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Bio-electro CO_2 recycling platform based on two separated steps

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ABSTRACT

The present work assessed the alliance of microbial electrochemical technologies (METs) and fermentation in a two-step process for the electro-bioconversion of carbon dioxide (CO_2) into elongated chemical building blocks. The electro bio-reduction of CO_2 into acetic acid and ethanol (EtOH:HAc) at a 1-to-1 ratio is linked to a subsequent elongation step to produce C_4 and C_6 compounds. Key operational conditions of each step were assessed. Key parameters considered in the first step were pH, and both hydrogen and CO_2 partial pressures. Concerning the second stage, selected parameters were pH, ethanol to acetate ratio, and hydrogen availability. The aim was to steer each stage's performance and to obtain higher value products. Reached EtOH:HAc proportion was over 1:1 when fed with CO_2, with H_2 availability and at pH around 5.3. Formed product reinforced the follow-up chain elongation processes. The fermentation step got up to C_6 compounds at pH 7.0, when fed with CO_2 and H_2. Outcomes demonstrated that pH was a crucial factor in the overall process. The overall process carbon conversion efficiency was 38% being the CO_2 transformation the limiting step. 1 kg of CO_2 fed in the system resulted in the production of 0.38 kg of elongated acids (C_4-C_6). In other words, 1 m^3 of CO_2 (normal conditions) captured resulted firstly in the production of 0.90 kg C_{C2} and then, 0.75 kg $C_{C4\text{-}C6}$ are finally acquired. The presented results pave the ground for improving the selectivity during the production of reduced commodity chemicals from CO_2 and electricity.

1. Introduction

Microbial Electrochemical Technologies (METs) are a potential resource recovery approach whereby different chemical building blocks can be produced from carbon dioxide (CO_2) and electricity as sole carbon and reducing power sources, respectively [13]. These chemical building blocks can be elongated to more valuable products through a fermentation process (i.e. reverse β-oxidation) where chain elongation takes place [9]. Ethanol is a key player in the elongation process [10], foremost a METs product [7]. CO_2 conversion in METs is usually a bio-hydrogen-mediated process [27].

Elongated compounds such as butyric and medium chain carboxylic (MCC) acids are more desired, due to their higher market value [11,20,34]. Bian and colleagues [6] highlighted a significant decrease in extraction costs after a chain elongation process, but these organic compounds are more challenging to obtain. To date, recent studies reported the production of MCC acids through METs [21]. Besides, product selectivity remains challenging [36]. Notwithstanding, C_2 compounds' relevance relies on their aptness as substrates for chain elongation reactions [7].

Acetic acid production is thermodynamically favorable, therefore solventogenesis appears to be the bottleneck in the achievement of higher C_2 proportions [2]; [13]. Consequently, it also limits the production yields of elongated acids [41]. Chain elongation from these products occurs by means of reverse β-oxidation pathway with acetic acid as carbon and ethanol as reducing power sources [10]. Here, two-carbon acetyl-CoA derived from ethanol is used to reduce the carboxylates and form longer-chain molecules [2].

Furthermore, proper operational conditions to steer acetogenesis and solventogenesis differ from the chain elongation suitable ones [14]. Thereby, a potential strategy to overcome this challenge may lie in coupling a MET system with a fermentation unit.

The present work assesses the alliance of METs and fermentation in a two-step process for the electro-bioconversion of CO_2 into elongated chemical building blocks. This conversion is driven by electricity while promoting optimal operational conditions to trigger each reaction and maximize production rates. The initial step consisted of the bio-electro CO_2 recycling into acetic acid and ethanol aiming at a 1–to–1 ratio. In

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the second stage, the produced compounds were used to feed a secondary fermenter to perform chain elongation and obtain larger commodity chemicals (mainly butyric – C_4 – and caproic – C_6 – compounds). Operational parameters such as pH, ethanol-to-acetic acid ratio and feeding gas regimes were tested to promote chain elongation processes and assessed both the product spectrum and selectivity towards target products.

