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Opinion

Production of Bio-alkanes from Biomass and CO₂Richen Lin ^{1,2}, Chen Deng ^{1,2,*}, Wuyuan Zhang ^{3,*}, Frank Hollmann ⁴ and Jerry D. Murphy ^{1,2,*}

Bioelectrochemical technologies such as electro-fermentation and microbial CO₂ electrosynthesis are emerging interdisciplinary technologies that can produce renewable fuels and chemicals (such as carboxylic acids). The benefits of electrically driven bioprocesses include improved production rate, selectivity, and carbon conversion efficiency. However, the accumulation of products can lead to inhibition of biocatalysts, necessitating further effort in separating products. The recent discovery of a new photoenzyme, capable of converting carboxylic acids to bio-alkanes, has offered an opportunity for system integration, providing a promising approach for simultaneous product separation and valorisation. Combining the strengths of photo/bio/electrochemical catalysis, we discuss an innovative circular cascading system that converts biomass and CO₂ to value-added bio-alkanes (C_nH_{2n+2}, n = 2 to 5) whilst achieving carbon circularity.

Carbon Neutrality in a Circular Bioeconomy

The transition to a climate neutral future requires innovative pathways that lead to net-zero carbon emission energy systems. Energy sectors such as light-duty transportation, heating, and electricity may be straightforward to decarbonise via renewable electricity (such as wind, wave, and solar), as they have witnessed significant development and associated cost reduction in the past decade. However, elements of the transportation sector such as aviation, long-distance haulage, and shipping are not ideally suited to electrification [1–3]. The European Union has proposed to reduce greenhouse gas emissions by at least 40% below 1990 levels by 2030 [4]. To meet this challenging target, **advanced biofuels** (see [Glossary](#)), including biomethane and longer C₂₊ **alkanes** from renewable feedstocks, can serve as high-quality sustainable transportation fuels in these difficult to decarbonise sectors.

Anaerobic digestion is an established biotechnology that can produce renewable C₁ methane from a wide range of organic wastes [5,6]; however, methane has a lower energy density and economic value compared with other longer chain alkanes. For example, pentane (C₅) presents a higher heating value (3507 kJ/mol) than that of methane (889 kJ/mol) (<https://webbook.nist.gov/chemistry/>). As such, producing alkanes with higher energy density is more attractive considering their use as drop-in advanced fuels. Nonetheless, high-efficiency production of longer carbon chain bio-alkanes via microorganisms is technically challenging and typically requires metabolic engineering of natural microorganisms [7]. Emerging interdisciplinary bio-based technologies such as **electro-fermentation (EF)**, **microbial CO₂ electrosynthesis (MES)**, and photo-biocatalysis at whole cell or enzymatic level may provide a promising platform for advanced biofuels and chemicals production. These technologies can be used either alone or combined to produce desired products, such as hydrogen, alkanes, alcohols, and **carboxylic acids**. EF can convert bio-based feedstock into hydrogen, CO₂, and **short chain carboxylic acids (SCCAs)**, C₁–C₅; such as acetic/butyric acid [8]. SCCAs together with CO₂ can be upgraded to value-added **medium chain carboxylic acids (MCCAs)**, C₆–C₁₂, such as caproic acid) through MES, ideally

Highlights

Electro-fermentation (EF) and microbial CO₂ electrosynthesis (MES) are emerging interdisciplinary technologies that can produce renewable carboxylic acids.

A newly discovered photo-decarboxylase offers an innovative route for bio-alkane production from carboxylic acids.

The cascading photo/bio/electrochemical system can produce advanced bio-alkanes (C_nH_{2n+2}, n = 2 to 5) from biomass and CO₂.

Systems will require optimisation to reduce economic cost and carbon footprint.

