacteriaceae* and *Ruminococcaceae* (Table 1). Although pure culture fermentation is conducive to investigate the microbial metabolic pathway, the use of pure cultures on a large-scale process is not cost effective, as the operation without contamination may become economically unfeasible. Furthermore, to date there have been no reports of pure culture methods outperforming mixed culture strategies in terms of performance (Candry and Gangué, 2021).

3.1.1 *Caproiciproducens* spp.

Recent studies have unveiled the remarkable ability of microorganisms belonging to the genus *Caproiciproducens* to produce MCFAs (Crognale et al. 2021; De Groof et al. 2021). The genus *Caproiciproducens* exhibits microbiological traits of strict anaerobic bacteria, forming rod-shaped cells with spores ($1-3 \times 0.4-0.8 \mu\text{m}$) that appear Gram-negative upon Gram staining, despite possessing a Gram-positive type of cell wall ultrastructure. The optimal growth conditions have been observed

Table 1 Summary of bacterial pure cultures involved in CE process. Strain designation, origin, pH and temperature growth conditions, ED used for CE process, fatty acids produced, and

the maximum achieved concentration of caproate are reported. n.a. not available

	pH range (optimum value)	Temperature range °C (opti- mum value)	EDs	Fatty acids	Maximum caproate concen- tration	References
<i>Caproiciproducens</i>	4.5–9 (5.2)	22.5–50 (30)	Saccharides	Acetate, butyrate, caproate	32 g/L	(Jeon et al. 2013; Kim et al. 2015; Flaiz et al. 2020; Esquivel-Elizondo et al. 2021)
<i>Clostridium</i>	6.0–7.5 (6.8)	19–37 (34)	Ethanol	Butyrate, caproate	12.8 g/L	(Barker and Taha 1942; Seedorf et al. 2008; Steinbusch et al. 2011; Weimer and Stevenson 2012)
<i>Megasphaera</i>	4.5–8 (7)	15–40 (37)	Lactate; saccharides	Propionate, butyrate, caproate	28.4 g/L	(Elsden et al. 1956; Choi et al. 2013) Lanjekar et al. 2014; Wang et al. 2022;
<i>Pseudoramibacter</i>	5–6 (5.5)	25–45 (35)	Lactate; saccharides	Acetate, butyrate, caproate	n.a	(Collins et al. 1994; Willems and Collins 2015; Scarborough et al. 2020b; Myers et al. 2023)
<i>Eubacteriaceae</i>	6–8 (7)	25–40 (39)	Lactate; saccharides	Acetate, propionate, butyrate, valerate, caproate	1.16 g/L	(Sharak Genthner et al. 1981; Wallace et al. 2003, 2004; Kim et al. 2022)
<i>Ruminococcaceae</i>	5–6.5 (5.5)	30–40 (40)	Lactate	Propionate, butyrate, caproate	16.6 g/L	(Zhu et al. 2017)

between 30 °C and 37 °C, while the pH range for growth lies between 5 and 9 (Flaiz et al. 2020). The *Caproiciproducens* sp. strain 7D4C2 exhibited proficiency in generating lactate, acetate, butyrate, caproate, and H₂/CO₂ from hexoses (Kim et al. 2015). Recently, in addition to sugars, this genus has been proved to utilize other substrates, such as food waste, to produce caproate (Tang et al. 2022). Based on the 16S rRNA gene sequence, strain 7D4C2 was found to be closely related to *Caproicibacter fermentans* (99.5%) and *Caproiciproducens galactitolivorans* (94.7%), bacteria known for the involvement in CE and lactate production. The specificity for caproate production (i.e., the amount of caproate

out of total fatty acids concentration) was around $60.9 \pm 1.5\%$ of total fatty acids by using fructose as substrate at controlled pH of 5.5 (Esquivel-Elizondo et al. 2021). In the same study the simultaneous use of fructose and butyrate as substrates significantly elevated the caproate specificity of *Caproiciproducens* sp. strain 7D4C2 reaching up to $83.1 \pm 0.4\%$ of total fatty acids. Overall, the highest caproate production ascribable to the genus *Caproiciproducens* was 32 g/L (Jeon et al. 2013) by using synthetic medium as substrate. When FW is used as substrate, *Caproiciproducens* resulted to be highly selected (range 32–72% of total reads) along with

Lactobacillus and produced up to 5.4 g/L (Contreras-Dávila et al. 2020).

