Coupled Electrochemical and Microbial Catalysis for the Production of Polymer Bricks

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Power-to-X technologies have the potential to pave the way towards a future resource-secure bioeconomy as they enable the exploitation of renewable resources and CO_2. Herein, the coupled electrocatalytic and microbial catalysis of the C_5-polymer precursors mesaconate and 2*S*-methylsuccinate from CO_2 and electric energy by in situ coupling electrochemical and microbial catalysis at 1 L-scale was developed. In the first phase, 6.1 ± 2.5 mM formate was produced by electrochemical CO_2 reduction. In the second phase, formate served as the substrate for microbial catalysis by an engineered strain of *Methylobacterium extorquens* AM-1 producing 7 ± 2 μM and 10 ± 5 μM of mesaconate and 2*S*-methylsuccinate, respectively. The proof of concept showed an overall conversion efficiency of 0.2% being 0.4% of the theoretical maximum.

Introduction

Technologies that allow the preservation of scarce fossil resources and the exploitation of renewable resources will form the foundation of a future resource-secure bioeconomy. This bioeconomy needs interweaving of the sector of production of chemicals and fuels and the sector of electric power generation and storage.[1] The threads for the interweaving are technologies using electricity-driven reactions that are currently referred to as Power-to-X technologies,[2] with the "X" denominating, for example, "Heat", "Chemicals", or "Fuels". When it comes to Power-to-Chemicals as well as Power-to-Fuels, however, the portfolios of feedstock and products are extremely narrow. Exceptions are, for instance, the chlorine production or the synthesis of the nylon-6,6 precursor adiponitrile.[3] Among others, one endeavor for a bioeconomy is the establishment of technologies for the exploitation of CO_2 as feedstock for carbon. However, CO_2 is a thermodynamically very stable molecule and thus needs to be activated.[4] Using (abiotic) electrochemical catalysis for CO_2 reduction does give access to complex molecules (i.e., $\geq C_2$) but suffers low selectivities and undesired side products.[5] Only recently, improved selectivities for molecules like *n*-propanol were achieved.[6] In contrast, using microbial catalysis for CO_2 conversions enables the formation of $> C_3$ such as butanol,[7] isobutyraldehyde,[8] or cellular biomass.[9] However, the biocatalytic routes are still limited by low productivities.[10] An alternative route that gives access to complex and high-value compounds for Power-to-Chemicals is based on the combination of electrochemical and microbial catalysis. Thereby the best of both types of catalysis are combined, that is, high efficiencies and rates of electrochemical catalysis[11] and the high selectivity and access to complex products by microbial catalysis.[1a] This has been demonstrated for the electrochemical CO_2 reduction (ECO_2R) with its product (e.g., formate or CO) serving as substrate for biosynthesis, or the generation of H_2 by electrolysis.[12] Using microbial substrates gained by ECO_2R has been shown for the production of higher alcohols and bioplastic precursors using *Cupriavidus necator*,[13] as well as amino acids synthesized by *Escherichia coli*.[14] Exploiting electrochemically produced H_2 allowed the chemolithoautotrophic synthesis of α-humulene involving microbial CO_2 fixation by using *C. necator*.[15] Herein, we describe the exploitation of CO_2 as feedstock by a combined electrochemical and microbial catalysis taking place in situ in a single vessel, allowing significant expansion of the spectrum of possible products. The proof of concept is exemplified for the production of mesaconate (MC) and 2*S*-methylsuccinate (MS). MC and MS are C_5-dicarboxylates that gained interest as novel precursors with industrial relevance.[16] MC is considered a precursor for the production of the bulk chemical methacrylic acid[17] that covers a worldwide annual production worth approximately $8.1 billion.[18] MS finds extensive applications in coatings, cosmetic solvents, and bioplastics[19] and is considered to be a promising precursor for tailor-made fuels.[20] A scheme of the process is shown in Figure 1. Using electrochemical catalysis CO_2 is electrochemically reduced to formate (Phase 1). Subsequently, formate served as microbial substrate being