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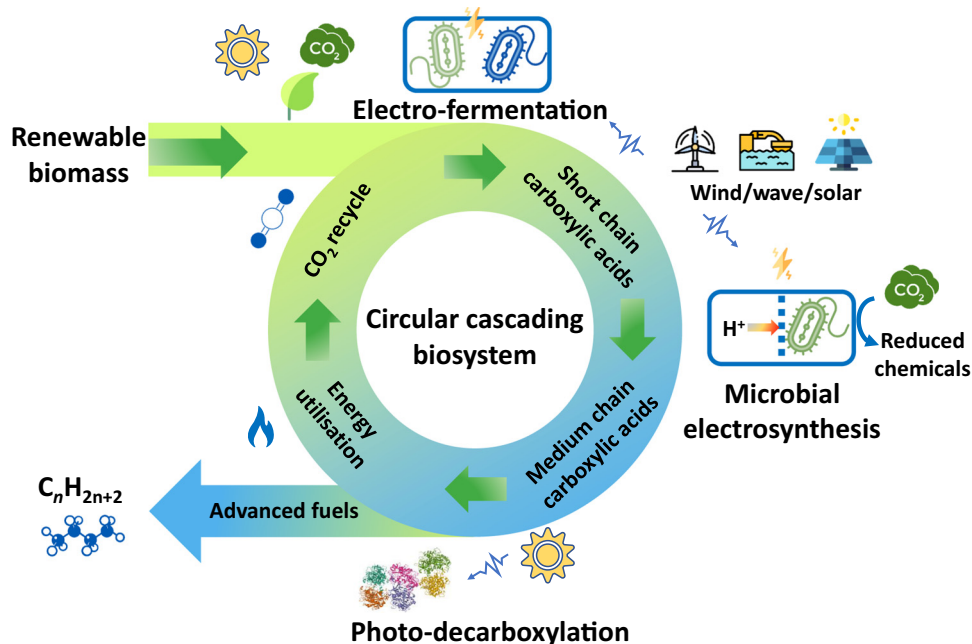
driven by surplus renewable electricity [9]. These MCCAs can be further valorised to lightweight bio-alkanes via photo-biocatalytic decarboxylation [10]. Among these biofuels, renewable alkanes are attractive due to their diverse use, valorisation opportunities, and high economic value [11]. The major sources of alkanes are currently produced through fossil fuel-based refining or Fischer-Tropsch synthesis [12]. Bio-based production of alkanes via metabolic engineering has been proposed as a sustainable way to replace fossil-based technologies [13]. However, current bio-based conversion poses two significant challenges: (i) fermentation is vulnerable to factors such as pH, microbial activity, and product inhibition, whilst a wide range of by-products may be produced due to diverse metabolic pathways [14]; and (ii) traditional linear conversion processes for biofuels can be limited in sustainability and efficiency, resulting in inefficient resource use and product generation [15]. Here, a unique opportunity lies in synergistically combining biocatalysis, bioelectrochemical synthesis, and photo-decarboxylation to innovate new technical pathways (Figure 1, Key Figure), which can enable desirable and tuneable bio-alkane (C_nH_{2n+2}) production from renewable biomass and CO_2 in a circular bioeconomy system.

EF for Carboxylic Acid Production

By introducing polarised electrodes, EF can provide renewable electricity by imposing an electrical field on the fermentation environment, thereby influencing microbial metabolism [8]. EF provides a hybrid metabolism in which external electrons are exchanged with solid electrodes,

Key Figure

Technological Pathways for Advanced Bio-alkanes Production from Biomass and CO_2



Glossary

Advanced biofuels: biofuels (gaseous or liquid) that are produced from various types of feedstocks that either do not use agricultural land or compete with food. Typical advanced fuels include those produced from animal manure, sewage sludge, food waste, lignocellulosic biomass, and algae.

Alkanes: compounds with the general chemical formula C_nH_{2n+2} and include for CH_4 (methane), C_2H_6 (ethane), C_3H_8 (propane), C_4H_{10} (butane), and C_5H_{12} (pentane).

Carboxylic acids: organic acids with the general formula $R-COOH$, with R referring to the alkyl group; include for formic acid ($HCOOH$), acetic acid (CH_3COOH), propionic acid (CH_3CH_2COOH), butyric acid ($CH_3(CH_2)_2COOH$), valeric acid ($CH_3(CH_2)_3COOH$) and caproic acid ($CH_3(CH_2)_4COOH$).

Electro-fermentation (EF): fermentation in which polarised electrodes are used as a driving force to boost the microbial metabolism through extracellular electron transfer.

Medium chain carboxylic acids

(MCCA): carboxylic acids with a straight carbon chain of six to 12 carbon atoms, such as n-caproic acid, which has a straight chain of six carbon atoms (C_6).

Microbial CO_2 electrosynthesis

(MES): an electrochemical process used to drive microbial metabolism for biofuel/biochemical production from the reduction of CO_2 .

Photoenzyme: an enzyme that catalyses photochemical reactions. A recently discovered fatty acid photo-decarboxylase can convert fatty acids into alkanes via decarboxylation in the presence of blue light.

Short chain carboxylic acids

(SCCA): carboxylic acids with fewer than six carbon atoms, such as acetic acid with two carbon atoms (C_2) and butyric acid with four carbon atoms (C_4).