3.1.2 *Clostridium* spp.

The genus *Clostridium* comprises the most identified and utilized microorganisms in the process of dark fermentative hydrogen production (Wang and Yin 2021). *Clostridium kluyveri* was the first microorganism described for its capability to elongate carbon chain (Seedorf et al. 2008). It belongs to the family *Clostridiaceae*, and the members of this species grow anaerobically using ethanol and acetate as the sole energy sources to produce a wide range of substrates, such as acetate, propionate, succinate, butyrate, caproate, and H₂ (Kenealy and Waselefsky 1985; Yin et al. 2017; Candry et al. 2020). *C. kluyveri* was initially isolated by Barker in 1937 from the mud of a canal in Delft, the Netherlands (Barker and Taha 1942) and then regularly found in microbiomes converting organic wastes to MCFAs (Steinbusch et al. 2011). This microorganism can grow within a pH range of 6.0–7.5, with the optimal pH being around 6.8. The optimal growth temperature falls between 19 °C and 37 °C, and lower temperatures were not subjected to testing. The most rapid growth was observed at approximately 34 °C (Barker and Taha 1942). *C. kluyveri* was used in various applications to produce MCFAs from different substrate sources. For example, it was involved in open-culture carbon conversion processes, such as combined culture with *Clostridium autoethanogenum* to produce MCFAs from syngas (Diender et al. 2019), with rumen microbiota for MCFAs production from cellulose biomass (Weimer et al. 2015), and for bioaugmentation of digested sludge to generate MCFAs from a mix of acids and sugars (Zagrodnik et al. 2020). Additionally, *C. kluyveri* predominated in fermentation systems specialized in MCFAs production from mixed acids and sugars (Steinbusch et al. 2011; Coma et al. 2016; Leng et al. 2017). The highest reported caproate production by *C. kluyveri* was 12.8 g/L (Weimer and Stevenson 2012) observed on synthetic medium as substrate. Few information regarding the role of *C. kluyveri* in the CE process from FW have been reported to date (Reddy et al. 2018).

3.1.3 *Megasphaera* spp.

The strain *Megasphaera elsdenii* LC3 was initially discovered in the rumen of sheep. Microorganisms affiliated with the genus *Megasphaera* have been reported for their capability to utilize saccharides and lactate to produce MCFAs (Elsden et al. 1956; Wang et al. 2022). These microorganisms grow at temperatures between 15 and 40 °C, with the best growth rate at 37 °C, cannot survive at temperature as high as 45 °C (Lanjekar et al. 2014) and can utilize a variety of carbohydrates that include glucose, lactose, cellobiose, rhamnose, galactose, and sucrose. Genus *Megasphaera*, although rarely found in mixed-culture fermentations of FW guided by lactate (Crognale et al. 2021), showed the highest caproate production (28.4 g/L) when tested in pure culture on sucrose-based medium with the addition of butyrate and extraction solvent (Choi et al. 2013).

3.1.4 *Pseudoramibacter* spp.

Microorganisms belonging to the genus *Pseudoramibacter* exhibit a characteristic rod-shaped morphology and are strict anaerobes, catalase-negative, gram-positive, and non-sporulating bacteria. Their growth can be stimulated by fermentable carbohydrates, resulting in the production of formate, acetate, butyrate, caproate, and gaseous hydrogen as end products of fermentation (Willems and Collins 2015). To date, only few evidence of MCFAs production from FW by *Pseudoramibacter* pure culture have been reported. A recent metatranscriptomic analyses focusing on *lactate dehydrogenase* activity have highlighted the ability of *Pseudoramibacter fermentans* to synthesize MCFAs from lactate (Scarborough et al. 2020b; Myers et al. 2023). Furthermore, thermodynamic investigations on *P. fermentans* have raised the possibility that glycerol may serve as an additional viable substrate (Scarborough et al. 2020b; Myers et al. 2023). The optimal pH conditions for growth with glucose as substrate have been observed to range between 5.0 and 5.6, occasionally extending to 6.0 (Collins et al. 1994). Regarding temperature preferences, most strains exhibit optimal growth between 35 and 37 °C, although several strains thrive at 30 °C, while others can adapt to both lower (25 °C) and higher (45 °C) temperatures (Collins et al. 1994). Unfortunately, no information about caproate

production by *P. fermentans* pure culture nor in terms of % out of the total fatty acids nor as absolute quantification (e.g., g/L) are available.

