ISBN: 978-628-96613-1-6. ISSN: 2414-6390. Digital Object Identifier: https://dx.doi.org/10.18687/LACCEI2025.1.1.1042

Biomass-Based Proven Techniques for Medium-Chain Fatty Acids: Innovative Methods

Joisleen Ramírez[1]; Génesis Rodríguez[2]; Arthur James Rivas[3]; Paolo Dessì [4]
[1,2,3] Universidad Tecnológica de Panamá, Panamá, joisleen.ramirez@utp.ac.pa, genesis.rodriguez1@utp.ac.pa, arthur.james@utp.ac.pa
[4] Università degli Studi di Napoli Federico II, Italia, *paolo.dessi@unina.it*.

Abstract*– Acid fermentation for the generation of medium-chain fatty acids (MCFA) through mixed culture is an alternative that has a high potentital for high-value biomaterials production. It has been studied with greater concurrence in recent years, obtaining promising results in the pharmaceutical industry and biorefineries. This has created a circular economy by using biomass from distilleries, livestock waste, food waste, and organic sludge. Therefore, this research aims to evaluate the state of the art of producing medium-chain fatty acids from biomass. During this acid fermentation process, electron donors are needed to promote chain elongation pathways, such as β-reverse oxidation, fatty acid synthesis, or other pathways, such as maintaining a high intracellular concentration of acetyl-Coenzyme A. On the other hand, emphasis is made on improved processes to produced medium-chain fatty acids, such as microbial electrolysis, metabolic engineering techniques, the use of new enzymes, and the effect of various carbon sources to increase microbial activity. The results were compared to control samples achieving higher MCFA concentration. The use of biomass for the generation of MCFA for chemical and pharmaceutical products, as well as for biorefining, is an innovative and constantly advancing application.

Keywords*-- medium-chain fatty acids, ethanol, biomass, chain elongation, electron donor.

I. Introduction

Biomass is all organic matter from plants, animals, or waste generated from natural or artificial transformation [1]. These wastes include agro-industrial wastes, which include plant and fruit wastes. In addition, animal wastes, such as manure, poultry manure, and pig slurry, as well as sludge from wastewater treatment, are also included [2]. Currently, due to the increasing food demand caused by population growth, the generation of agro-industrial waste has been increasing, which is causing waste management problems in cities and countries [3]. This problem occurs especially in countries that produce fruits, vegetables, meats, among others. An example of this overgeneration of waste in Panama is rice husks and rice straw. These constitute one of the biggest environmental problems in terms of waste management [4]. It favors the generation of greenhouse gases and respiratory diseases [5], since its "management" makes use of indiscriminate burning by producers. Therefore, researchers have studied the use of biomass for the generation of biofuels and chemical products through thermal, thermochemical, and biological digestion processes [6], [7].

Biological decomposition is divided into anaerobic and aerobic decomposition. Anaerobic decomposition occurs in the absence of oxygen, while aerobic digestion occurs in the presence of oxygen [8]. Anaerobic digestion is a process in which organic substrates are converted into biogas and a nutrient-rich digestate. Instead, anaerobic fermentation results in the production of biohydrogen, biol (liquid organic fertilizer), short-chain fatty acids (SCFA), and medium-chain fatty acids (MCFA), among other by-products [4], [8], [9].

Medium-chain fatty acids (MCFA) can be generated from anaerobic fermentation. This has been the most widely applied technology since it can generate renewable energy and can reduce and take advantage of organic wastes, such as agro-industrial waste, wastewater, food wastes, among others [10], [11], [12]. Medium-chain fatty acids are products with wide applications in sectors of the food, pharmaceutical, chemical, and biofuel industries [13], [14], [15]. Currently, most of these products are generated from fossil materials, which is why it is considered an unsustainable and polluting process due to the generation of greenhouse gases [15], [16]. MCFA are obtained through the chain elongation process [17].

The elongation process for MCFA production is a redox reaction that requires short medium-chain fatty acids as electron acceptors and electron donors, such as ethanol or other reduction agents [18]. Throughout this process, anaerobic organisms use electron donor oxidation to produce acetyl-CoA. This relates to the electron acceptor by donating two carbon atoms from the electron donor during each cycle of reverse β-oxidation producing, then MCFA [12], [19], [20].

Medium-chain fatty acids (MCFA) are organic acids containing a carboxylic group attached to a hydrocarbon chain, with chain lengths between six and twelve carbon atoms (C6-C12) [21]. These MCFAs include caproate, heptylate, caprylate, nonanoate, decanoate, undecanoate, and dodecanoate [22]. MCFAs are bioproducts with wide application in biorefineries and circular economy. However, producing and recovering MCFAs from biomass is a difficult process and is under study. These carboxylic acids are currently obtained from fossil or vegetal precursors, such as coconut and palm oil production. However, both approaches are unsustainable due either to high carbon emissions or to competition with the food industry [23].

Medium-chain fatty acids have different industrial applications such as antimicrobial agents, comestible additives, animal feed, and biofuels [24]. Biofuels derived from fatty acids depend on the length of the chain generated. For example, gas can be obtained through the C1-C4 carbon chain. Meanwhile, gasoline is produced through the C4-C9 chain. Jet fuel, diesel, and lubricants through the C8-C16, C10-C18, and C16-C30 carbon chain [25]. Hydrocarbons generated from medium-chain fatty acids (C6-C14) are favorable substitutes for jet fuel and gasoline [26].

Due to their high carbon/oxygen (C/O) ratio, methyl esters of MCFA have a higher energy density than ethanol, so they can be used or applied in the process of the generation of liquid fuels. In addition, it is a highly hydrophobic product [21]. The initial pH of the process has been evaluated through various investigations using different biomasses. In addition, the working temperatures during the acid fermentation process and the C/N ratio for the generation of MCFA have been evaluated. However, the available information is still limited, and this process is still under study [27]. Accordingly, this project aims to evaluate the state of the art of producing medium-chain fatty acids from biomass.

II. PRODUCTION OF MEDIUM-CHAIN FATTY ACIDS FROM DIFFERENT SUBSTRATE

Livestock waste, organic sludge, and food waste are the most used biomasses for MCFA production.

A. Livestock waste

Livestock waste include animal manure, poultry manure, straw, and husks from animal bedding [28]. These wastes emit greenhouse gases into the environment [29]. Steed and Tibbo [30] mentioned that livestock contributes between 59% and 63% of the world's greenhouse gas emissions. In addition, they contribute 84% of nitrous oxide (N_2O) emissions and 54% of methane (CH_4) emissions. Rekleitis et al. [28] also mentioned that methane generation is high from this waste. Another study [20] mentions that cattle manure biomass generates a higher percentage of caproate than other biomasses, such as activated sludge. This is due to the high presence of chains elongating microorganisms inherent to the biomass, resulting in high MCFA production efficiency.

Liu et al. [31] used cattle manure as a substrate in their two-stage assays, where the first stage corresponded to the acidification process of the material, and the second stage involved the addition of the electron donor (lactic acid). To enhance the fermentation process, they added N2 nanobubble water at the second stage of the process. This enhanced caproic acid production by 55.03% compared to the control group. This was because nanobubbles can break down recalcitrant material from the substrate. Which enhances the presence of microorganisms associated with lignocellulose degradation, increasing carbohydrate degradation, and enhancing the fatty acid cycle pathway during chain elongation [31]. The anaerobic fermentation of swine manure and corn stalks under thermophilic conditions improved lactic acid production, which resulted in the generation of MCFA by chain elongation under mesophilic conditions. A maximum productivity of 1890 mg_{COD}/L/d and 1290 mg_{COD}/L/d of MCFA and n-caproate was obtained, respectively [32]. Another study [33] reported the production of 6.7 g_{COD}/L/d of MCFA using swine manure as substrate, where 55.1% corresponds to caproic acid (C6) and only 6.1% to heptanoic acid (C7).

B. Organic sludge

Organic sludge is the solid waste generated by wastewater treatment plants. High organic matter concentrations characterize this raw material. It also contains nutrients such as nitrogen (N), phosphorus (P), calcium (Ca), potassium (K), copper (Cu) and zinc (Zn) [34], [35]. In this sense, the hydrolysis of sewage sludge to obtain amino acids (as an in-situ electron donor) through the acid fermentation process is a treatment to obtain MCFA. In their study, Wu et al. used this technique to obtain a production of up to 3.01 g/L of caproate [36]. Organic sludge from treatment plants is a substrate used to obtain MCFAs because it has a high content of microorganisms and extracellular hydrolytic enzymes. Sludge has been used as a substrate, and inoculum mixed with ethanol as an electron donor, obtaining a maximum of 7173.1 mg_{COD}/L of MCFA [37]. Another study produced a 61.3% conversion rate of short-chain fatty acids (SCFA) to MCFA. This study used liquor-making wastewater such as substrate and ethanol as the electron donor for chain elongation [38].