Figure 1. Biomass and CO_2 are converted to advanced bio-alkanes (C_nH_{2n+2} , $n = 2$ to 5) in a circular cascading system. Electro-fermentation and microbial electrosynthesis are parallel options, which both produce carboxylic acids.

which act as alternative oxidising/reducing equivalents [16]. Depending on the use of electrodes as electron sinks or donors, the configuration of EF can be categorised as cathodic or anodic EF, respectively (Box 1). EF conditions can be electrochemically controlled to optimise the redox condition, favour microbial growth, regulate microbial metabolism, and promote extracellular electron transfer [8]; this leads to a higher yield and selectivity of products, such as hydrogen, methane, alcohols (ethanol, butanol), and carboxylic acids (acetic acid, butyric acid, and caproic acid). Among these products, carboxylic acids not only have a higher economic value as compared with biogas or biomethane, but also are versatile precursors for even higher-valued chemical synthesis [17], such as MCCA, polyhydroxyalkanoates, single cell proteins, and multicarbon alkanes (for use as transportation fuels for heavy goods vehicles).

Table 1 summarises the production of various carboxylic acids by EF applications. By regulating redox potential through different electrode working potentials at -1.0 , -0.6 , and -0.2 V (versus Ag/AgCl), tuneable fermentation products (SCCA, C_2 – C_4) can be obtained from glucose [17]. External stimulation of microbial catalysts in EF can enhance the production of SCCA (such as C_2 acetic acid) from food waste; this is accompanied by enrichment of acidogenic bacteria [18]. Production of longer MCCA (such as C_6 caproic acid) typically requires external electron donors (such as ethanol and lactic acid) to initiate chain elongation reaction through the reverse β -oxidation pathway; for example, $(2C_2H_5OH + CH_3COO^- = C_6H_{11}COO^- + 2H_2O, \Delta G^0 = -79.0 \text{ kJ/mol})$. Despite the progress in EF technology, to achieve higher carboxylic acid production, more investigation needs to be performed to understand the role of important variables, such as applied potential, activities of regulatory enzymes, and functional microbial communities. To quantify the efficiency of EF, Moscoviz and colleagues introduced the indicator of 'EF coefficient', which can be calculated

Box 1. Electro-Fermentation (EF) versus Traditional Fermentation

What Is Anodic or Cathodic EF?

The presence of electrodes in EF provides a flexible way to consume or supply electrons in fermentation (Figure 1). In anodic EF, the working electrode acts as an anode and is used to dissipate excess electrons during fermentation; as such the final product is more oxidised than the substrate (for example ethanol from glycerol). By contrast, in a cathodic EF, the working electrode supplies electrons for a reduced final product (for example acetic acid from glucose). The electric current during EF is not the main energy source nor the targeted product, but is a stimulator allowing fermentation to take place in unbalanced conditions for an enhanced product selectivity and production rate.

What Are the Advantages of EF over Traditional Fermentation?

The stability of traditional fermentation is dependent on many process factors (such as microbial activity, oxidation–reduction potential, media buffering capacity, and product accumulation), leading to constrained economic feasibility and environmental sustainability. EF with an optimised current density can provide surplus renewable electricity for energy storage in fuels/chemicals, regulate fermentation pathways by affecting redox condition ($NADH/NAD^+$ balance), facilitate anodic/cathodic EF reactions by consuming/supplying electrons, and achieve tuneable fermentation products simply by varying working potential.

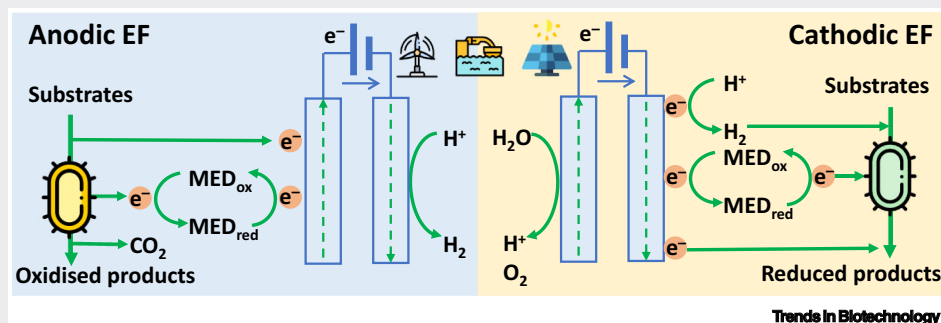


Figure 1. Schematic of Anodic and Cathodic Electro-Fermentation (EF). Abbreviations: MED_{ox} , oxidised mediator; MED_{red} , reduced mediator.