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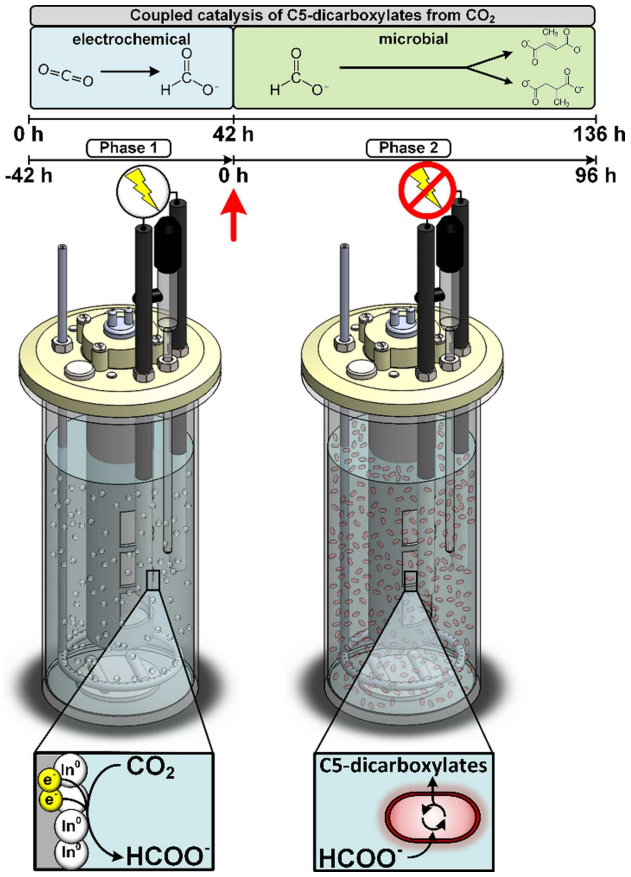

Figure 1. Scheme of the coupled electrochemical and microbial catalysis of C_5-dicarboxylates from CO_2. Phase 1: ECO_2R to formate in 0.03 M carbonate buffer electrolyte solution using indium electrodes poised at −1.6 V vs. Ag/AgCl; Phase 2: microbial catalysis of mesaconate and 2*S*-methylsuccinate by *Methylobacterium extorquens* AM-1 pCM160_RBS-*yciA*. The coupled catalysis was carried out in a 1 L-electrobioreactor with a working volume of 800 mL. The red arrow in the timeline indicates the point in time of the inoculation of *M. extorquens* AM-1 from a pre-culture grown in shake flasks. Before inoculation, the ECO_2R was stopped. Further details are described in the Supporting Information section SI 1.