C. Food waste

Population growth has caused the inadequate use of natural resources since it is necessary to cover people's basic needs, such as food, electricity, and transportation. This has caused land, water, and air to show a continuous deterioration [39], [40]. This population increase leads to more food production, which increases the generation of municipal organic waste (OMW). According to the World Bank, in growing and low-income countries, 93% of municipal solid waste is incinerated or deposited in open fields, roads, waterways, and bodies of water. In high-income countries, only 2% of municipal solid waste is incinerated [41]. Large-scale waste management continues to be a problem for developing countries [42].

Food waste has been used in anaerobic fermentation to produce MCFA and long-chain alcohols. Food waste represents a good substrate for the formation of MCFA. In one study, these were inoculated with yeast (*Saccharomyces cerevisiae*) to obtain ethanol, which is an electron donor. This helped to generate 533 mg_{COD}/L of MCFA *[43]. In their study, Fernando-Foncillas et al. [44] used a 40/60 ratio of sewage sludge/municipal organic waste (restaurant, supermarket, and household waste) to produce MCFA. Without external addition of ethanol. They generated 1870 mg/L caproate and 823 mg/L heptanoate.* Ethanol is the most used electron donor for chain elongation [19].

Ethanol is the sole electron donor in the system, and CO_2 is required to promote the growth of MCFA generating organisms. This is for the stimulation of syntrophic ethanol oxidation, which increases production costs [12], [45], [46]. Therefore, in situ electron donor generation is recommended to alleviate the cost problem [36], [47], [48].

In their study, Wua et al. used food waste as a substrate. These were inoculated by anaerobic digestion sludge (sewage sludge). They added an anaerobic yeast (Saccharomyces cerevisiae) to produce ethanol as an electron donor [43]. This yeast was added in the form of dry cell powder. They showed that adding the yeast improves the formation of MCFA by chain elongation, 27.44% MCFA against 2.97% of the control [47].

By using expired beverage and dairy wastes for chain elongation by lactate or lactic acid (as electron donor), >500 mMC of caproate and >300 mMC of butyrate were produced, with 85% caproate selectivity [49]. Lactic acid and ethanol as electron donors allow the increase of carbons in the electron acceptor chain to produce MCFA [21]. On the other hand, a high ratio of lactic acid/volatile fatty acids promotes electron transfer from the electron donor to the carbon chain because volatile fatty acids are the electron acceptors [32].

III. Strategies for Medium-Chain Fatty Acid Production

The conversion of biomass into medium-chain fatty acids (MCFAs) has been widely studied due to its potential to transform organic waste into high-value products. Among the most consolidated strategies is anaerobic fermentation, which has proven to be highly effective for this purpose [80]. In this regard, Wu et al. [81] evaluated the application of this technology using waste activated sludge (WAS) as a substrate, demonstrating that these residues, when anaerobically fermented, can significantly increase the production of n-caproate, one of the most valued MCFAs [98]. Similarly, De

TABLE I
Parameters and Operational Conditions for the Production of Medium-Chain Fatty Acids.

Fermented Digested Biomass	Process Size	Process Characteristics	Digestive Temperature	pH	Fatty Acid Production	Electron Donor	Electron Donor Volume	Ref
Food Waste	Bioreactor (1 L)	- A continuous chain elongation reactor was used. - The system is maintained under anaerobic conditions. - CO_2 flow is controlled to enhance protein synthesis of MCFA-producing microorganisms and to prevent excessive ethanol oxidation to acetate. - Reactor feeding consists of leachate from the acidification stage and chemical-grade ethanol, which is continuously added to promote fatty acid chain elongation. - The Hydraulic Retention Time is maintained at 11 hours, allowing sufficient time for fatty acid chain elongation while limiting acetate consumption by acetotrophic methanogens.	30 °C (Mesophilic)	The pH is regulated between 6.5 and 7.0 using a pH controller and 5 M NaOH solution from day 102 onwards.	Medium-Chain Fatty Acids (MCFA): Caproic, Caprylic of 512 ± 72 mg DQO/g SV	Ethanol	The electron donor volume varies between 9 and 22 g/L (with a minimum concentration of 18.4 g/L from day 123 onwards).	[56]
Food Waste	Discontinuous reactor (20 L)	- The process is carried out at atmospheric pressure (1 atm), simplifying operation and reducing costs compared to pressurized systems. - The organic loading rate (OLR) represents the amount of organic matter (expressed as Chemical Oxygen Demand, COD) introduced daily into the reactor. - The system's pH is regulated by adding sodium hydroxide (NaOH) to neutralize acidity generated by fermentation, or hydrochloric acid (HCl) if pH reduction is needed.	35 °C (Mesophilic)	Adjusted to pH 5.5	Medium-chain fatty acid production: 5.5 ± 0.4 g/L - Caproic acid (C6): ~4.0 g/L - Caprylic acid (C8): ~1.2 g/L - Capric acid (C10): ~0.3 g/L	Ethanol	~4.0% (w/w) (~40 g/L, ~640 g per reactor)	[57]
Fermented Activated Sludge	Batch / Anaerobic Reactor	- Acidification is controlled until short-chain fatty acid (SCFA) concentrations no longer increase. Samples are typically collected every 2-3 days to measure acid concentrations. - 10.5 g/L of sodium 2-bromoethanesulfonate is added to inhibit methanogen activity, allowing acidic products to accumulate without being converted into methane. - The chain elongation process can last up to 33 days, depending on fatty acid concentrations and other parameters. Sampling is performed every 2-3 days to monitor progress and MCFA production. - No external microbial inoculum was added in this study, as the wastewater sludge contains endogenous microorganisms sufficient for fatty acid production. The microorganisms in the sludge participate in biomass hydrolysis and subsequent fermentation to produce SCFA and MCFA.	35 °C (Mesophilic)	Adjusted to pH 6.0 - 8.0	Medium-Chain Fatty Acids (MCFA): 4079 mg/L (max with 20 g/L ZVI) Caproic acid (C6): 3719.6 mg/L Heptylic acid (C7): 211.1 mg/L Caprylic acid (C8): 148.3 mg/L	Ethanol,	The electron donor volume 4555 mg/L (~5 - 20% v/v ethanol) | 100 mL (in 250 mL serum bottles) |	[58]

Groof et al. [82] reported that the fermentation of complex waste using mixed microbial cultures is effective in producing medium-chain carboxylic acids (MCCAs), positioning it as a competitive alternative to traditional technologies [83][82]. On the other hand, emerging methods such as microaerophilic fermentation have begun to attract interest. Although less studied, this technique has shown interesting potential to improve product selectivity by reducing the formation of undesired compounds, as demonstrated by Zhang et al. [84] These results suggest a possible complementary role to anaerobic fermentation, although it still faces technical challenges such as the need for precise oxygen control and the lack of large-scale validation studies [99].

Fatty acid chain elongation is a biochemical process involving carbon units to short-chain fatty acids, converting them to middle-chain fatty acids. This process occurs intracellularly [50], [51]. An effective strategy for chain elongation is using acidified and hydrolyzed feeds containing either ethanol or lactic acid to convert them to n-caproate. The operation of a reactor with a long hydraulic retention time of 4 days was more effective than 1 day in promoting chain lengthening from MCFA and ethanol. in a higher concentration of MCFA. In addition, it leads to more efficient use of ethanol and substrates (fermentation medium or substrate for microorganism cultivation) [52].

Another strategy for chain elongation is using metabolic engineering by genetically engineering the essential enzymes to promote MCFA production [28]. A study provided evidence that metabolic engineering is critical to designing better pathways. For example, Rigouin et al., the role of the protein used (YALI0F0654 (Yl ELO1)) in the elongation of exogenous or de novo synthesized C14 into C16 and C18 can be seen [53].

Metagenomics is also defined as a strategy used for chain elongation. A study investigated how ammonia affects chain elongation, giving insights into the mechanism of ammonia tolerance [53]. Fernandez-Blanco et al. [54] conducted studies that established optimal conditions for bacterial growth. In addition, they conducted studies in batch bioreactors to observe the fermentation of lactate, which is the electron donor, and acetate (the electron acceptor), as a result, confirmed consistent production profiles of C4 - C8 fatty acids.

Another process used for chain elongation is the use of precursor substrates. For example, Smit et al. used propionate and methanol as electron donors for chain elongation to n-valerate in an open-culture bioreactor. This proved that increasing the propionate substrate concentration provided a consumption between 10 and 30%, and increasing the methanol substrate concentration in the effluent (from 250 to 400 mM) resulted in higher productivity (from 45 to 58 mmol C/L/day) [55].

Table 1 analyzes various biomass fermentation processes for fatty acid production, detailing the operational characteristics of each system, including the type of biomass used, reactor configuration, temperature, and pH conditions. It also highlights the role of electron donors in optimizing substrate conversion into value-added products. The study examines different biomass types, such as the acidified organic fraction of municipal solid waste (MSW), leachates, and fermented activated sludge, each undergoing specific processes with variations in reactor scale and design.