Table 1. Production of Various Carboxylic Acids by Electro-Fermentation (EF) Applications

Substrate	Main carboxylic acid production	Working potential (V versus SHE ^a)	Temperature (°C)	Microbial culture	EF performance compared with traditional fermentation	Refs
Glycerol	Acetic acid	0.2	37	Engineered <i>Escherichia coli</i>	Increased glycerol consumption rate (anodic EF)	[55]
Glucose	Acetic/propionic/butyric acid	0/−0.4/−0.8 ^b	35	Mixed culture	Tuneable production of mixed carboxylic acids (cathodic EF)	[17]
Food waste	Acetic/propionic/butyric acid	−0.6 (reference electrode not indicated)	28	Mixed culture	Total carboxylic acids production increased by 72.3% (cathodic EF)	[18]
Sucrose	Butyric acid	−0.2 ^b	37	<i>Clostridium tyrobutyricum</i>	35% increase in butyrate production yield (cathodic EF)	[56]
Glucose, acetate, and ethanol	Iso-butyric acid	−0.7	25	Mixed culture	Almost 20-fold increase in the yield of iso-butyrate (compared with open circuit controls) (cathodic EF)	[57]
Acetate, ethanol	Caproic acid	−0.9 ^b	30	Mixed culture	Caproate production selectivity increased by 28% (cathodic EF)	[58]

^aSHE, standard hydrogen electrode.

^bCalculated based on the standard electrode potential of the Ag/AgCl (saturated KCl) reference electrode against SHE is +0.2 V.

as the ratio of the charge transferred through the electric circuit to the total charge in the desired product [16]. The currently achieved EF coefficient varies between 0.01 and 0.38 using pure strains or mixed cultures [16]. The effectiveness of EF can be affected by different process conditions, including both dissolved redox mediators and interactions between the electrode surface and microorganisms via extracellular electron transfer [16]. In a typical syntrophy, the reaction occurs close to the thermodynamic equilibrium, as such a minor disturbance in substrates/products concentration can shift the metabolic pathway. Recent research has found that direct interspecies electron transfer via conductive materials (for example, graphene [19,20], carbon nanotubes [21], and biochar [22,23]) has significant potential to overcome delicate thermodynamic balances inherent in conventional mediated hydrogen transfer. This suggests new opportunities to advance electron transfer in EF, through which electroactive bacteria may be enriched, thereby resulting in enhanced EF efficiency [24].

Since EF is built based on ‘classical’ established fermentation and has shown potential to enhance carboxylic acid yields, its technological maturity defined by technology readiness level (TRL) can be regarded as somewhere between basic and industrial research (TRL of 3–4). Improving the TRL of EF requires further research to reveal the interaction between electrode and microorganisms, thereby developing optimisation strategies to improve product selectivity. A detailed techno-economic assessment of EF would also be needed to facilitate scale-up and industrial implementation of the process.

MES for Carboxylic Acid Production

Both MES and EF use electrochemical mechanisms to affect microbial metabolic activities to enhance production of targeted products. The main differences between MES and EF include the following: the reactions in MES are nonspontaneous without external electrical driving force; the working electrode in MES is a cathode conducting reducing reactions (such as from CO₂ to acetic acid), while the working electrode of EF can be either anode or cathode; and the electric current density of EF (0.001–10 A/m²) is typically lower than that of MES (0.01–200 A/m²) (which uses the electrode solely to provide the reducing power) [25]. Microbial electrosynthesis can produce diverse products from CO₂ (Table 2 and Box 2). Acetic acid is mainly produced; other C₂₊ products such as (iso)butyric/caproic acid are also produced

Table 2. Production of Various Carboxylic Acids (C₂₊) by Microbial CO₂ Electrosynthesis

Cathode material	Cathode potential (V versus SHE ^a)	Temperature (°C)	Microbial culture	Main products (g/l)	Refs
Gas diffusion biocathode	−1.1 to −1.3	30	Enriched anaerobic sludge	Butyric acid 0.1	[32]
Carbon felt	−0.85	32	Enriched mixed culture	Butyric acid 2.85 Caproic acid 1.05	[27]
Carbon felt	−1.02	32	Enriched mixed culture	Butyric acid 9.3 Caproic acid 3.1	[48]
MXene-coated carbon felt	−0.6	35	Mixed culture	Propionic acid 1.57 Butyric acid 0.87	[59]
Graphite granules	−0.8	35	Mixed culture	Butyric acid 3.1 Iso-butyric acid 1.6 Caproic acid 1.2	[60]

^aSHE, standard hydrogen electrode.