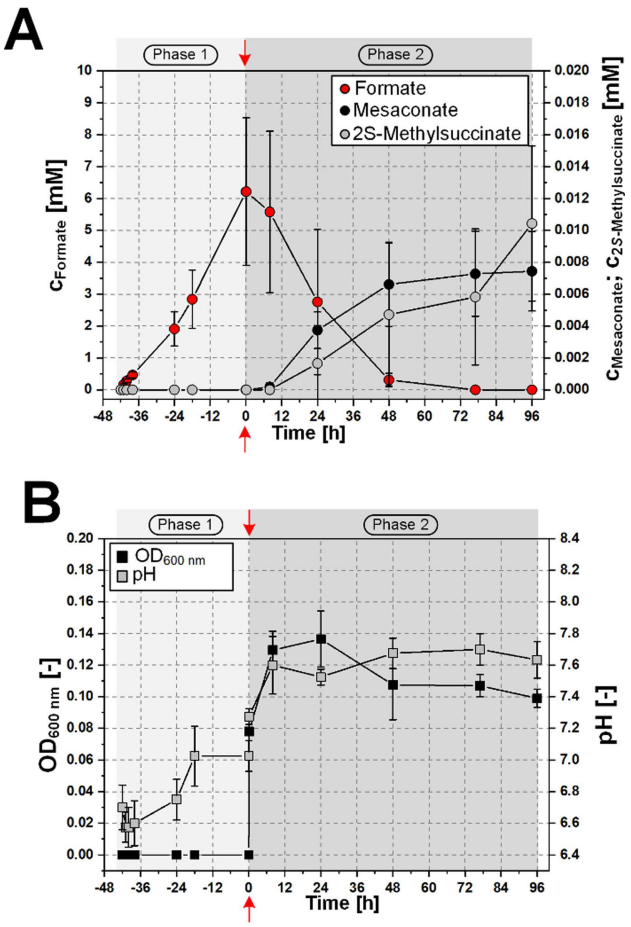

Figure 2. A) ECO_2R to formate in Phase 1 and its subsequent conversion to mesaconate and 2*S*-methylsuccinate in Phase 2. **B)** $OD_{600\,nm}$ and pH during coupled electrochemical and microbial catalysis. The event of ECO_2R-stop and inoculation of *M. extorquens* AM-1_pCM160-RBS-*yciA* is indicated by the arrows at $t = 0$ h. Reported values are mean values and the error bars represent the standard deviation of $n = 4$. The single electrobioreactor runs are compiled in Figure S3.

converted to the C_5-dicarboxylates by a genetically modified *Methylobacterium extorquens* AM-1 strain (Phase 2).[21]

Results and Discussion

In Phase 1, up to 6.1 ± 2.5 mM formate was produced within 42 h from CO_2 via ECO_2R (Figure 2A). Within the first two hours the coulombic efficiency (CE), as well as formate production rate, increased from 6.8 ± 4.5 to 16.2 ± 6.0 % and 0.0007 ± 0.004 to 0.0017 ± 0.0008 mmol cm^{-2} h^{-1}. While the CE remained stable until the end of Phase 1, the formate production rate further increased to 0.0029 ± 0.0014 mmol cm^{-2} h^{-1} (Figure S4A). This can be explained with an increase of the conductivity (κ) of the carbonate buffered electrolyte solution due to the charge balancing ion transfer (Figure S4B). The indium (In) catalyst layer is stable for a period of at least 48 h as the In^{3+} concentration in solution was only 3.8×10^{-5} ppm. The loss of In from the electrode backbone (i.e., 0.3×10^{-3} %, Figure S5B), therefore, is negligible. The average CE for formate production in Phase 1 was 12.9 ± 3.8 % (Table 1). Due to the proton-consuming reactions of formate production (Eq. (SI 28)) and hydrogen evolution reaction (HER, Eq. (SI 29)) being the competing electrode reaction, the pH slightly increases (Figure 2B). As proven here and shown in a previous study H_2 was the only side product (Figure S5A).[22] Thereby it is of note that H_2 is no waste product. H_2 can be further collected and used as fuel (Power-to-Fuel). Moreover, the electrochemically produced H_2 can serve within the electrobioreactor itself as microbial electron donor.

In Phase 2, the ECO_2R was stopped by switching the 1 L-electrobioreactor to open circuit (OC). Within 48 h after inoculation with *M. extorquens* AM-1 pCM160_RBS-*yciA* formate was almost completely consumed (Figure 2A). An increase of the An increase of the optical density ($OD_{600\,nm}$) clearly shows that formate was the substrate for microbial growth and microbial catalysis (Figure 2B) as this was not observed for *M.*

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Table 1. Conversion efficiency in Phase 1 and Phase 2 of the coupled electro- and biocatalysis of C_5-dicarboxylates from CO_2. Reported values are mean values ± standard deviation of $n = 4$.

Process phase	Parameter	Value	Max. theoretical value	Value relative to max. theoretical value
1	CE of ECO_2R [%]	12.9 ± 3.8	100	12.9 ± 3.8
2	formate conversion efficiency[a] $\eta_{C5/formate}$ [%]	1.6 ± 0.8	52.3[b]	3.1 ± 1.6
1 + 2	overall conversion efficiency η [%]	0.2 ± 0.1	52.3	0.4 ± 0.1

[a] Takes into account that formate acts as C-source and e^--source for the formation of the target C_5-dicarboxylates. [b] Based on the experimental ratio of MC to MS of 0.7.