Gyebi Arhin et al. utilized food waste in a 1 L bioreactor, operating a continuous anaerobic system with acidified leachate and ethanol at mesophilic temperatures (see Table 1). The pH was controlled between 6.5 and 7.0, with a retention time of 11 hours, resulting in a production of 512 ± 72 mg COD/g VS of caproic (C6) and caprylic (C8) acids [56]. In a 20 L batch reactor, Lan Wu et al. investigated food waste fermentation at 35 °C and a pH of 5.5 (see Table 1). Ethanol was added at approximately 40 g/L (~640 g per reactor), yielding a total MCFA production of 5.5 ± 0.4 g/L. Of this, caproic acid (C6) accounted for 4.0 g/L, caprylic acid (C8) for 1.2 g/L, and capric acid (C10) for 0.3 g/L [57]. On the other hand, the investigation focused on fermented activated sludge in an anaerobic reactor. T. Lou et al. maintained a temperature of 35 °C, with the pH adjusted between 6.0 and 8.0 (see Table 1). Ethanol was added at a concentration of 4555 mg/L (5-20% v/v), leading to a maximum MCFA concentration of 4079 mg/L. Among the fatty acids produced, caproic acid (C6) reached 3719.6 mg/L, followed by heptylic acid (C7) at 211.1 mg/L and caprylic acid (C8) at 148.3 mg/L [58].

IV. Strategies for the Improvement of the Medium-Chain Fatty Acid Production Process

Several strategies have been proposed for improving chain elongation, such as feedstock optimization using microbial electrolysis (ME), biomass fermentation, and co-fermentation to produce MCFA [59]. Chua et al., in their review, refer to ME as using microbes as catalytic electrodes for chemical production from CO2 [60]. He also mentioned that ME can be used for in-situ ammonia and sulfur recovery, resulting in lower microbial toxicity and competing reactions, thus increasing MCFA production [61]. A study explored the co-fermentation of sludge and lignocellulosic biomass (fallen leaves and grass). This significantly accelerated caproate production, showing a 41.73 % improvement [21].

Zerovalent iron addition resulted in an MCFA production of 15.4 g, which was 5.3 times higher than the control [62]. Wu Lin et al. conducted a study using biochar and activated carbon in fermentation to improve MCFA production. The results show that using fine biochar of 75-150 µm improved MCFA production with a higher electron transfer efficiency of 92.6% [63].

Another strategy employed to improve MCFA production is the use of metabolic engineering and multidimensional engineering techniques for the modification of microorganisms [64],[65]. In a study by Zhiwei et al., an orthogonal Type I bacterial fatty acid synthase (FAS) was engineered to produce medium-chain fatty acids (MCFA) in the yeast *Saccharomyces cerevisiae*. Multidimensional engineering was carried out to increase cellular tolerance to toxic MCFA, targeting the

TABLE II
EFFECTS OF BIOCHAR ON MEDIUM-CHAIN FATTY ACID PRODUCTION FROM BIOMASS

Fermented Digested Biomass	Process Size	Digestive Temperature	Process Characteristics	pH	Fatty Acid Production	Biochar	Biocarbon Volume	Ref
Anaerobic Digestion and Fermentation of Sludge	Pilot Scale (1-10L)	30-37°C (Mesophilic)	-Anaerobic controlled process with electron addition (ethanol). -Biochars improve microbial activity, with precise temperature control and constant agitation. Continuous gas composition monitoring.	6.0-7.0	Medium-chain fatty acids (e.g., caproate), ~10-20 g/L	Biochar derived from anaerobically digested sludge	Generation from waste, 5-10% of processed biomass volume (~50-100 g/L)	[77]
Anaerobic Biomass Treatment of Sludge	Laboratory Scale	35°C (Mesophilic)	-Fermentation with constant temperature, pH adjustment, and control during the process. - Microbiota adaptation to maximize fatty acid production, with biocarbon inclusion to optimize fermentation. - Monitoring key parameters like residence time.	6.5	Caproic acid (C6), ~5-8 g/L	Biochar derived from anaerobically digested sludge	Biochar for microbial enhancement, 7% of biomass volume (~70 g/L)	[77]
Organic Waste Digestion (Food Waste)	Industrial Scale	37°C (Mesophilic)	-Continuous reactor process with strict temperature and pH control. -Biochar is used as an adsorbent to improve substrate conversion, monitoring of gases, and optimization of reaction time for maximum fatty acid production.	7.0	C6-C10 fatty acids, ~15-25 g/L	Biochar derived from anaerobically digested sludge	Biochar optimizes production, 10% of biomass volume (~100 g/L)	[77]
Sewage Sludge (Low Carbon)	Laboratory Scale	35-37°C (Mesophilic)	-Improved carbon conversion and electron transfer efficiency in EC. - Biochar dose affects medium-chain fatty acid production. - Different doses impact microbiological genera. Dominant pathways in EC related to MCFA production. - High biocarbon doses stimulate acidifying genera.	6.8-7.2	Medium-chain fatty acids: Caproate (3347.95 mg/L) and caprylate (612.36 mg/L)	Biocarbon (10-20 g/L) (It is not mentioned)	Biochar (10-20 g/L), 10-20% of biomass volume	[78]
Sewage Sludge (Low Carbon)	Laboratory Scale (Serum Bottles)	35-37°C (Mesophilic)	-Biocarbon increases MCFA accumulation by more than 114%. It also improves carbon conversion efficiency (134.66%) and electron transfer efficiency (94.22%). -The addition of biocarbon before acidification improves the activity of chain elongation bacteria (Paraclostridium). -Biocarbon enhances solubilization of the sludge, achieving a SCOD of 18,425 mg/L at terminal acidification.	6.68	Medium-chain fatty acids: Caproate (3347.95 mg/L) and caprylate (612.36 mg/L)	Wood Biocarbon	Wood biocarbon (10-20 g/L), 10-20% of biomass vo	[79]

evolution of the Type I membrane transporter and adaptive laboratory evolution of the strain. This combination of individual yeast selection and optimized cultivation resulted in a 1.3 ± 0.3-fold and 1.7 ± 0.2-fold increase in MCFA production, respectively [64]. In addition, mention should be made of metabolic engineering of the biosynthesis pathway. This is intended to generate a cell factory that produces cost-effective molecules on an industrial scale [65]. Separately, such modifications have been shown to increase accumulation up to fivefold and result in extracellular heaping of free fatty acids [66].

Fatty acid optimization utilizes modified metabolic strains, and to achieve this, genes encoding thioesterase (TE) protein were expressed [9]. Liu Jin et al. demonstrated that TE from *Corynebacterium glutamicum* (CG) can potentially improve fatty acids. The results showed that the total production of FAs increased to 180.52 mg/g dry molecular weight [67]. Also, among the strategies for improving MCFA production is the engineering of the transport system. A few years ago, endogenous genes related to transporters responsible for the export of MCFA in *E. coli* were investigated [68]. These endogenous genes are related to medium-chain fatty acid transporters to identify those responsible for fatty acid exports in *E. coli*. As a result, it was shown that the modified strain evidenced a twofold increase compared to the original strain. The basis of this research was based on a strain that overexpressed the accre genes, mdtE, and mdtC, and had the cmr gene deleted to achieve a combinatorial effect [69].

Exploiting new enzymes to enhance the production of MCFA is one way to improve the production of MCFA in microbial strains [70]. Computational methods have been developed that accurately predict enzyme numbers from protein sequences and have the desired yield for improved production of MCFAs [68],[71]. More recently, AI (Artificial Intelligence) computational designs have been developed to suggest practical strategies and reduce iterative experiments to obtain synthetic enzymes with desired catalytic functions [52], [68].