2. Materials and methods

2.1. METs setup and operation

Two tubular METs were constructed (named as R1 and R2, Figure S1) and consisted of two concentric chambers separated by a 580 cm2 tubular cation exchange membrane (CMI-1875 T, Membranes international, USA) (Fig. 1). The cathode chamber was placed in the inner space. The cathode material consisted of commercial granular graphite (model 00514, diameter 1.5–5 mm, EnViro-cell, Germany). This material was chosen to allow the electricity flow and sustain the biofilm growth above an entire electrode surface of 0.361 m2. A stainless-steel wire was placed in between the granules bed to allocate electrons equally onwards the chamber and to facilitate the connection with the potentiostat (BioLogic, Model VSP, France). The anode consisted of a carbon cloth sheet (140 cm2, working area of 280 cm2; Thickness 490 μm; NuVant's ELAT, LT2400 W, FuelCellsEtc, USA) connected to a titanium plate (3.78 cm2 Ti-MMO, 15 ×40 mm, 2 mm light path and 1 mm wire diameter, NMT electrodes, South Africa) and a stainless-steel wire. Cathode and anode working volumes were 0.3 L each. Both METs were operated in a three-electrode configuration with a potentiostat that monitored the current density and poised a cathode potential of − 0.8 V vs. Standard Hydrogen Electrode (SHE) to promote H2 production [27]. An Ag/AgCl electrode (+0.197 V vs. SHE, model SE11-S Sensortechnik, Meinsberg, Germany) placed in the cathode chamber worked as the reference electrode.

Both chambers were connected to buffer tanks that served as feeding and sampling points, in the case of the cathodic one, achieving a total chamber volume of 1 L. The liquid was continuously recirculated (4.5 L h^{-1}), as presented in Fig. 1. The headspace of the cathodic buffer tank was 0.2 L. Both METs were operated in batch mode at 25 ± 1 ºC and kept in the dark to avoid the growth of phototrophic microorganisms'.

Each chamber was filled with inorganic modified ATCC 1754 PETC medium [5], containing 0.1 g KH_2PO_4, 0.8 g NaCl, 1 g NH_4Cl, 0.2 g $MgCl_2.6H_2O$, 0.1 KCl, 0.02 $CaCl_2.2H_2O$, 1.95 g commercial MES hydrate (employed as a medium buffer), 0.4 g Cysteine-HCl L^{-1}, 1 mL of vitamin solution and 1 mL L^{-1} of trace element solution (Table S1). The medium was prepared anaerobically with a final pH of 5.40.

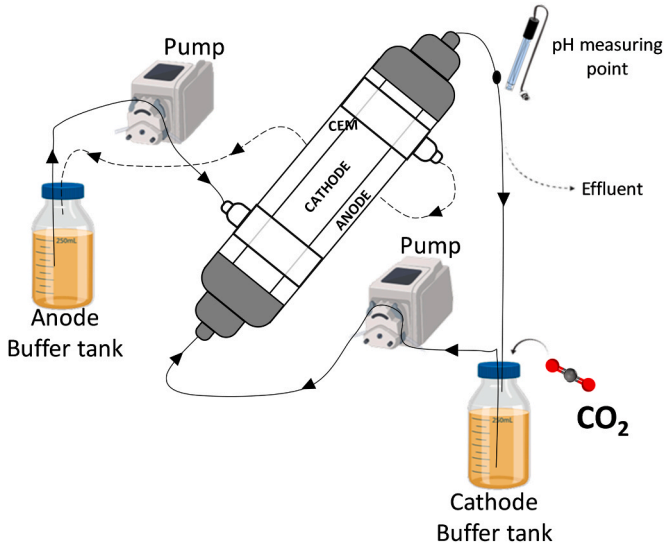

Fig. 1. Schematic representation of the first-step METs setup. CEM: Cationic exchange membrane.

Cathodes were inoculated with a 20% (v/v) of a mixed culture enriched with an isolated named I-19 at the late exponential phase. The isolate was identified before as *Eubacterium limosum* [29], which showed the ability to produce acetic acid and ethanol at 37 ºC when fed with syngas ($CO:H_2:N_2:CO_2$ [32:32:28:8% *v/v*]). Additionally, this isolate showed an equimolar production of acetic acid and ethanol in METs when fed with CO_2 at 25 ºC [7].

Pure CO_2 (99.9%, Praxair, Spain) was periodically sparged every 2–3 days for 5 min into the cathode buffer tank. pH was monitored during each sampling event, ranging 5.0–6.5 along the experimental period.