but with relatively low concentrations. The highest acetate production rate of 1330 g/m² electrode/day has been achieved by improved electrode design and optimal operational conditions [26]. In comparison, the production rates of n-butyrate (nC₄) and n-caproate (nC₆) were 160 and 46 g/m² electrode/day, respectively, with carbon felt as the cathode [27]. The performance of MES may be affected by several factors, including cathode material/architecture, cathode potential, current density, and microbial inoculum, all of which can affect the fundamental electron transfer towards targeted products [28]. Production of longer C_{4–6} carboxylic acids in MES is not straightforward and requires a chain elongation process (typically with internally produced ethanol or hydrogen as an intermediate) through the reverse β-oxidation pathway. Electron transfer needs significant modulation for practical production of caproic acid, considering in theory the reduction of CO₂ to one mole of caproate requires 32 electrons ($6\text{HCO}_3^- + 37\text{H}^+ + 32\text{e}^- \rightarrow \text{CH}_3(\text{CH}_2)_4\text{COO}^- + 16\text{H}_2\text{O}$), while only eight electrons are needed to convert CO₂ to acetate ($2\text{HCO}_3^- + 9\text{H}^+ + 8\text{e}^- \rightarrow \text{C}_2\text{H}_3\text{O}_2^- + 4\text{H}_2\text{O}$). As such, longer chain acid formation is restricted by the increased kinetic challenge of proton coupled electron transfer reactions, which inherently require large applied potentials.

The cathode is the core of MES as it governs electroactivity and biocompatibility. This necessitates the design of cathodes with high electrical conductivity, low cost, and large biocompatible surface area for microbial biofilm formation/cell adherence [29–31]. Previous research has focused on the improvements of MCCA production with respect to cathode surface modifications, cathode architecture, and supply of CO₂ gas (concentration and bubble size). Carbon-based materials are mostly investigated to improve CO₂ conversion to carboxylic acids. A cathode with a 3D structure (such as a gas diffusion electrode) providing a gas–liquid–solid interface for CO₂ diffusion can significantly increase the electrode surface area and deliver enhanced CO₂ mass transfer [32,33]. A biofilm-driven microbial electrochemical system can significantly enhance the electron consumption rate with enhanced biocatalytic activity, leading to the production of acetate, n-butyrate, and n-caproate at the same applied potential [27]. According to Jourdin and colleagues, over 12.8% of electrons can be transformed to n-caproate in the most productive period during a continuous operation [27]. It is postulated that porous composite materials with high conductivity and biocompatibility show most promise for future applications [34]. Despite the successful production of SCCA from MES, the production of longer MCCA is less selective at lower production rates as compared with conventional chain-elongation fermentation with external electron donors (such as ethanol and lactic acid) [27].

Box 2. Microbial CO₂ Electrosynthesis (MES) for Producing Carboxylic Acids

Modified Cathode Structure

The interface of cathode with microorganisms plays a key role in affecting the rate of microbial CO₂ reduction and the selectivity of product formation. The cathode properties such as biocompatibility, surface area, and chemical stability have been investigated to develop an effective MES cathode configuration. The methods developed for the surface modification of cathodes are shown in Figure 1 and include improved 3D porous structure and surface area, functional group attachment for improving biofilm growth, and positively charged surface for microorganism adsorption, the optimisation of which can deliver improved cathode–microorganism interactions and bioelectrochemical activity.

Electron Transfer Mechanism

The cathodic electron transfer mechanisms include indirect electron transfer (IET) with hydrogen or other redox chemicals as the mediator and direct electron transfer (DET) via extracellular cytochromes or nanowires (Figure 1). IET requires diffusible extracellular electron carriers such as hydrogen to transfer electrons between the electrode and microorganisms; the concentration gradient is regarded as the rate-limiting parameter in IET based on the Fick's diffusion law. In comparison, DET requires physical contact between the electrode and microorganisms via either outer membrane c-type cytochromes, microbial nanowire, or biocompatible conductive materials; however, the underlying molecular mechanisms by which the associated microbes accept electrons are not clear [61,62].

Metabolic Pathways for Carboxylic Acid Production

The dominant compound produced from MES has been acetic acid (C₂H₄O₂) since the first proof-of-concept [63]. Recently, carbon elongation to longer chain carboxylic acids such as butyric (C₄H₈O₂) or caproic acid (C₆H₁₂O₂) and their respective alcohols has shown to be possible, but in relatively low rates and selectivity [30]. Figure 1 shows the possible metabolic pathways for different longer chain C₂₊ platform chemicals production, which depends on the operational conditions, predominant microbes, and thermodynamic hierarchy. The synthesis of these chemicals originates from acetyl-CoA, which is the precursor to produce intermediates such as acetic acid (C₂), ethanol (C₂), propionic acid (C₃), and lactic acid (C₃). Subsequent elongation to butyric acid (C₄) may occur via acetate with CO₂ or two moles of acetate without CO₂. Caproic acid (C₆) may be produced with butyric acid and acetate (or CO₂). Direct chain elongation of CO₂ to butyric/caproic acid is thermodynamically possible [48]; however, this has not been proved with experimental evidence.