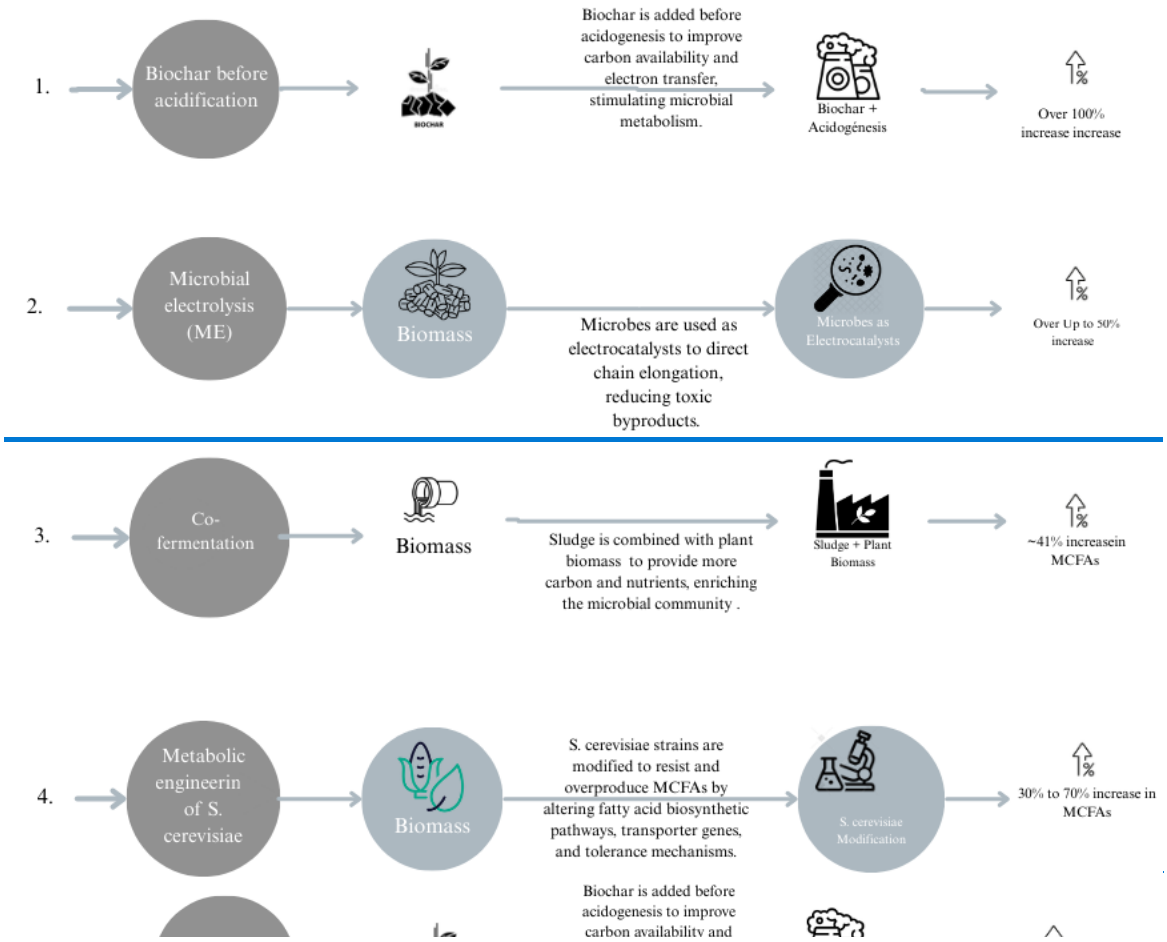
Engineering the transport system in MCFA is a useful and effective strategy to improve cellular acid resistance in MCFA producers [68]. Cultures producing MCFAs become acidic due to their accumulation. Thus, acids easily enter the cell, causing intracellular acidity [72]. The transport engineering strategy to

maintain intracellular pH is to identify proton pumps. This is because as they consume ATP (adenosine triphosphate) to export protons, supplementation of auxiliary energy substrates and increased oxidative phosphorylation enhance cellular regulation and thus increase the yield of MCFA production [54], [74].

The effect of carbon sources on the production of MCFA plays an important role in fermentation. To evaluate the suitable source for optimal growth, three different carbon sources combined with coconut oil (3%) and palm oil (3%) were used [65], [75]. It was then found in this research by Hussain et al. higher fatty acid contents (%FA) were obtained at 26 °C, with mixed palm oil medium, and the highest overall lipid productivity (8.6 g/L-d) with coconut oil supplementation [76].

Table 2 summarizes strategies for MCFA production at different scales, under mesophilic temperatures (30-37 °C) and pH adjustment to optimize microbial activity. The use of biochar is highlighted for enhancing substrate conversion and electron transfer. The first three cases presented in Table 2 are from research conducted by Lin Wu et al. They performed three experiments using biochar derived from digested sludge and organic waste. The first experiment was carried out at a pilot scale (1-10 L) under mesophilic conditions (30-37 °C) with controlled pH between 6.0 and 7.0 (see Table 2). Additionally, they used biochar derived from anaerobically digested sludge at a dosage of 5-10% of the biomass volume (~50-100 g/L). This approach enhanced microbial activity and produced caproate (C6) concentrations ranging from 10 to 20 g/L. The second experiment involved anaerobic sludge treatment at a laboratory scale, conducted at 35 °C with a pH adjusted to 6.5 (see Table 2). They included biochar derived from digested sludge at a concentration of 7% of the biomass volume (~70 g/L), facilitating caproic acid (C6) production within a range of 5-8 g/L. For the industrial-scale study, they operated a continuous reactor at 37 °C with a pH of 7.0. The addition of biochar represented 10% of the biomass volume (~100 g/L), optimizing substrate conversion and enabling the production of C6-C10 fatty acids in concentrations ranging from 15 to 25 g/L [77].

Laboratory-scale sludge treatment with low carbon content was carried out by Tianru Lou et al. They conducted the process at mesophilic temperatures ranging from 35 to 37 °C, with a pH between 6.8 and 7.2 (see Table 2). A biochar dosage of 10-20 g/L (10-20% of the biomass volume) was applied, which enhanced the production of caproate (3347.95 mg/L) and caprylate (612.36 mg/L) while also stimulating acidogenic bacterial genera associated with MCFA production [78]. Also, Tianru Lou et al. conducted experiments using serum bottles at 35-37 °C under mesophilic conditions, with a pH of 6.68 (see Table 2). They reported that adding biochar before the acidification process led to an increase of over 114% in MCFA accumulation, improving carbon conversion efficiency by 134.66% and electron transfer efficiency by 94.22%. As a



result, a SCOD of 18,425 mg/L was achieved. In this system, wood biochar was applied at 10-20 g/L concentrations, representing 10-20% of the biomass volume [79]. Overall, biochar enhances process efficiency by increasing carbon conversion and promoting the growth of key microorganisms. Its dosage ranges between 5% and 20% of the biomass volume, depending on the type of waste treated.

V. Challenges, Opportunities, and Economic Feasibility in MCFA Production

The use of residual biomass to produce medium-chain fatty acids (MCFAs) stands out as an emerging strategy aligned with circular economy principles [85]. Being regarded as waste, discarded or used products can be repurposed as raw materials to produce high-value compounds [86]. However, its implementation is limited by several factors, among which the variability of raw material composition, such as municipal organic waste, sewage sludge, or lignocellulosic waste, is one of the main challenges [87]. This is largely due to the partial utilization of the substrate, the lack of electron donors, the generation of secondary compounds, the toxic inhibition caused by MCFAs in their undissociated form, and by ethanol. Additionally, the low performance of the microbiome limits the process efficiency, making it necessary to apply specific pretreatments to favor effective fermentation [88], [89]. It is also essential to optimize operational parameters such as pH, temperature, and the ratio between substrate and electron donor, as well as maintain the stability of the microbial consortium responsible for chain elongation [90], [91].

Given the challenges in MCFA production, it is important to highlight the opportunities that enhance this biotechnological pathway. One such opportunity is the use of biochar, which not only serves as a support for microbial growth but also improves process stability by buffering pH fluctuations and adsorbing toxic compounds [87], [92]. Metabolic engineering also plays a crucial role in optimizing biosynthetic pathways in microorganisms, improving production efficiency by modifying key genes, reducing the formation of by-products, and facilitating the design of more productive strains for industrial applications [93].

The economic feasibility of producing MCFAs from biomass remains a challenge for scalability. While the use of low-cost waste such as sludge and food waste has proven to reduce operational costs, challenges such as pretreatment, collection, and optimization of conditions like pH, temperature, and organic load persist [94], [95]. Studies such as that by Scarborough et al., which conducted a techno-economic assessment (TEA) based on lignocellulosic vinasse and simulations with ASPEN, have demonstrated that, at the pilot level, productivity remains insufficient to reach cost parity with the market [96]. On the other hand, although strategies like the addition of biochar and the use of residual ethanol as an electron donor have improved conversion, there is still a need to increase process efficiency and reduce investment costs (CAPEX) to achieve viable and sustainable MCFA production on an industrial scale [97].

In this regard, future research should focus on specific areas such as the detailed characterization of microbiomes specialized in chain elongation, with an emphasis on highly productive bacterial strains that are tolerant to inhibitors like caproate and capable of adapting to a wide variety of complex substrates [100]. The exploration of synthetic microbiomes designed through synthetic biology tools could enable the creation of more efficient and stable microbial communities [102],[103]. Moreover, emerging technologies such as bioelectrochemical systems integrated with anaerobic fermentations could offer new pathways to enhance energy recovery and the production of high-value compounds [101]. Another key strategy is the use of modular and scalable bioreactor configurations, adaptable to different types of waste, along with the evaluation of new technologies for the in-situ separation and purification of MCFAs, to reduce their toxicity during fermentation [104][105]. These lines of research are essential to overcome current technical and economic challenges, move toward efficient industrial implementation, and strengthen waste valorisation within a circular bioeconomy [94].