3.1.5 *Eubacteriaceae*

Eubacteriaceae is a family of microorganisms that can utilize lactate and saccharides as EDs in their metabolic processes, unfortunately not genus level identification of these caproate-producing microorganisms has been reported to date. Bacteria affiliated with this family display an extensive metabolic repertoire, leading to the production of various organic compounds, notably including acetate, propionate, butyrate, valerate, and caproate (Kim et al. 2022). Among these, caproate production is particularly noteworthy within the family. These microorganisms optimally live within a pH range of 6–8, with an ideal pH around 7, facilitating their breakdown of organic substrates and subsequent fermentation into various MCFAs. Regarding temperature, *Eubacteriaceae* typically thrive in mesophilic conditions, favoring a temperature range between 25 and 40 °C (Sharak Genthner et al. 1981). Their adaptability to these temperatures enhances their ability to efficiently metabolize substrates, contributing to the production of various MCFAs and alcohols in their metabolic pathways. Recently, microorganisms affiliated with this family have been found at low extent (representing on average 5% of total community) in mixed microbial communities involved in the production of caproate from FW (Gazzola et al. 2022). *Eubacteriaceae* can produce a significant amount of caproate, with reports suggesting concentration up to 1.2 g/L (Wallace et al. 2003, 2004).

3.1.6 *Ruminococcaceae*

Microorganisms affiliated with family *Ruminococcaceae* belong to the *Clostridium* cluster IV. Optimal growth conditions were determined to be at acidic initial pH levels ranging from 5.0–6.5, along with a temperature range of 30–40 °C (Zhu et al. 2017). Recently, the potentialities of *Ruminococcaceae* CPB6 strains in caproate production through lactate utilization have been documented (Zhu et al. 2017; Duber et al. 2018; Nzeteu et al. 2018; Sträuber et al. 2016). Furthermore, a recent investigation focusing on fed-batch fermentation, utilizing non-sterilized organic wastewater containing lactate, revealed

promising results for caproate synthesis by these microorganisms in pure cultures (Zhu et al. 2017). The caproate production reached up to 16.6 g/L (from an initial lactate concentration of 45.1 g/L), with a peak productivity of 5.3 g/L/d (Zhu et al. 2017). These findings underscored the feasible application of *Ruminococcaceae* CPB6 strains in sustainable caproate production from lactate-rich feedstocks; however, few applications of these microorganisms with FW substrate have been tested so far.

3.2 Microbial ecology model for caproate production from organic waste

An elevated microbial diversity can contribute to enhance the stability and efficiency of CE process under dynamic conditions (Han et al. 2018). Additionally, the use of mixed cultures does not require sterile conditions, thus decreasing operative costs and increasing the feasibility of the CE process with real substrates such as FW. The microbiome selected in systems designed for CE process can show a great genomic diversity (Candry and Ganigué, 2021). To effectively convert a complex substrate, such as FW, the synergistic activities of various microbial taxa are essential in establishing a trophic network. The knowledge regarding the mechanisms underlying CE systems in mixed culture conditions, as for example the effect of environmental parameters and biotic factors on microbial diversity, metabolism and growth kinetics, can help in fostering large-scale application and maximize caproate production.

As emerging trend, the use of mixed microbial culture to produce medium-chain carboxylic acids have been reported from different organic wastes such as sewage sludge (Wu et al. 2019a, 2019b), pit-mud (Wu et al. 2019b), leachate (Nzeteu et al. 2018), rumen fluid (Weimer et al. 2015), and stillage (Scarborough et al. 2018b). Among the organic wastes, FW, rich in carbohydrates and proteins, allowed the in-situ generation of the EDs (mainly lactate) and the presence of electron acceptors (EAs) required for the MCFAs production (Wang et al. 2020b), avoiding any external addition and improving cost-effectiveness of the process. The CE process with FW showed better performances than that with other organic wastes (Wang et al. 2023a) and, although the composition of FW can widely vary depending on different sources, caproate was always revealed as the main