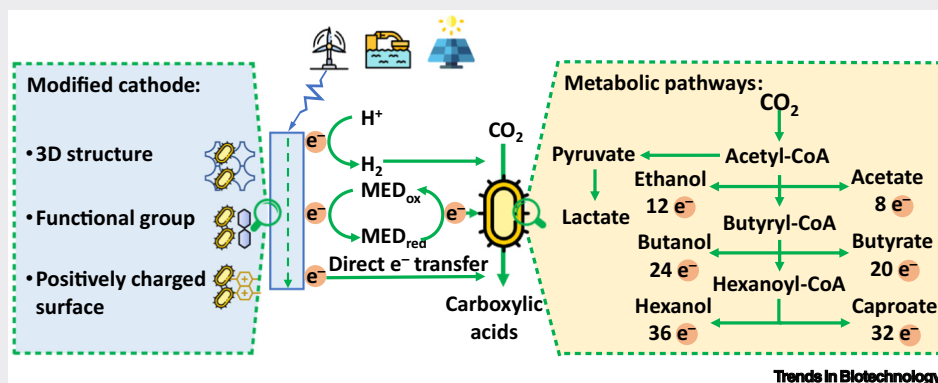


Figure 1. Schematic of Microbial Pathways for C₂₊ Carboxylic Acid Production from CO₂ with Modified Cathode Structures. Abbreviations: MED_{ox}, oxidised mediator; MED_{red}, reduced mediator.

MES has been established at lab scale to produce primarily acetate and a mixture of longer carboxylic acids. MES has a lower TRL of 1–3, with technical challenges including nonselective production, low production rates, and low energy conversion efficiency (from electricity to target products). The advance of MES technology should focus on understanding microbial electron transfer mechanisms, steering electron flow to targeted products, and enhancing CO₂ conversion efficiency.

Bio-alkanes from Cascading Photo-Bioelectrochemical Systems

Bioelectrochemical technologies principally give access to MCCA. However, the specific calorific value of carboxylic acids is somewhat less than of fuels used for transport. Furthermore, the toxic

effect of MCCA on the biocatalysts used in EF/MES, and issues concerning their isolation from aqueous media [35], further reduce their attractiveness.

Both issues may be solved by removing the carboxylic acid functionality (namely carbonyl group). Esterification into methyl or ethyl esters (FAMES or FAEEs, respectively), resulting in classical biodiesel, is one option. The reversible nature of the esterification reaction together with its low thermodynamic driving force, however, requires high molar surpluses of the short chain alcohols to shift the equilibrium. Reduction of carboxylic acids into the corresponding aldehydes and alcohols is another option but yields relatively low caloric value products [36,37]. Another possibility is to oxidatively remove the carboxyl group yielding terminal alkenes [38,39].

An interesting alternative approach comprises the photoenzymatic decarboxylation of carboxylic acids using photoactivated decarboxylases recently reported by Sorigué and coworkers [10]. The photoactivated carboxylic acid decarboxylase from *Chlorella variabilis* NC64A (CvFAP) only needs photochemical activation and therefore is fully compatible with EF and MES. The wild type **photoenzyme** displays only poor activity with MCCA; this can be addressed using a decoy molecule strategy [40] or via protein engineering [41,42]. Although the selectivity of the enzyme and the mild reaction conditions make it an interesting candidate to produce advanced bio-alkanes, as per Figure 1, issues related to low activity still need to be addressed [40].

The decarboxylase shows an evident preference for longer chain fatty acids (C₁₆–C₁₇) compared with shorter chain acids [10]. To increase the bioactivity for shorter chain acids, Zhang and coworkers proposed the use of decoy molecules to fill up the vacant substrate channel of the enzyme and successfully accelerated the reaction rate by 1.2–7.3-fold with selectivity of almost 100% to the corresponding alkanes [40]. Huijbers and colleagues [64] proposed a two-step enzymatic cascade comprising lipase-catalysed hydrolysis and photo-decarboxylation, which enabled the synthesis of biodiesel (transforming triglycerides to alkanes) with a conversion efficiency of 83% and reaction turnover number of over 8000 (product concentration divided by enzyme concentration). Further enzymatic cascade reactions have also been demonstrated to expand the application of this photoenzyme [43,44]. However, enzyme-based processes are not self-regenerating and are unstable, thereby losing their biocatalytic activity over time. The laborious purification of enzymes may further constrain their practical application.

Achieving whole cell photo-biocatalysis may reduce operational complexity and improve process stability by preserving innate replication and healing mechanisms [45,46]. Amer and colleagues recently constructed bioengineered strains of *Escherichia coli* and *Halomonas* expressing the genes encoding CvFAP and demonstrated that the whole cell of recombinant strains could produce propane (C₃) and butane (C₄) from the corresponding C₄ and C₅ carboxylic acids [41]. Therefore, tuneable production of bio-alkane gases using different compositions of carboxylic acids is possible [41]. Based on this key technical route, multiple systems for bio-alkane production can be designed depending on renewable biomass supply, bioreactor design, and process integration.