Conclusions

The conversion of biomass, such as agroindustrial waste, sewage sludge, and other feedstocks, into medium-chain fatty acids (MCFA), represents a strategy to sustainably harness the growing amount of organic waste. Employing biological processes like anaerobic fermentation and chain elongation enables the production of high-value-added products for the food, pharmaceutical, and energy industries, alongside the study of operational condition optimization (pH, temperature, nutrients, and electron donors). This makes it possible to create increasingly specialized bioproducts with greater added value. Moreover, incorporating advanced methodologies, ranging from adding biochar and zerovalent metals to metabolic engineering techniques and artificial intelligence tools, has demonstrated the potential to increase MCFA productivity and selectivity. However, areas still need further study, such as variability in substrate composition, production scale, the development of systems resistant to inhibitions, and the reduction of operating costs. Consequently, deepening research and designing efficient processes for MCFA production is crucial to drive the circular economy and reduce the carbon footprint by leveraging organic waste, which remains a significant global challenge in waste management today.

Acknowledgment

The authors thank the National Secretariat of Science, Technology, and Innovation (SENACYT) for its financial

contribution to this work, which is part of Project FID2024-118, "Evaluation of organic waste for its conversion into medium-chain fatty acids for transformation into biofuels for the maritime and aviation sectors." In addition, the authors thank the National Research System (SNI) of the Republic of Panama.

REFERENCES

[1] M.-A. Perea-Moreno, E. Samerón-Manzano, and A.-J. Perea-Moreno, "Biomass as Renewable Energy: Worldwide Research Trends," *Sustainability*, vol. 11, no. 3, p. 863, Feb. 2019, doi: 10.3390/su11030863.

[2] E. A. Reyes Aguilera, "Generación de biogás mediante el proceso de digestión anaerobia, a partir del aprovechamiento de sustratos orgánicos," *Revista Científica de FAREM-Estelí*, no. 24, pp. 60–81, Jan. 2018, doi: 10.5377/farem.v0i24.5552.

[3] K. Cury R, Y. Aguas M, A. Martinez M, R. Olivero V, and L. Chams Ch, "Residuos agroindustriales su impacto, manejo y aprovechamiento," *Revista Colombiana de Ciencia Animal - RECIA*, vol. 9, no. S1, pp. 122–132, May 2017, doi: 10.24188/recia.v9.nS.2017.530.

[4] B. Bharathiraja, T. Sudharsana, J. Jayamuthunagai, R. Praveenkumar, S. Chozhavendhan, and J. Iyyappan, "RETRACTED: Biogas production–A review on composition, fuel properties, feed stock and principles of anaerobic digestion," *Renewable and Sustainable Energy Reviews*, vol. 90, pp. 570–582, Jul. 2018, doi: 10.1016/j.rser.2018.03.093.

[5] M. Romero Placeres, F. Diego Olite, and M. Álvarez Toste, "La contaminación del aire: su repercusión como problema de salud," *Scielo*, vol. 44, no. 2, 2006, [Online]. Available: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1561-30032006000200008

[6] J. Ramírez, E. Deago, and A. M. C. James Rivas, "Effect of Biochar on Anaerobic Co-Digestion of Untreated Sewage Sludge with Municipal Organic Waste under Mesophilic Conditions," *Energies (Basel)*, vol. 17, no. 10, p. 2393, May 2024, doi: 10.3390/en17102393.

[7] J. Prens, Z. Kurt, A. M. James Rivas, and J. Chen, "Production and Characterization of Wild Sugarcane (Saccharum spontaneum L.) Biochar for Atrazine Adsorption in Aqueous Media," *Agronomy*, vol. 13, no. 1, p. 27, Dec. 2022, doi: 10.3390/agronomy13010027.

[8] Y. Acosta Lorenzo and M. C. Obaaya Abreu, "La digestión anaerobia. Aspectos teóricos. Parte I," *ICIDCA*, vol. 39, no. 1, pp. 35–48, 2005.

[9] Yanan Yin, Yuming Hu, and Jianlong Wang, "Co-fermentation of sewage sludge and lignocellulosic biomass for production of medium-chain fatty acids," *Bioresour Technol*, vol. 361, Oct. 2022.

[10] Q. Liu, L. Li, X. Zhao, and K. Song, "An evaluation of the effects of nanoplastics on the removal of activated-sludge nutrients and production of short chain fatty acid," *Process Safety and Environmental Protection*, vol. 148, pp. 1070–1076, Apr. 2021, doi: 10.1016/j.psep.2021.02.029.

[11] L. Li, Z. Li, K. Song, Y. Gu, X. Gao, and X. Zhao, "Short-chain fatty acids resource recovery potential from algal sludge via anaerobic fermentation under various pH values," *Chemosphere*, vol. 275, p. 129954, Jul. 2021, doi: 10.1016/j.chemosphere.2021.129954.

[12] S.-L. Wu, Y. Long, W. Wei, X. Shi, D. Shen, and B.-J. Ni, "Co-electron donors driven medium-chain fatty acids production: Roles of electron donors, reaction kinetics and metabolic pathways," *Chemosphere*, vol. 338, p. 139515, Oct. 2023, doi: 10.1016/j.chemosphere.2023.139515.

[13] A. Naresh Kumar and V. M. S., "Acidogenesis of waste activated sludge—Biohydrogen production with simultaneous short chain carboxylic acids," *J Environ Chem Eng*, vol. 6, no. 2, pp. 2983–2991, Apr. 2018, doi: 10.1016/j.jece.2018.04.032.

[14] W. de A. Cavalcante, R. C. Leitão, T. A. Gehring, L. T. Angenent, and S. T. Santaella, "Anaerobic fermentation for n-caproic acid production: A review," *Process Biochemistry*, vol. 54, pp. 106–119, Mar. 2017, doi: 10.1016/j.procbio.2016.12.024.

[15] O. Sarkar, U. Rova, P. Christakopoulos, and L. Matsakas, "Ethanol addition promotes elongation of short-chain fatty acids to medium-chain fatty acids using brewery spent grains as substrate," *J Environ Chem Eng*, vol. 9, no. 5, p. 105990, Oct. 2021, doi: 10.1016/j.jece.2021.105990.

[16] K. L. Wasewar and D. Z. Shende, "Extraction of caproic acid using tri-n-butyl phosphate in benzene and toluene at 301 K," *J Chem Eng Data*, vol. 55, no. 9, pp. 4121–4125, Sep. 2010, doi: 10.1021/je100337m.

[17] M. Roghair *et al.*, "Effect of n-Caproate Concentration on Chain Elongation and Competing Processes," *ACS Sustain Chem Eng*, vol. 6, no. 6, pp. 7499–7506, Jun. 2018, doi: 10.1021/acssuschemeng.8b00200.

[18] S. O-Thong, X. Zhu, I. Angelidaki, S. Zhang, and G. Luo, "Chapter Two - Medium chain fatty acids production by microbial chain elongation: Recent advances," vol. 5, 2020, pp. 63–99. doi: 10.1016/bs.aibe.2020.04.002.

[19] L. T. Angenent *et al.*, "Chain Elongation with Reactor Microbiomes: Open-Culture Biotechnology To Produce Biochemicals," *Environ Sci Technol*, vol. 50, no. 6, pp. 2796–2810, Mar. 2016, doi: 10.1021/acs.est.5b04847.

[20] Q. Yin, Q. Wen, L. Tan, A. Watson, G. Erickson, and X. Li, "Combining chain elongation and oleogel extraction for the production and recovery of caproate from beef cattle manure wastewater," *Resour Conserv Recycl*, vol. 202, p. 107405, Mar. 2024, doi: 10.1016/j.resconrec.2023.107405.

[21] J. Ho Ahn, K. Hwan Jung, E. Seok Lim, S. Min Kim, S. Ok Han, and Y. Um, "Recent advances in microbial production of medium chain fatty acid from renewable carbon resources: A comprehensive review," *Bioresour Technol*, vol. 381, p. 129147, Aug. 2023, doi: 10.1016/j.biortech.2023.129147.

[22] Q. Wu, Y. Jiang, Y. Chen, M. Liu, X. Bao, and W. Guo, "Opportunities and challenges in microbial medium chain fatty acids production from waste biomass," *Bioresour Technol*, vol. 340, p. 125633, Nov. 2021, doi: 10.1016/j.biortech.2021.125633.

[23] S. C. McDowall, M. Braune, and R. Nitzsche, "Recovery of bio-based medium-chain fatty acids with membrane filtration," *Sep Purif Technol*, vol. 286, p. 120430, Apr. 2022, doi: 10.1016/j.seppur.2021.120430.

[24] N. Chu *et al.*, "Microbial Electrosynthesis for Producing Medium Chain Fatty Acids," *Engineering*, vol. 16, pp. 141–153, Sep. 2022, doi: 10.1016/j.eng.2021.03.025.