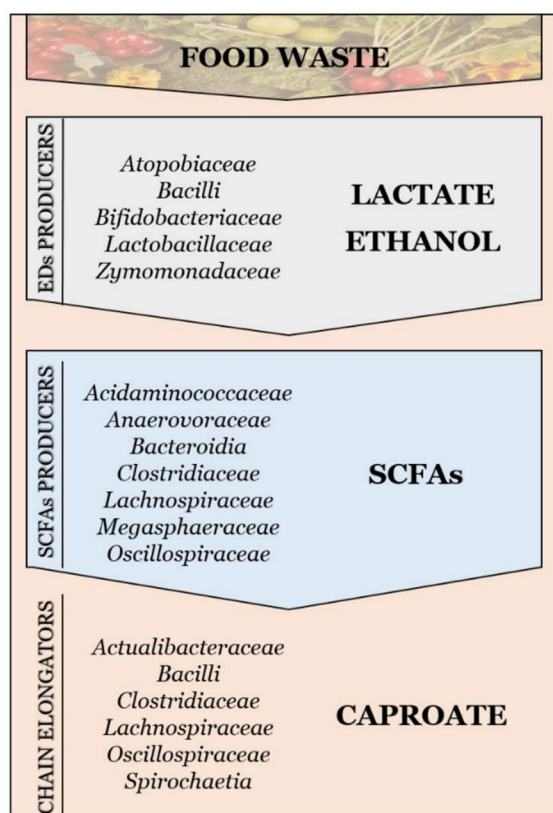


Fig. 2 General scheme of steps involved in EDs, SCFAs and caproate production from FW

MCFA produced (Wang and Yin 2022). Generally, FW exhibited globally applicable characteristics, with a moisture content ranging from 74 to 90%, a high volatile solids fraction of approximately $85 \pm 5\%$, and an average acidic pH of 5.1 ± 0.7 (Zhang et al. 2007; Fisgativa et al. 2016). Typical FW is predominantly composed by degradable carbohydrates (41–62%), proteins (15–25%), and lipids (13–30%) (Braguglia et al. 2018).

Recently, Crognalet et al. (2023) and Myers et al. (2023) proposed a microbial ecology model for caproate production from organic waste (i.e., FW and agro-industrial residues) providing an overview of the different metabolic functions of various microbial taxa involved in the CE process. This model identified different roles, mutually complementary with each other within the microbiome (Fig. 2). In detail, some microorganisms (e.g., members of families *Lactobacillaceae*, *Bifidobacteriaceae*, and *Atopobiaceae*) are responsible for primary fermentation by

converting carbohydrates into intermediates such as lactic acid or ethanol, but do not directly participate in the elongation of fatty acids. At the same time, another microbial group, generally composed by members of families *Megasphaeraceae*, *Acidaminococcaceae*, *Clostridiaceae*, *Anaerovoracaceae*, and *Eubacteriaceae*, act as catalyst in the CE by using lactic acid or ethanol as electron source. Finally, a third group of microorganisms (i.e., *Lachnospiraceae* and *Acutalibacteraceae* families) can directly elongate carbon chain from saccharides to MCFAs (Scarborough et al. 2018a; Myers et al. 2023). These insights contributed to delineating a general understanding of the microbial dynamics involved in CE process and the importance of different EDs for the MCFAs production. Considering the metabolism of lactic acid/ethanol and SCFAs/MCFAs producing bacteria, the coexistence of various microorganisms interplaying in CE systems enables different types of metabolic interactions as well for example the competition for the same substrate (i.e., sugars). Furthermore, the different microorganisms can have different growth strategies with lactic acid/ethanol bacteria resembling that of a r-strategist and chain-elongating bacteria resembling that of a K-strategist (Ulčar et al. 2024). Insight into the main functional groups responsible of the caproate production from FW can allow to promote future bioprocess design and optimization.

Taken as a whole, the use of biomolecular techniques has facilitated the identification of the most efficient microbiome involved in the caproate production from FW. Primarily, the Illumina platform-based amplicon sequencing, specifically high-throughput sequencing of V1–V3 region of bacterial 16S rRNA gene, has been utilized for profiling microbial communities. In addition, very few studies applied shotgun metagenome analysis to examine the complete genome of the microbial community. This approach has enabled the identification not only the main taxa present but also their potential functional capabilities involved in converting FW to caproate.