Photoactivated decarboxylation is currently at lab scale with a lower TRL of 1–3. Considering the nearly 100% product selectivity (from carboxylic acids to alkanes) achieved in previous proof-of-concept research, further efforts should focus on developing recombinant whole-cell biocatalysts to reduce operational complexity and improve process stability compared with enzyme-level process. Standalone photo-decarboxylation of longer chain carboxylic acids requires efforts in reaction engineering (such as implementation of bi-phasic solvent reaction) to address the solubility issues of both longer carbon chain substrates and products. This solubility issue is a common challenge in the field of biosynthesis and biocatalysis.

Concluding Remarks and Future Perspectives

Anaerobic digestion is a viable biotechnology to produce C_1 methane from biomass. Natural microbial fermentation to produce carboxylic acids (such as acetic/butyric acid) has been known for over 100 years. Advances in electrochemistry powered by renewable electricity have enabled and stimulated bio-electrochemical production of higher-molecular mass carboxylic acids via EF or MES using either organic wastes or CO_2 as feedstock. However, product toxicity presents a common challenge that limits the efficacy of these processes to achieve maximal product concentration and production rate. Here we propose and assess a cascading circular photo/bio/electrochemical system to convert organic wastes and CO_2 value-added bio-alkanes with tuneable compositions. Box 3 provides a theoretical evaluation of carbon conversion efficiency (CCE) of the envisioned systems. In comparison with the traditional fossil-based refining for alkane production, the advantages of the photo-bioelectrochemical system as outlined in Figure 1 include the flexibility to convert complex and varied substrates (such as organic wastes) in EF, the ability to produce a tuneable MCCA composition in MES of CO_2 , the excellent selectivity (nearly 100%) to target bio-alkanes via the photo-decarboxylation reaction, and the ability to expand the application of versatile photo/bio/electrochemical strategies. These merits, combined with ambient temperature/pressure operation, can facilitate the development of more sustainable and versatile approaches for valuable C_nH_{2n+2} fuels/chemicals production from renewable biomass and CO_2 . Furthermore, our proposed cascading circular system is a flexible interdisciplinary platform and the general concept of combining microbial, electrochemical, and photocatalysis has opened a whole new research frontier, which can be expanded and diversified with tailored designs with respect to feedstocks, intermediates, and targeted products. For example, in addition to producing alkanes, the produced intermediated carboxylic acids can be valorised to terminal alkenes, fatty amines, and alcohols, which are very important compounds in the field of polymers and surfactants [38,46,47].

Outstanding Questions

How can we maximise the net energy gain from electro-fermentation (EF) and microbial CO_2 electrosynthesis (MES)?

How do we enhance the production rate and selectivity of the most valuable products in EF and MES?

Will the intermittent supply of renewable electricity affect the performance of bioelectrochemical systems?

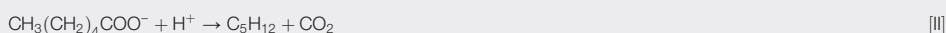
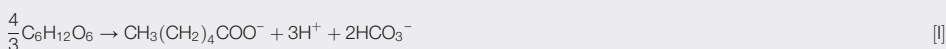
By what means can we improve the photo-decarboxylation reaction rate from carboxylic acids to alkanes?

What can we do to lower the production cost of bio-alkanes from biomass and CO_2 ?

Box 3. Theoretical Carbon Conversion Efficiency (CCE) of the Envisioned Systems

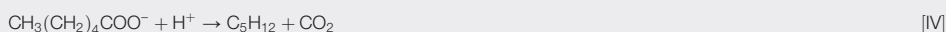
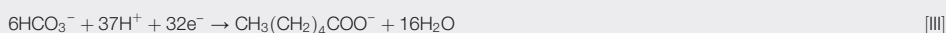
To understand the theoretical CCE of the envisioned system using EF/MES with photo-decarboxylation, we have considered two typical conversion scenarios: (i) from biomass ($C_6H_{12}O_6$) to pentane (C_5H_{12}), and (ii) from CO_2 (bicarbonate) to pentane (C_5H_{12}). CCE is defined here as the number of carbon atoms in one mole of pentane relative to that in biomass or CO_2 as per the stoichiometric reactions. To simplify the calculations, the following assumptions are adopted: (i) the composition of biomass is assumed as glucose ($C_6H_{12}O_6$) for EF, and (ii) the only carboxylic acid produced from both EF and MES is caproic acid.