[25] M.-K. Kang, Y. J. Zhou, N. A. Buijs, and J. Nielsen, "Functional screening of aldehyde decarbonylases for long-chain alkane production by Saccharomyces cerevisiae," *Microb Cell Fact*, vol. 16, no. 1, p. 74, Dec. 2017, doi: 10.1186/s12934-017-0683-z.

[26] R. Lu, T.-Q. Shi, L. Lin, R. Ledesma-Amaro, X.-J. Ji, and H. Huang, "Advances in metabolic engineering of yeasts for the production of fatty acid-derived hydrocarbon fuels," *Green Chemical Engineering*, vol. 3, no. 4, pp. 289–303, Dec. 2022, doi: 10.1016/j.gce.2022.07.008.

[27] Syed Ammar Hussain, Yusuf Nazir, Ahsan Hameed, Wu Yang, Kiren Mustafa, and Yuanda Song, "Optimization of Diverse Carbon Sources and Cultivation Conditions for Enhanced Growth and Lipid and Medium-Chain Fatty Acid (MCFA) Production by Mucor circinelloides," *Safety and Microbiological Quality*, vol. 5, p. 35, Apr. 2019, Accessed: Jun. 29, 2024. [Online]. Available: https://www.mdpi.com/2311-5637/5/2/35

[28] G. Rekleitis, K.-J. Haralambous, M. Loizidou, and K. Aravossis, "Utilization of Agricultural and Livestock Waste in Anaerobic Digestion (A.D): Applying the Biorefinery Concept in a Circular Economy," *Energies (Basel)*, vol. 13, no. 17, p. 4428, Aug. 2020, doi: 10.3390/en13174428.

[29] J. Felippe Cury Marinho Mathias, "Manure as a Resource: Livestock Waste Management from Anaerobic Digestion, Opportunities and Challenges for Brazil," *International Food and Agribusiness Management Review*, vol. 17, no. 4, pp. 87–110, 2014, Accessed: Jun. 01, 2022. [Online]. Available: http://purl.umn.edu/188711

[30] J. Van De Steeg and M. Tibbo, Livestock and Climate Change in the Near East Region Measures to adapt to and mitigate climate change. Food and Agriculture Organization of the United Nations. Regional Office for the Near East, Cairo. 2012. Accessed: Jun. 01, 2022. [Online]. Available: https://www.fao.org/3/i2714e/i2714e.pdf

[31] Y. Liu *et al.*, "Effect of nanobubble water on medium chain carboxylic acids production in anaerobic digestion of cow manure," *Waste Management*, vol. 184, pp. 37–51, Jul. 2024, doi: 10.1016/j.wasman.2024.05.029.

[32] W. Zhang *et al.*, "Medium-chain carboxylates production from co-fermentation of swine manure and corn stalk silage via lactic acid: Without

external electron donors," *Chemical Engineering Journal*, vol. 439, p. 135751, Jul. 2022, doi: 10.1016/j.cej.2022.135751.
[33] W. Zhang *et al.*, "Produce individual medium chain carboxylic acids (MCCA) from swine manure: Performance evaluation and economic analysis," *Waste Management*, vol. 144, pp. 255–262, May 2022, doi: 10.1016/j.wasman.2022.04.001.
[34] E. González-Flores, M. A. Tornero-Campante, E. Sandoval-Castro, A. Pérez-Magaña, and A. J. Gordillo-Martínez, "Biodisponibilidad y fraccionamiento de metales pesados en suelos agrícolas enmendados con biosólidos de origen municipal," *Revista Internacional de Contaminacion Ambiental*, vol. 27, no. 4, pp. 291–301, 2011.
[35] M. K. Hossain, V. Strezov, K. Y. Chan, A. Ziolkowski, and P. F. Nelson, "Influence of pyrolysis temperature on production and nutrient properties of wastewater sludge biochar," *J Environ Manage*, vol. 92, no. 1, pp. 223–228, Jan. 2011, doi: 10.1016/j.jenvman.2010.09.008.
[36] P. Wu *et al.*, "Amino acids as in-situ electron donors drive medium chain fatty acids production from sludge acidogenic fermentation liquid by electro-fermentation enhancement," *Chemical Engineering Journal*, vol. 476, p. 146537, Nov. 2023, doi: 10.1016/j.cej.2023.146537.
[37] Y. Wang *et al.*, "Peracetic acid (PAA)-based pretreatment effectively improves medium-chain fatty acids (MCFAs) production from sewage sludge," *Environmental Science and Ecotechnology*, vol. 20, p. 100355, Jul. 2024, doi: 10.1016/j.ese.2023.100355.
[38] W. Zhang *et al.*, "Bioconversion of swine manure into high-value products of medium chain fatty acids," *Waste Management*, vol. 113, pp. 478–487, Jul. 2020, doi: 10.1016/j.wasman.2020.06.021.
[39] V. Stoehrel, "The impacts of climate change upon food security in the world : The interaction between physical, biological and societal systems," *Theoria*, vol. 20, no. 1, pp. 51–63, 2011.
[40] R. Bhagawati, S. Paul, and A. Dambale, "ROLE OF AGROFORESTRY SYSTEM IN CARBON SEQUESTRATION FOR REGULATING CLIMATE CHANGE," in *Agriculture Development and Economic Transformation in Global Scenario*, Part 1., Mahima Research Foundation and Social Welfare, 2020, ch. ROLE OF AG, pp. 35–42.
[41] D. Barne and D. Wadhwa, "Resumen anual: El año 2018 en 14 gráficos," Dec. 2018. Accessed: Jun. 29, 2023. [Online]. Available: https://www.bancomundial.org/es/news/feature/2018/12/21/year-in-review-2018-in-14-charts
[42] F. F. Franceschi, J. S. Castillo, J. A. Cherni, A. Acosta-González, and M. F. Gómez, "ADPMDesign: The use of a Participatory Methodology to design a dry anaerobic digestion power plant for municipal solid waste treatment," *Energy for Sustainable Development*, vol. 74, pp. 173–184, Jun. 2023, doi: 10.1016/j.esd.2023.03.017.
[43] L. Wu, W. Wei, J. Qian, X. Chen, and B.-J. Ni, "Converting food waste into high-value medium chain fatty acids and long chain alcohols *via* chain elongation with an internally produced electron donor," *Green Chemistry*, vol. 25, no. 24, pp. 10567–10575, 2023, doi: 10.1039/D3GC03614F.
[44] C. Fernando-Foncillas, C. I. Cabrera-Rodríguez, F. Caparrós-Salvador, C. Varrone, and A. J. J. Straathof, "Highly selective recovery of medium chain carboxylates from co-fermented organic wastes using anion exchange with carbon dioxide expanded methanol desorption," *Bioresour Technol*, vol. 319, p. 124178, Jan. 2021, doi: 10.1016/j.biortech.2020.124178.
[45] Q. Wu *et al.*, "Upgrading liquor-making wastewater into medium chain fatty acid: Insights into co-electron donors, key microflora, and energy harvest," *Water Res*, vol. 145, pp. 650–659, Nov. 2018, doi: 10.1016/j.watres.2018.08.046.
[46] S. G. Arhin, A. Cesaro, F. Di Capua, and G. Esposito, "Acidogenic fermentation of food waste to generate electron acceptors and donors towards medium-chain carboxylic acids production," *J Environ Manage*, vol. 348, p. 119379, Dec. 2023, doi: 10.1016/j.jenvman.2023.119379.
[47] L. Wu, W. Wei, Z. Chen, X. Shi, J. Qian, and B.-J. Ni, "Novel anaerobic fermentation paradigm of producing medium-chain fatty acids from food wastes with self-produced ethanol as electron donor," *Chemical Engineering Journal*, vol. 483, p. 149236, Mar. 2024, doi: 10.1016/j.cej.2024.149236.
[48] L. Wu, W. Wei, J. Qian, X. Chen, and B.-J. Ni, "Converting food waste into high-value medium chain fatty acids and long chain alcohols *via* chain elongation with an internally produced electron donor," *Green Chemistry*, vol. 25, no. 24, pp. 10567–10575, 2023, doi: 10.1039/D3GC03614F.
[49] B. Bian *et al.*, "Lactate-mediated medium-chain fatty acid production from expired dairy and beverage waste," *Environmental Science and Ecotechnology*, vol. 21, p. 100424, Sep. 2024, doi: 10.1016/j.ese.2024.100424.
[50] Rutger S. van der Hoeven and John C. Steffens, "Biosynthesis and Elongation of Short- and Medium-Chain-Length Fatty Acids," *Plant Physiol*, vol. 122, no. 1, pp. 275–282, Jan. 2000, doi: https://doi.org/10.1104/pp.122.1.275.
[51] Mark RoghairMark Roghair, Yuchen LiuYuchen Liu, David PTB Huelga, Ruud A. Weusthuis, Marieke E. Bruins, and Cees J. N. Buisman, "Development of an Effective Chain Elongation Process From Acidified Food Waste and Ethanol Into n-Caproate," *Waste Biorefineries: Future Energy, Green Products and Waste Treatment*, vol. 6, Apr. 2018, doi: https://doi.org/10.3389/fbioe.2018.00050.
[52] Joseph P. Torella, Tyler J. Ford, Scott N. Kim, Amanda M. Chen, Jeffrey C. Way, and Pamela A. Silver, "Tailored fatty acid synthesis via dynamic control of fatty acid elongation," *The Scripps Research Institute, La Jolla, CA, and accepted by the Editorial Board*, vol. 110, no. 28, pp. 11290–11295, May 2013, doi: https://doi.org/10.1073/pnas.1307129110.
[53] Zheng Chen *et al.*, "Metagenomic analysis towards understanding the effects of ammonia on chain elongation process for medium chain fatty acids production," *Bioresour Technol*, vol. 395, p. 130413, Mar. 2024, doi: https://doi.org/10.1016/j.biortech.2024.130413.
[54] Carla Fernández-Blanco, María C. Veiga, and Christian Kennes, "Effect of pH and medium composition on chain elongation with Megasphaera hexanoica producing C4-C8 fatty acids," *Front. Microbiol.:Sec. Microbial Physiology and Metabolism*, vol. 14, Nov. 2023, doi: https://doi.org/10.3389/fmicb.2023.1281103.
[55] Sanne M. de Smit, Kasper D. de Leeuw, Cees J. N. Buisman, and David Strik, "Continuous n-valerate formation from propionate and methanol in an anaerobic chain elongation open-culture bioreactor," *Biotechnology for Biofuels* , vol. 12, no. 132, 2019, doi: https://doi.org/10.1186/s13068-019-1468-x.
[56] Samuel Gyebi Arhin, Alessandra Cesaro, Francesco Di Capua b, and Giovanni Esposito a, "Acidogenic fermentation of food waste to generate electron acceptors and donors towards medium-chain carboxylic acids production ," *Journal of Environmental Management* , vol. 348, pp. 119–379, 2023.
[57] Lan Wu, Wei We, Zhijie Chen, Xingdong shi, Jin Qian, and Bing-Jie Ni, "Novel anaerobic fermentation paradigm of producing medium-chain fatty acids from food wastes with self-produced ethanol as electron donor ," *Chemical Engineering Journal* , vol. 483, pp. 149–236, 2024.
[58] T. Lou, Y. Yin, and J. Wang, "Enhancement of medium-chain fatty acids production from sewage sludge fermentation by zero-valent iron," *Chemosphere*, vol. 370, p. 143912, Feb. 2025, doi: 10.1016/j.chemosphere.2024.143912.
[59] W. H. Na Chua , Q. J. L. Qinglian Wuc , Yong Jiang, Peng Liang, and Zhiyong Jason Ren, "Microbial Electrosynthesis for Producing Medium Chain Fatty Acids," vol. 16, pp. 1–153, Sep. 2022, Accessed: Jun. 26, 2024. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S209580992100309X
[60] Yong Jiang, Harold D. May, Lu Lu, Peng Liang, Xia Huang, and Zhiyong Jason Ren, "Carbon dioxide and organic waste valorization by microbial electrosynthesis and electro-fermentation," *Water Res*, vol. 14+, pp. 42–55, 2019, doi: https://doi.org/10.1016/j.watres.2018.10.092.
[61] Korneel Rabaey and René A. Rozendal, "Microbial electrosynthesis — revisiting the electrical route for microbial production.," *Nat Rev Microbiol*, vol. 8, no. 10, pp. 706–716, 2010, doi: https://doi.org/10.1038/nrmicro2422.
[62] A. Mishra, M. Kumar, N. S. Bolan, R. Kapley Atya and Kumar, and L. Singh, "Multidimensional approaches of biogas production and up-gradation: Opportunities and challenges," *Bioresour Technol*, vol. 338, Oct. 2021, doi: 10.1016/j.biortech.2021.125514.
[63] Shu-Lin Wu *et al.*, "Revealing the Mechanism of Biochar Enhancing the Production of Medium Chain Fatty Acids from Waste Activated Sludge Alkaline Fermentation Liquor," *ACS EST Water*, vol. 1, pp. 1014–1024, 2021, doi: https://doi.org/10.1021/acsestwater.0c00278.
[64] Quinn Zhu and Ethel N Jackson, "Metabolic Engineering," *Comprehensive Biotechnology*, vol. 3, 2019, Accessed: Jun. 28, 2024.