Biomolecular analysis proved the complexity of microbial interactions in the production of caproate from FW, suggesting a clear division of tasks between different members of the microbiome. The identification of these microbial functional groups has provided valuable insights for optimizing production process and yields. Unraveling the abiotic/biotic interactions within mixed microbial communities can provide

Table 2 Summary of mixed microbial communities involved in caproate production from FW. Predominant microorganisms, caproate yield/content, process configuration, volume, temperature and pH, and total days of operation are reported. n.a. not analyzed; n.r. not reported

Predominant microorganisms	Caproate yield/content	Process configuration	V (L)	T (°C)	pH	days	References
<i>Clostridia</i> , <i>Sphingobacteriales</i> , <i>Desulfobacteraceae</i> , <i>Bacillus</i> spp.	8.1 g/L	Batch	0.12	37	6.7–6.9	91	Reddy et al. 2018
<i>Clostridiales</i> , <i>Lactobacillales</i>	8.2 g/L	Batch	0.5	37	7.7	10	Tampio et al. 2019
n.a	11.4 g/L	Batch	0.6	35	10	2	Dahiya and Mohan 2019
<i>Propionibacterium</i>	0.5 g/g (dry weight of FW)	Batch	1	30	6	21	He et al. 2019
<i>Clostridium kluyveri</i>	14.9 g/L	Batch	1	35	6.3	70	Yu et al. 2019
<i>Caproiciproducens</i>	0.2 gCOD/gVS	Batch	0.5	37	7	13	Ma et al. 2021
<i>Clostridium_sensu_stricto_2</i> , <i>Pseudoramibacter</i> , <i>Romboutsia</i> , <i>Paraclostridium</i> , <i>Proteiniphilum</i> , <i>Clostridium_sensu_stricto_1</i> , <i>Terrisporobacter</i> , <i>Sporanaerobacter</i>	0.7 ± 0.04 gCOD/gVS	Batch	0.25	35	4.5/6/6.75	8	Wang et al. 2022
<i>Clostridium kluyveri</i> , <i>Clostridium_sensu_stricto</i> , <i>Caproiciproducens</i>	9.7 g/L	Batch	1.5	35	5.5/7.5	18	Huo et al. 2022
n.a	18.5 g/L	Fed-batch	0.6	37	7.4	144h	Zhang et al. 2021
<i>Clostridium</i> , <i>Sporanaerobacter</i> , <i>Tissierella</i> , <i>Bacillus</i>	0.9 gCOD/gVS	Semicontinuous	0.5	40	5.2/6.4	30	Wu et al. 2016
<i>Clostridia</i> , <i>Bacteroidia</i> , <i>Bacilli</i>	n.r	Semicontinuous	1	35	7	45	Zhang et al. 2020
<i>Caproiciproducens</i> spp., <i>Secundilactobacillus</i> spp., <i>Lactobacillus</i> spp.	13.6 gCOD/L	Semicontinuous	2	35	5.9	30	De Groof et al. 2021
<i>Bifidobacterium</i> , <i>Olsenella</i> , <i>Atopobium</i> , <i>Proteiniphilum</i> , <i>Caproiciproducens</i> , <i>Synergistetes</i> , <i>Chloroflexi</i>	10.7 gCOD/L	Semicontinuous	1	37	6 ± 0.5	90	Zhang et al. 2022a
<i>Caproiciproducens</i> , <i>Rummeliibacillus</i> , <i>Clostridium_sensu_stricto_12</i>	10.2 gCOD/L	Semicontinuous	1	55	6	90	Zhang et al. 2022b
<i>Caproiciproducens</i> , <i>Clostridium_sensu_stricto_12</i> , <i>Rummeliibacillus</i> , <i>Oscillibacter</i>	2 g/L	n.r	1.8	35	n.r	98	Li et al. 2023

Table 2 (continued)

Predominant microorganisms	Caproate yield/content	Process configuration	V (L)	T (°C)	pH	days	References
<i>Olsenella</i> , Anaerostipes, <i>Clostridium sensu stricto</i> 7, <i>C. sensu stricto</i> 12, Corynebacterium, Erysipelotrichaceae UCG-004, F0332, Leuconostoc, <i>Caproiciproducens</i>	7 g/L	Continuous/semicontinuous	3	37	6	n.r	Crognale et al. 2023
<i>Clostridium cochlearium</i> , <i>Clostridium</i> spp.	10 g/L	Continuous	3	37	7	21	Nzeteu et al. 2018
<i>Lactobacillus</i> spp., <i>Caproiciproducens</i> spp.	5.4 g/L	Continuous	1	35	6	41	Contreras-Dávila et al. 2020
<i>Actinomyces</i> , Atopobium, <i>Olsenella</i> , <i>Pseudoramibacter</i>	4.8 g/L	Continuous	3	37	6	39	Crognale et al. 2021
<i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Sporanaerobacter</i> , <i>Caproiciproducens</i>	28.2 gCOD/L	Continuous	1	40	5.78±0.43	120	Zhang et al. 2023
<i>Bifidobacterium</i> , <i>Clostridium</i> , <i>Bacteroides</i> , <i>Dysgonomonas</i>	28.6 gCOD/L	Leachate bed reactor	7.5	22	6/7/8	14	Xiong et al. 2019

control, diagnostic and prevision tools for bioprocess monitoring (Cabrol and Malhautier [2011](#)). Indeed, the complex interplays between biotic interactions and operation conditions is crucial for enhancing the performance improvement and engineering of the system up to large-scale applications.