Scenario 1: from biomass ($C_6H_{12}O_6$) to C_5H_{12} via cascading EF and photo-decarboxylation.



$$CCE = 5/8 = 62.5\%$$

Scenario 2: from CO_2 (bicarbonate) to C_5H_{12} via cascading MES and photo-decarboxylation.



$$CCE = 5/6 = 83\%$$

A higher theoretical CCE of 83% is expected in Scenario 2 compared with that of 62.5% in Scenario 1. This is due to the fact that the production of caproic acid in EF is accompanied by CO_2 formation and these carbon atoms cannot be recovered into pentane in the subsequent photo-decarboxylation reaction. However, it should be noted that the theoretical CCE for the EF/MES systems is dependent on several factors, including the composition of biomass used in EF and targeted final product formation (namely C_2 – C_5 alkanes). Furthermore, the practical CCE can be much lower than the theoretical CCE, as the production of carboxylic acids in EF and MES can be very diverse and less selective.

However, many challenges still exist. First, the fundamental understanding on how external voltage/current affects the behaviours of specific electroactive microbes and interspecies interactions in EF needs to be improved to optimise product selectivity, effect precise process control, and enable rational bioreactor design. Furthermore, although the additional electric energy in EF can be sourced from renewable electricity, the net gains from desirable products have to offset this energy investment (see Outstanding Questions).

Second, MES faces challenges to achieve economically viable performance metrics (such as production rate, selectivity, final product concentration, and energy conversion efficiency) [9]. For example, although 63.6% of carbon atoms in CO₂ were recovered in butyric and caproic acid in a continuous MES system (suggesting a higher product selectivity of C₄ and C₆ products than that of C₂ acetate), the maximum concentrations of butyric and caproic acid to date achieved are only 9.3 and 3.1 g/l, respectively [48]. These low concentrations highlight the need for further research into increasing production rate and selectivity of the most valuable products, which will in turn reduce the process energy input. Future strategies may include microbial and process engineering and novel combinations of electrochemistry and biology [9].

Third, the major concern for practical application of the newly discovered photo-decarboxylase is associated with its low biocatalyst activity. Possibly, new reactor concepts comprising internal illumination will increase the activity of the enzyme [49]. Also, the issue of photoinactivation of CvFAP remains to be elucidated as to whether it poses a significant constraint to the robustness of the overall process [50,51]. The latter may be overcome by continuous replenishment of fresh CvFAP with live cell culture.

A fourth challenge relates to the cascading photo/bio/electrochemical system. This versatile platform has shown feasible applications for bio-alkane production using various carboxylic acids as the feedstock, derived from the fermentation of renewable biomass or MES of CO₂. Although each process can be optimised to ensure maximal performance of that process, the efficacy of the integrated approach largely relies on mutual compatibility and kinetic matching of each step.

Due to the complexity of biomass compositions, EF tends to produce a wide range of carboxylic acids dominated by acetic and butyric acid. Caproic acid can be produced through microbial chain elongation in the presence of additional electron donors (such as ethanol). In comparison, MES currently can convert CO₂ to acetic acid with promising production rates, although the production of mixed carboxylic acids seems common. The reduction of CO₂ to caproic acid is far more difficult, thus the production rate of acetic acid can be tenfold higher than that of caproic acid, depending on the variations in microorganisms, working potentials, and reactor types [25]. Therefore, it is recommended that EF may be more suitable for integration to produce a mixture of alkanes (C₂–C₅), while MES has a higher theoretical CCE when producing targeted longer alkanes.

It would be valuable to develop a system that can produce C>5 alkanes as more valuable drop-in advanced fuels. Longer chain n-caprylate (C₈) can be synthesised in low productivities through microbial chain elongation processes with additional electron donors (such as ethanol) [52,53], thereby heptane (C₇) production is achievable through photo-decarboxylation. Using electrochemical reactions subsequent to EF/MES may provide another innovative route for energy-dense C>5 alkane production. Urban and colleagues demonstrated that combining microbial and electrochemical reactions (Kolbe electrolysis, for example:

$2\text{C}_6\text{H}_{12}\text{O}_2 \leftrightarrow 2\text{C}_6\text{H}_{11}\text{O}_2^- \rightarrow \text{C}_{10}\text{H}_{22}$) allowed production of longer alkanes and alkenes from biomass wastes [54].

Progressing to future applicable systems for longer bio-alkane production requires further efforts to improve product yield and reduce economic cost. For example, a recent techno-economic analysis on a cascading photo-biocatalysis system predicted a propane production cost of approximately 2 USD/kg at scale [41], compared with approximately 0.3 USD/kg wholesale price based on chemical refining processes (https://www.eia.gov/dnav/pet/pet_pri_wfr_dcus_nus_m.htm). We suggest that to ensure a successful implementation of the proposed circular cascading system, further assessments are required in terms of improving carbon circularity efficiency, net energy output, material streams reuse, and techno-economic feasibility.

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