[Online]. Available: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/metabolic-engineering
[65] Paul Handke, Sean A. Lynch, and Ryan T. Gill, "Application and engineering of fatty acid biosynthesis in Escherichia coli for advanced fuels and chemicals," *Metab Eng*, vol. 13, no. 1, pp. 28–37, Jan. 2011, doi: https://doi.org/10.1016/j.ymben.2010.10.007.
[66] Pu Xue *et al.*, "A mass spectrometry-based high-throughput screening method for engineering fatty acid synthases with improved production of medium-chain fatty acids," *Biotechnol Bioeng*, vol. 117, no. 7, Mar. 202AD.
[67] Jin Liu, Mandlaa, Jia Wang, Ziyu Sun, and Zhongjun Chen, "A strategy to enhance and modify fatty acid synthesis in Corynebacterium glutamicum and Escherichia coli: overexpression of acyl-CoA thioesterases," *Microb Cell Fact*, vol. 22, p. 191, 2023, doi: https://doi.org/10.1186/s12934-023-02189-w.
[68] Junjun Wu, Zhe Wang, Xia Zhang, Peng Zhou, Xiudong Xia, and Mingsheng Dong, "Improving medium chain fatty acid production in Escherichia coli by multiple transporter engineering," *Food Chem*, vol. 272, pp. 628–634, Jan. 2019, Accessed: Jun. 29, 2024. [Online]. Available: https://www.sciencedirect.com/science/article/abs/pii/S0308814618315127
[69] Ningzi Guan and Liu largo, "Microbial response to acid stress: mechanisms and applications," *Microbiol Biotechnol* , vol. 104, pp. 51–65, Jan. 2020, Accessed: Jun. 29, 2024. [Online]. Available: https://link.springer.com/article/10.1007/s00253-019-10226-1#citeas
[70] A. Madhavan, R. Sindhu, P. Binod, R. K. Sukumaran, and A. Pandey, "Strategies for design of improved biocatalysts for industrial applications," *Bioresour Technol*, vol. 245, no. part B, pp. 1304–1313, Dec. 2017, doi: 10.1016/j.biortech.2017.05.031.
[71] Yameng Xu *et al.*, "Design and construction of novel biocatalyst for bioprocessing: Recent advances and future outlook," *Bioresour Technol*, vol. 332, Jul. 2021, Accessed: Jun. 29, 2024. [Online]. Available: https://www.sciencedirect.com/science/article/abs/pii/S0960852421004107
[72] P. K. Jain, V. Jain, A. K. Singh, A. hauhan, and S. Sinha, "Evaluation on the responses of succinate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase and glucose-6-phosphate dehydrogenase to acid shock generated acid tolerance in Escherichia coli," *Adv Biomed Res*, vol. 2, p. 75, Oct. 2012, Accessed: Jun. 29, 2024. [Online]. Available: https://journals.lww.com/adbm/fulltext/2013/02000/evaluation_on_the_responses_of_succinate.75.aspx
[73] Yinan Wu, Aysha Jameel, Xin-Hui Xing, and Chong Zhang, "Advanced strategies and tools to facilitate and streamline microbial adaptive laboratory evolution," *Trends Biotechnol*, vol. 40, no. 1, pp. 38–59, Jan. 2022, Accessed: Jun. 29, 2024. [Online]. Available: https://www.sciencedirect.com/science/article/abs/pii/S0167799210008334
[74] Ron Caspi *et al.*, "The MetaCyc database of metabolic pathways and enzymes - a 2019 update," *Nucleic Acids Res*, vol. 48, no. D1, pp. D445–D453, Jan. 2020, Accessed: Jun. 29, 2024. [Online]. Available: https://academic.oup.com/nar/article/48/D1/D445/5581728
[75] Michela Cerone and Terry K. Smith, "A Brief Journey into the History of and Future Sources and Uses of Fatty Acids," *Biomedical Sciences Research Complex*, vol. 8, Jul. 2021, doi: https://doi.org/10.3389/fnut.2021.570401.
[76] Xinyi Zan, Xin Tang, Linfang Chu, and Yuanda Song, "Characteristics of cell growth and lipid accumulation of high and low lipid-producing strains of Mucor circinelloides grown on different glucose-oil mixed media," *Process Biochemistry*, vol. 72, pp. 31–40, Sep. 2018, doi: https://doi.org/10.1016/j.procbio.2018.06.012.
[77] Shu Lin Wu, Wei Wei, and Qiuxiang XuXiaochen HuangSol JingXiaohu DaiBing Jie Ni*, "Revealing the Mechanism of Biochar Enhancing the Production of Medium Chain Fatty Acids from Waste Activated Sludge Alkaline Fermentation Liquor," *Agua ACS ES&T*, vol. 1, no. 4, 2021.
[78] Y. Y. a, J. W. Tianru Lou a, "Improved production of medium-chain fatty acids by biochar: Effect of biochar dosage and mechanism," *J Clean Prod*, vol. 450, p. 142051, Apr. 2024.
[79] Y. Y. J. W. Tianru Lou, "Influence of adding strategy of biochar on medium-chain fatty acids production from sewage sludge," vol. 354, pp. 141–660, 2024.
[80] Shu-Lin Wu, Gang Luo, Jing Sun, Wei Wei, Lan Song, and Lan Song, "Medium chain fatty acids production from anaerobic fermentation of waste activated sludge," *J Clean Prod*, vol. 279, no. 123482, Jan. 2021, doi: https://doi.org/10.1016/j.jclepro.2020.123482.
[81] Lan Wu, Wei Wei, Zhijie Chen, and Bing-Jie Ni, "Medium-chain carboxylate productions through open-culture fermentation of organic wastes," *J Clean Prod*, vol. 373, no. 133911, Nov. 2022, doi: https://doi.org/10.1016/j.jclepro.2022.133911.
[82] Vicky De Groof, Marta Coma, Tom Arnot, David J Leak, and Ana B Lanham, "Medium Chain Carboxylic Acids from Complex Organic Feedstocks by Mixed Culture Fermentation," *Molecules*, vol. 24, no. 3, 2019, doi: https://doi.org/10.3390/molecules24030398.
[83] Panagiota Stamatopoulou, Juliet Malkowski, Leandro Conrado, Kennedy Brown, and Matthew Scarborough, "Fermentation of Organic Residues to Beneficial Chemicals: A Review of Medium-Chain Fatty Acid Production," *Processes*, vol. 8, no. 12, 2020, doi: https://doi.org/10.3390/pr8121571.
[84] Na Chu *et al.*, "Microbial Electrosynthesis for Producing Medium Chain Fatty Acids".
[85] David P. B. T. B. Strik and Ramon Ganigue, "Microbial chain elongation—carbon recovering biorefineries for the circular economy," *Sec. Bioprocess Engineering*, vol. 12, 2024, doi: https://doi.org/10.3389/fbioe.2024.1448975.
[86] Beatriz Angulo Cabeza, "Transformación de derivados de ácidos grasos para la obtención de productos de interés industrial," Universidad de Zaragoz, Zaragoza, 2018.
[87] Yuhao Liu *et al.*, "Research on the Resource Recovery of Medium-Chain Fatty Acids from Municipal Sludge: Current State and Future Prospects," *Microorganisms*, vol. 12, no. 6, p. 680, 2024, doi: https://doi.org/10.3390/microorganisms12040680.
[88] Qinglian Wu, Yong Jiang, Ying Chen, Min Liu, Xian Bao, and Wanqian Guo, "Opportunities and challenges in microbial medium chain fatty acids production from waste biomass," *Bioresour Technol*, vol. 340, 2021, doi: https://doi.org/10.1016/j.biortech.2021.125633.
[89] Jung Ho Ahn, Kweon Hwan Jung, Eui Seok Lim, Sang Min Kim, Sung Ok Han, and Youngsoon Um, "Recent advances in microbial production of medium chain fatty acid from renewable carbon resources: A comprehensive review," *Bioresour Technol*, vol. 381, 2023, doi: https://doi.org/10.1016/j.biortech.2023.129147.
[90] Lan Wu *et al.*, "Medium chain fatty acids production from anaerobic fermentation of food wastes: The role of fermentation pH in metabolic pathways," *Chemical Engineering Journal*, vol. 472, 2023, doi: https://doi.org/10.1016/j.cej.2023.144824.
[91] Yun Wang, Wei Wei, Xiaohu Dai, Lan Wu, Xueming Chen, and Bing-Jie Ni, "Different Electron Donors Drive the Variation in the Performance of Medium-Chain Fatty Acid Production from Waste-Activated Sludge," ACS Publications, vol. 4, no. 3, pp. 650–659, 2023, doi: https://doi.org/10.1021/acsestengg.3c00423.
[92] Tianru Lou, Yanan Yin, and Jianlong Wang, "Improved production of medium-chain fatty acids by biochar: Effect of biochar dosage and mechanism," *J Clean Prod*, vol. 450, 2024, doi: https://doi.org/10.1016/j.jclepro.2024.142051.
[93] Ting Wei Tee, Anupam Chowdhury, Costas D. Maranas, and Jacqueline V. Shanks, "Systems metabolic engineering design: Fatty acid production as an emerging case study," Biotechnol Bioeng, vol. 111, no. 5, pp. 849–857, 2014, doi: https://doi.org/10.1002/bit.25205.
[94] Wanqin Zhang et al., "Produce individual medium chain carboxylic acids (MCCA) from swine manure: Performance evaluation and economic analysis," Waste Management, vol. 144, pp. 255–262, 2022, doi: https://doi.org/10.1016/j.wasman.2022.04.001Getrightsandcontent.
[95] Jihua Zhao, Hongzhi Ma, Ming Gao, Dayi Qian, and Su Shiung Lam, "Advancements in medium chain fatty acids production through chain elongation: Key mechanisms and innovative solutions for overcoming rate-limiting steps," Bioresour Technol, vol. 408, 2024, doi: https://doi.org/10.1016/j.biortech.2024.131133.