extorquens AM-1_pCM160-RBS-*yciA* in the absence of formate (Figure S7). Formate was consumed at an average rate (q_f) of 0.139 ± 0.026 mM h^{-1} (Table 2) and converted into the target C_5-dicarboxylates until the end of the experiment. According to the stoichiometry Eq. (SI 8) and Eq. (SI 11), the theoretical maximum formate carbon recovery is 40% (i.e., two carbons from formate and three carbons from CO_2 for five C-mole of each C_5-dicarboxylate). However, formate does not only provide carbon for the carbon backbone of the dicarboxylates but is also oxidized to CO_2 to gain electrons (e^-)[9a] that are needed for the regeneration of reducing equivalents (i.e., nicotinamide adenine dinucleotide (phosphate), NAD(P)H). These are driving the reduction reactions needed for carbon backbone formation (Figure S6). Thus, the maximum theoretical formate conversion efficiency ($\eta^{theoretical}_{C5/formate}$) for the here achieved 1:0.7 ratio of MS and MC is 52.3% (SI 1.5.4). The finally achieved titer ($t = 96$ h) of MC and MS was 7 ± 2 and 10 ± 5 μM, respectively. This corresponds to $\eta_{C5/formate}$ of 1.6%, which is 3.1% of $\eta^{theoretical}_{C5/formate}$ (Table 1). Looking at the overall process (i.e., Phase 1 and Phase 2), the conversion efficiency of electrons to C_5-dicarboxylates (η) is 0.2 ± 0.1%, being 0.4% of the theoretical maximum.

Noteworthy, here we show for the first time the production of these C_5-dicarboxylates from formate with a C_5-dicarboxylate yield ($Y_{C5/formate}$) of 0.009 g g^{-1} (Table 2). To the best of our knowledge, the C_5-dicarboxylates have so far only been produced by the *M. extorquens* AM-1 strain exploiting methanol as C_1-compound[21,23] where Sonntag *et al.* achieved a $Y_{C5/methanol}$ of 0.17 g g^{-1}.[23] The yield based on formate is 20 times lower, which can be expected by the higher degree of reduction (DoR = 6) of methanol compared to formate (DoR = 2)[24] (Table S1). Nevertheless, $Y_{MC/formate}$ and $Y_{MS/formate}$ were only 1.1 and 2.0% of the theoretically possible yields (Table S1). The low yields can be partially attributed to formate conversion to cellular biomass as indicated by $OD_{600\ nm}$ increase demonstrating the considerable optimization potential for process engineering. For optimization of electrobiotechnological processes design of experiments (DoE) can be used.[25] For Phase 1 optimization parameters include electrode materials and geometry as well as process engineering, for example by reaction medium optimization.[25] Optimization approaches for Phase 2 may include both genetic engineering of the production strain[26] and process engineering (e.g., reaction medium optimization).

Table 2. Kinetic and economic parameters of Phase 2. Reported values are mean values ± standard deviation of $n = 4$.

Parameter	Value
growth rate μ [h^{-1}]	0.023 ± 0.006
formate consumption rate q_f[a] [mM h^{-1}]	0.139 ± 0.026
mesaconate production rate $r_{MC,\ max}$ [μM h^{-1}]	0.179 ± 0.029
2*S*-methylsuccinate production rate $r_{MS,\ max}$ [μM h^{-1}]	0.129 ± 0.105
$Y_{MC/formate}$ [mol mol^{-1}]	0.0012 ± 0.0001
$Y_{MS/formate}$ [mol mol^{-1}]	0.0020 ± 0.0015
$Y_{C5/formate}$ [mol mol^{-1}]	0.0032 ± 0.0016
$Y_{MC/formate}$ [g g^{-1}]	0.0033 ± 0.0004
$Y_{MS/formate}$ [g g^{-1}]	0.0058 ± 0.0043
$Y_{C5/formate}$ [g g^{-1}]	0.0091 ± 0.0047

[a] Within the first 48 h after inoculation.

The electrochemical formate production rate achieved in Phase 1 (Figure S4A) matches the formate consumption rate of *M. extorquens* AM-1_pCM160-RBS-*yciA* using a volume-related geometric electrode surface area of 0.4 $m^2\,dm^{-3}$. Higher formate production rates are easily achievable, for example, by using 3D electrode geometries (e.g., gas diffusion electrodes (GDEs)) and materials such as reticulated vitreous carbon[27] that exhibit a more than ten times higher volume-specific electrode surface area of 6.5 $m^2\,dm^{-3}$. Moreover, for GDEs current densities of up to 500 times higher than reached in this study are reported (Figure S4A).[28] Thus, application-relevant formate production rates of 1 g $L^{-1}\,h^{-1}$ (i.e., 22 mmol $L^{-1}\,h^{-1}$) are in reach. At the same time, the 3D electrode would occupy approximately only 0.25‰ of the reactor volume showing that further process development is not limited by formate production rates.