3.3 Mixed microbial communities involved in caproate production from food waste

Several research efforts have contributed to exploring different approaches to convert FW into MCFAs mainly utilizing mixed microbial cultures selected in batch, fed-batch, semi-continuous and continuous process configurations (Table 2). The different applied configurations and experimental conditions resulted in significant differences in microbiome selection and caproate production yield. These differences are in detail discussed in this paper.

3.3.1 Microbiome selected in batch reactors

Several studies investigated caproate production from FW in batch conditions. In some cases, the obtained caproate production was approximately 8.2 g/L in correspondence with a microbial community mainly composed by members of *Clostridia* and *Bacillus* taxa (Reddy et al. [2018](#); Tampio et al. [2019](#)). Differently, *Clostridium kluyveri* was found as the predominant microorganism in a mesophilic batch reactor with a volume of 1 L and pH 6.3, achieving a caproate content of 14.9 g/L (Yu et al. [2019](#)). He et al. ([2019](#)) showed the capability of a mixed community mainly composed by *Propionibacterium* to produce caproate with a content up to 0.54 g/g dry weight of FW in a batch reactor at pH 6. The works of Ma et al. ([2021](#)) and Huo et al. ([2022](#)) highlighted the presence of *Caproiciproducens* in batch reactors with volumes of 0.5 L and 1.5 L, respectively, and pH values of 7 and 7.5, reaching a caproate content of 9.67 g/L and a production yield of 0.21 gCOD/gVS. In other batch tests (35 °C, pH 4.5–6.75) experiments,

microbial communities, mainly composed of *Clostridium_sensu_stricto_2*, *Pseudoramibacter*, *Romboutsia*, *Paraclostridium*, *Proteiniphilum*, *Clostridium_sensu_stricto_1*, and *Terrisporobacter* allowed to obtain a maximum caproate yield of 0.71 ± 0.04 gCOD/gVS_{fed} (Wang et al. 2022). In other experiments using FW in batch reactor (Dahiya and Mohan 2019; Zhang et al. 2021), a caproate content ranging between 11.4 g/L and 18.5 g/L was achieved but, unfortunately, the composition of the selected microbial community was not analyzed.

3.3.2 Microbiome selected under semi-continuous and continuous conditions

Researchers have explored several methods to investigate caproate production from FW including the assessment of semi-continuous and continuous processes and exploring the diversity of selected microbiomes.

For example, a microbiome mainly composed by members of *Clostridia*, *Bacteroidia*, and *Bacilli* was involved in a two-stage process where FW was first pretreated using a hydrolysis/fermentation bioreactor, then fed into a MCFAs-producing bioreactor (Zhang et al. 2020). In other cases, microbiomes mainly composed of microorganisms belonging to genera *Clostridium* and *Caproiciproducens* drove the CE process (Nzeteu et al. 2018; Tampio et al. 2019; Huo et al. 2022; Crognale et al. 2023), whereas *Lactobacillus* and *Olsenella* species took on primary roles in the fermentative process (Contreras-Dávila et al. 2020; Crognale et al. 2021; De Groof et al. 2021). Most of these studies tested various pH and temperature conditions, demonstrating that a slightly acidic pH and mesophilic regime can promote the production of MCFAs (Reddy et al. 2018; Huo et al. 2022; Wang et al. 2022).

In most studies, a temperature range between 28 and 40 °C has been utilized, highlighting the advantages of mesophilic temperature in terms of process and cost-effectiveness. However, exceptions include a semi-continuous reactor with a thermophilic temperature of 55 °C (Zhang et al. 2022b) and a leachate bed reactor at 22 °C (Xiong et al. 2019). In the first case, a microbiome mainly composed by *Caproiciproducens*, *Rummeliibacillus* and *Clostridium_sensu_stricto_12*, allowed to obtain a content of 10.2 gCOD/L of caproate in the fermentation broth; in the second case,

up to 28.6 gCOD/L of caproate was found within the fermentation broth with a microbial biomass mainly composed by *Bifidobacterium*, *Clostridium*, *Bacteroides*, and *Dysgonomonas*.