[96] Matthew J. Scarborough, Griffin Lynch, Mitch Dickson, Mick McGee, Timothy J. Donohue, and Daniel R. Noguera, "Increasing the economic value of lignocellulosic stillage through medium-chain fatty acid production," *Biotechnol Biofuels*, vol. 11, 2018.

[97] Pieterjan Debergh and Miet Van Dael, "Production of caproic acid from acetate and ethanol through microbial chain elongation: A techno-economic assessment," Bioresour Technol Rep, vol. 18, 2022, doi: https://doi.org/10.1016/j.biteb.2022.101055.

[98] V. De Groof, M. Coma, T. Arnot, D. J. Leak, y A. B. Lanham, "Medium Chain Carboxylic Acids from Complex Organic Feedstocks by Mixed Culture Fermentation," *Molecules*, vol. 24, no. 3, p. 398, Jan. 2019, doi: 10.3390/molecules24030398.

[99] Y. Zhang, Y. Wang, H. Wang, y Y. Li, "Effects of substrate concentrations and fermentation modes on caproate production from liquor fermentation waste," *Journal of Cleaner Production*, vol. 278, p. 123805, Jan. 2021, doi: 10.1016/j.jclepro.2020.123805.

[100] W. Han, P. He, L. Shao, y F. Lü, "Metabolic Interactions of a Chain Elongation Microbiome," *Applied and Environmental Microbiology*, vol. 84, no. 22, p. e01614-18, Nov. 2018, doi: 10.1128/AEM.01614-18.

[101] C. N. Reddy, S. Kondaveeti, G. Mohanakrishna, and B. Min, "Application of bioelectrochemical systems to regulate and accelerate the anaerobic digestion processes," *Chemosphere*, vol. 287, p. 132299, 2022. DOI: 10.1016/j.chemosphere.2021.132299.

[102] M. J. Scarborough, C. E. Lawson, J. J. Hamilton, T. J. Donohue, and D. R. Noguera, "Metatranscriptomic and thermodynamic insights into medium-chain fatty acid production using an anaerobic microbiome," *mSystems*, vol. 3, no. 4, e00221-18, 2018. DOI: 10.1128/msystems.00221-18.

[103] Q. Wu, W. Ren, W. Guo, y N. Ren, "Effect of substrate structure on medium chain fatty acids production and reactor microbiome," *Environmental Research*, vol. 204, p. 111947, Jan. 2022. doi: 10.1016/j.envres.2021.111947.

[104] P. T. Quosai, *Improving Leach Bed Reactor Design for Medium-Chain Fatty Acid Production from Food Waste at Room Temperature*, Tesis de Maestría, Univ. of Waterloo, Waterloo, ON, Canadá, 2023.

[105] F. Palladino, P. R. F. Marcelino, A. E. Schlogl, Á. H. M. José, R. de C. L. B. Rodrigues, D. L. Fabrino, I. J. B. Santos, y C. A. Rosa, "Bioreactors: Applications and Innovations for a Sustainable and Healthy Future—A Critical Review," *Applied Sciences*, vol. 14, núm. 20, art. 9346, 2024, doi: 10.3390/app14209346.