In Phase 2, a major limitation of feeding formate as the microbial substrate is its cytotoxicity.[26] In this matter, the in situ approach presented here could be advantageous, as the electrochemical formate production can also take place under bioprocess-compatible conditions,[22,29] making its spatially and temporally homogeneously distributed on-demand supply possible. This is particularly important as spatial and temporal formate gradients can lead to undesired substrate inhibition or long lag phases.[26] Carrying out ECO_2R and microbial catalysis simultaneously inevitably leads to electrode poisoning and consequently decrease of performance of ECO_2R. Thus, strategies to avoid the negative effects of electrode poisoning must be applied (see below). Furthermore, a control experiment, in which formate was solely provided by the addition of sodium formate (Figure S7) confirmed that providing formate electrochemically does not negatively influence C_5-dicarboxylate production (Table S2). However, microbial catalysis based on

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formate is so far largely unexplored.[26] Metabolically engineered strains of *M. extorquens* should be used to generate a better understanding of the formate metabolism. Four natively occurring pathways (i.e., Calvin cycle, ribulose monophosphate cycle, dihydroxyacetone cycle, and serine cycle) including several of their variants are known to support formate (and methanol) utilization at aerobic conditions.[26] Based thereon, plenty of synthetic variants for implementation also in non-formatotrophic hosts. For example, the above-mentioned hydrogenotrophic *C. necator* has been suggested but so far only partially demonstrated.[26] Hydrogenotrophic microorganisms can utilize H_2 as e^--donor to drive the metabolism. Thus, also H_2 as the only side product of Phase 1 can be exploited for the microbial catalysis, ultimately leading to an improvement of η.

For providing proof of concept, the process was run sequentially in only a single vessel, allowing a seamless flow between the processes while maintaining sterile conditions. Generally, a reduction of operating units of a process is economically favored.[20] However, a parallel process mode or a repetitive sequential process mode may improve kinetics and, thus, the process economy. A further approach is to spatially separate Phase 1 and Phase 2 in separate reaction vessels. Supporting Information section SI 2.9 contains an illustration (Figure S8) and further details of the suggested different process modes. In an integrated parallel process electrochemical catalysis is connected with microbial catalysis via the reaction medium being electrolyte solution and microbial medium at the same time.[30] However, metal catalysts, such as In used in this study, are prone to mixed potential formation and inactivation by deposits of microbial medium compounds such as trace metals.[20,31] We have shown in a previous study that mixed potential formation negatively affects the performance of the ECO_2R to formate.[32] However, this can be overcome by optimizing the reaction media.[25] Furthermore, using periodically-pulsed electrochemical catalysis by applying reduction and oxidation potentials alternately may provide a strategy to stabilize the electrode performance, as it has been shown for Cu- and Pd-based electrodes.[33]

A simplified economic evaluation of the coupled electrochemical and microbial catalysis of C_5-dicarboxylates from CO_2 in the 1 L-electrobioreactor that (i) only considers the costs for electricity (0.025 € kWh^{-1}[13b]) and (ii) excludes capital expenditures (capex) and further operating expenditures (opex) than for electric energy shows that formate can be produced at costs of 0.34 € kg^{-1}. This is only half of the formic acid market price (Table 3). It can be assumed that the price difference is mainly due to the simplified analysis only considering electric energy as opex and not accounting for capex, as at the present technology readiness level of the entire field no honest capex calculation can be performed.[36] Providing formate at this price as the substrate for the microbial catalysis, MC and MS are produced at costs of 99.03 and 90.35 € kg^{-1}, respectively. The fermentative production of MC is estimated to reach the level of itaconic acid production as the yields are similar.[35] As a comparison, on a global scale, the annual production volume of itaconic acid is expected to reach 50,000 tons.[37] Based on a techno-economic assessment Lundberg *et al.* proposed a MC market price of 0.91 € kg^{-1} if produced in a full-scale plant with an annual production of 50,000 tons.[35] This is only about two orders of magnitude lower as calculated for the herein presented process at 1 L-scale (Table 3), which still has extensive optimization potential. An evaluation for MS is much more difficult as market prices for industrial-scale production are not available. Currently, MS can be purchase in kg-scale for approximately 45 € kg^{-1}.[38] For production in a full-scale plant, we assume the market price to be two orders of magnitude lower. As a result and similar to MC, the MS market price is then two orders of magnitude lower as calculated for the herein presented process at 1 L-scale (Table 3). Of course, this treatise does not provide a profound techno-economic assessment. However, it highlights that the proposed technology for the production of C_5-dicarboxylates from CO_2 represents a potential alternative production route.