In some semi-continuous investigations, the presence of microorganisms such as *Clostridium*, *Sporanaerobacter*, *Tissierella*, and *Bacillus* enabled a caproate yield of 0.9 gCOD/gVS_{fed} (Wu et al. 2016). Furthermore, a microbiome constituted by *Clostridia*, *Bacteroidia*, and *Bacilli* resulted to be able to achieve 0.3 gVFA/gVS_{fed} in a reactor operated at 35 °C and pH 7 (Zhang et al. 2020). Furthermore, bacteria affiliated with the genera *Actinomyces*, *Atopobium*, *Olsenella*, and *Pseudoramibacter* were revealed in a reactor treating the liquid extract of FW at 37 °C and pH 6, reaching a caproate content of 4.8 g/L in the fermentation broth (Crognale et al. 2021).

4 Outlook and perspectives

Research efforts to find new ways for generating chemicals compounds from renewable sources, in particular organic waste, are an important step towards a sustainable bio-based economy that decrease society's dependence on conventional fossil sources. From this point of view, the use of FW for caproate production resulted to be convenient for two main reasons. On the one hand, management and disposal issues related to FW amount (1.3 billion tons per year) can be reduced; on the other hand, a compound with a high added value as well as caproate can be produced through an eco-sustainable process.

The biological production of caproate from FW is an innovative process that offers an ecologically sustainable approach to MCFAs production from waste sources. Nevertheless, several obstacles must be overcome before a widespread large-scale industrialization of this biological process. Composition, structures as well as the metabolic pathways and functional stability of microbial communities play a crucial role in the caproate production. Functional stability, in terms of resistance and resilience of microorganisms, is essential in biotechnological applications. In the case of caproate production from FW, it ensures consistent and reliable performance of the microbial community in the caproate production process, even under varying environmental conditions and potential stresses. This reliability is a key point

for scaling up from laboratory to large-scale industrial processes, where fluctuations in conditions frequently occur. Understanding the factors that most influence the stability of the microbial community, allows for adjustments of processes to support these conditions, thereby enhancing the overall efficiency and output of caproate production. Moreover, functional stability allows for adjustments to the resilience of the microbial community. A stable microbial community can withstand disturbances without significant losses in productivity, which is critical for maintaining efficient production rates over time. This resilience is often a result of the diversity and interactions within the microbial community, where different species can compensate for each other's functions if some members are inhibited or removed. Finally, functional stability can impact the economic and environmental viability of the fermentation process. A stable process reduces the need for frequent interventions and adjustments, lowering operational costs and minimizing resource wastage. It also ensures that the process is robust enough to handle the complexities and variations inherent in using food waste as a feedstock. However, research on these aspects needs more in-depth studies. For instance, how the biotic interactions affect microbial functional stability in terms of resistance and resilience of respiration and growth rate of bacteria in CE systems remain ambiguous, the economic and environmental viability of the fermentation process at large-scale requires validation, and the absence of a comprehensive operational model still hampers process guidance. The use of continuous or semi-continuous reactors had provided understanding of fermentation process, allowing for precise control of operational conditions. This approach would enable simulating more realistic large-scale production conditions and evaluating the efficiency of microbial communities in a continuous environment, more representative of industrial conditions. Despite of the importance of the role of microorganisms in this biological process, less than half works reporting so far, the use of FW for caproate production described the diversity and dynamics of microbial communities involved in CE.

It is therefore clear how much important could be to further expand knowledge about the chain elongating microbiome. To date, less than 20 microorganisms able to perform chain elongation have been isolated. Different approaches and various medium

conditions were reported in literature for the isolation of pure cultures, anyway the success in obtaining CE isolates was limited (Jeon et al. 2010; Candry et al. 2023; Ulčar et al. 2024). The isolation of microorganisms from the reactor biomass could lead to a more accurate understanding of the microbial communities involved in the fermentation process. This could help identifying and characterizing specific key microbial strains or consortia contributing to caproate production. The isolation of chain elongating bacteria remains an open challenge for future research.

Recently, the research spotlights have turned on the development of new tools to construct synthetic microbial consortia and to program their behaviors for expanding the functions that can be performed by microbial mixed communities (McCarty and Ledesma-Amaro 2019). The engineering of microbial consortia for diverse applications, including the bioproduction of chemicals from waste sources, is an emerging approach for biotechnological applications that suggest future research efforts. The construction of synthetic microbial communities allows the generation of defined systems with reduced complexity compared to natural mixed microbial communities with often unknown functions (Großkopf and Soyer 2014). Overall, microbiome design can follow two different approaches (Lawson et al. 2019). The top-down approach tries to predict how ecosystem level controls can create a microbiome with desired functions. At the same time, recent advances in multi-omics have provided opportunities to design microbiomes from the bottom up by predicting how the control of metabolic networks and their interactions can create a microbiome with desired functions (Lawson et al. 2019). A combination of these approaches can offer complementary strategies to design microbiomes for caproate production from FW. Indeed, synthetic microbiome engineering could design and build tailored microbial communities to optimize the production of specific compounds. Using synthetic microbiomes may allow for more precise control over the composition and functions of the microorganisms involved in the fermentation process, enabling the design of highly specialized microbial communities for MCFAs production. Recent advances in systems and synthetic biology as well as metabolic engineering of microbial strains and consortia can be an innovative approach for efficient resource recovery. These strategies aim to enhance the conversion of diverse

renewable feedstocks (e.g., FW, agricultural residues) into value-added biocompounds. Future research needs to focus on optimizing both single-strain and consortium-based approaches and maximizing their synergy for higher efficiency and scalability of CE process. In a search of future application, analyses of the economic viability and environmental sustainability of these technologies will be necessary.

Overall, the CE pathway involves the iterative repetition of condensation, reduction, dehydration, and reduction steps to gradually increase the carbon chain length of the fatty acids. The cooperation between different enzymes and microbial species in this pathway allows for the production of MCFAs from SCFAs in a controlled and sequential manner. Understanding the specific enzymes and their respective expression in the CE pathway is essential for optimizing MCFAs production and engineering microbial consortia with enhanced CE capabilities.

A multi-omics approach can allow to largely improve comprehension of the genetic potential and diversity of microorganisms selected in the CE process. To date, few studies applied meta-omics methodologies for studying MCFAs production from organic wastes (Scarborough et al. 2018a, 2022; Myers et al. 2023) and even fewer focused on FW (Crognale et al. 2021). Nevertheless, metagenomic and metatranscriptomic data can provide insights into the functional potentialities and activities of chain elongating bacteria very helpful for the optimization and scale-up of the caproate production from FW. Indeed, the analysis of the genetic content of microorganisms allow defining metabolic pathways and gene regulatory networks involved in CE process. Most of the studies performed so far have applied amplicon sequencing (usually Illumina sequencing of 16S rRNA gene) for estimating diversity and dynamics of microbial communities. It is worth to noting that molecular ecology studies based on long reads sequencing, as well as Oxford Nanopore Technologies (Wang et al. 2021), is completely lacking in this field and required further research efforts.

A deep understanding of bacteria responsible for CE process within a mixed community can allow the identification of members of satellite community, whose role and contribution in carbon and electron losses is not well clarified. For this purpose, advanced molecular sequencing techniques combined with stable isotope probing (i.e. DNA-SIP, RNA-SIP) could

help to identify activity of community members with different substrates. Proteomic-SIP can determine how different microorganisms metabolize different substrates in CE process from FW, especially when combined with metagenome-assembled genomes from the complex microbiome (Bryson et al. 2016; Candry and Ganigué, 2021). Such knowledge could be combined with fluxomic analyses or condensed into mathematical models (e.g. stoichiometric or kinetic models) to unravel the flows of substrates and products between interacting community members (Winter and Krömer 2013; Chokkathukalam et al. 2014; Scarborough et al. 2020a).

Finally, the use of advanced biomolecular techniques for a deep characterization of microorganisms involved in CE process from FW can allow designing better bioprocesses by finding right operational conditions to favor chain elongators. The integration of ecological, physiological and metabolic data in biokinetic and/or thermodynamic models may help to find the right operational condition for optimizing and scaling-up the process (Crognale et al. 2021; Montecchio et al. 2024). A combined approach based on biomolecular exploration of microbial diversity and metabolic pathways and mathematical models can contribute to the advancement of this emerging field and promote the utilization of FW as a valuable resource to produce MCFAs at large scale.

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