Conclusions

We have presented the production of C_5-polymer precursors such as mesaconate (MC) and 2*S*-methylsuccinate (MS) from CO_2 and electric energy for the first time. This aligns to and advances previous studies, for example by the Palkovits and Tessonnier groups, from (i) the sustainability point of view as the starting materials were no complex molecules such as glucose,[20,31b,39] and (ii) the process engineering point of view as the gap between electrochemical and microbial catalysis was

Table 3. Simplified economic assessment of coupled electrochemical and microbial catalysis of C_5-dicarboxylates from CO_2. Reported values are mean values ± standard deviation of $n = 4$. Further details are stated in SI 2.8.

Phase	Parameter	Formate	Mesaconate	Methylsuccinate
1[a]	formate market price [€ kg^{-1}]	0.68[34]		
	$P_{formate}$ based on cathodic half cell reaction [kWh kg^{-1}]	13.75 ± 4.20		
	price per kWh [€ kWh^{-1}]	0.025[13b]		
	$Cost_{formate,\ ECO_2R}$ of this study [€ kg^{-1}]	0.34 ± 0.10		
2[b]	product market price [€ kg^{-1}]		0.91[35]	0.45[c]
	price of the product based on $Cost_{formate,\ ECO_2R}$ (SI 2.8) and $Y^{theoretical}_{product/formate}$ (SI 1.5.3) [€ kg^{-1}]		1.07 ± 0.32	1.17 ± 0.31
	price of the product based on $Cost_{formate,\ ECO_2R}$ and achieved $Y_{product/formate}$ (Table 2) [€ kg^{-1}]		99.03 ± 20.50	90.35 ± 62.96

[a] Phase 1: ECO_2R to formate. [b] Phase 2: microbial conversion of formate to C_5-dicarboxylates. [c] Based on the current market price for MS sold as 25 kg-containers and the assumption that MS production in a full-scale plant would drop this price by two orders of magnitude.

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seamlessly bridged in the 1 L-electrobioreactor. Economically viable production costs as reached in studies by Tessonnier and co-workers[40] could not be achieved yet. However, a simplified economic evaluation highlighted the promising nature and the significant growth potential of the exemplarily C_5-polymer precursors. Furthermore, the potential of formate, which can be gained by using renewable electricity, to serve as a platform chemical for biobased processes was shown. Formate can become a key compound for the establishment of a sustainable C_1-based bioeconomy.[26] Especially compared to methanol, another key component of C_1-bioeconomy, formate is suitable as a microbial substrate for aerobic microbial catalysis due to its high solubility in aqueous media and low volatility into the gas phase. The electrochemical production of formate in situ in the base microbial medium as shown here as a proof-of-concept, in the long run, can avoid downstream processing. This enables a coupled one-pot catalysis under sterile conditions to use biocatalysis based on tailor-made pure cultures and can be advantageous compared to the cascades of a multi-stage process (i.e., ECO_2R to formate production, subsequent formate downstream processing, and finally biocatalytic conversion). Particularly future work will focus on the optimization of the coulombic efficiency for ECO_2R in the electrobioreactor as well as on the expansion of the producible polymer precursor spectra by testing different production strains. The here introduced production line may represent a blueprint for electrobiorefineries[1a] and can serve as an important thread of a future biobased economy.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: biosynthesis · dicarboxylates · electrochemical CO_2 reduction · electrochemistry · formate

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