Gas- and liquid- samples were gathered from cathode and anode buffer tanks right before feeding with CO_2 to analyze the gas composition and the presence of volatile fatty acids (VFAs) and alcohols in the liquid phase. The exact withdrawn volume for the liquid phase analyses was subsequently replaced with a fresh medium.

2.2. Anaerobic fermentation setup and operation

Eight tests were conducted during the elongation stage experiments, which corresponds to the second step proposed based on fermentation of the C_2 compounds in presence of CO_2 and H_2. These fermenters consisted of 0.12 L serum bottles whose working volume was 0.05 L. All fermenters were filled with the effluent of the bioelectrochemical reactors. Acetic acid and ethanol total concentrations and their corresponding ratios were initially adjusted as shown in Table 1, obtaining final concentrations of 1 g L^{-1} of acetic acid and 3 g L^{-1} (T1, T2, T5 and T6) or 1 g L^{-1} (T3, T4, T7 and T8) of ethanol. Concentrations were adjusted in the first group of tests (EtOH: HAc 1: 3) to assess the effect of higher ratios. Acetic acid and/or ethanol were added when required to adjust compounds concentrations to the desired ratio in each fermenter. Flasks were all sealed and capped with butyl caps and aluminum crimp caps, respectively.

Bottles were incubated at 25 ± 1 ºC in a rotating shaker (100 rpm) stored horizontally to enhance gas-liquid mass transfer and maintain the biomass suspended in the medium.

0.5 L of the catholyte from the first step MET was used as inoculum for the second step. Cells were pelleted by centrifugation (20 min, 4.400 rpm at 0ºC). The supernatant was discarded, and the pellet was resuspended into 25 mL of fresh medium. Finally, 1 mL of the new solution was added to each fermenter as inoculum.

Operational conditions were established at the beginning of the experimental procedure (Table 1). The pH of the media was set at 5.5 (tests 1–4) or 7.0 (tests 5–8) and maintained along the operational period (18 days) by means of the addition of 5 M sodium hydroxide or

Table 1
Experimental setup. All tests were performed in triplicate. EtOH: Ethanol. HAc: Undissociated Acetic acid.

	pH		EtOH: HAc Ratio		Feeding gas*	
Test	5.5	7.0	3:1	1:1	CO_2:H_2 (80:20%)	CO_2 (99.9%)
T 1	✓	–	✓	–	✓	–
T 2	✓	–	✓	–	–	✓
T 3	✓	–	–	✓	✓	–
T 4	✓	–	–	✓	–	✓
T 5	–	✓	✓	–	✓	–
T 6	–	✓	✓	–	–	✓
T 7	–	✓	–	✓	✓	–
T 8	–	✓	–	✓	–	✓

* Gas percentages were 80:20% v/v in case of CO_2:H_2 and 99.9% v/v in case of CO_2.

1 M hydrochloric acid. Operational pHs were selected based on optimal pH values suggested by previous research [10,34].

The production of elongated commodity chemicals and the resulting product spectrum, from acetic acid and ethanol as substrates, were tested under different operational conditions. Studied conditions were: (i) with or without H_2 input as reducing power, depending on the feeding gas composition, (ii) different ethanol: acetic acid (EtOH: HAc) substrate ratios and (iii) the optimal pHs.

Fermenters were fed by sparging CO_2:H_2 (80:20% *v/v*, Praxair, Spain) or CO_2 (99.9%, Praxair, Spain) depending on the treatment, twice a week for 3 min. Initial overpressure of the fed gas was 1 bar in the fermenters. The gas feeding was fixed at 3–4 days to enhance microbial growth, coinciding with sampling times. Total pressure and pH were measured when sampling, while pH was adjusted to the initial settled conditions in case it was required. Withdrawn volumes while sampling was not subsequently replaced to avoid perturbations in the experimental design.

All tests were carried out in triplicates. Therefore, the results obtained were expressed as mean values and included the standard deviation of the three measurements.

2.3. Analyses and calculations

Gas- and liquid- samples were taken twice a week in both METs and fermenter experiments to determine the gas composition and the concentration of organic compounds (volatile fatty acids and alcohols), respectively. All procedures and analyses were carried out similarly.

The total pressure in the headspace of the system was measured with a digital pressure sensor (differential pressure gauge, Testo 512, Spain). Gas samples were analyzed using a gas micro-chromatography (μGC; 490 Micro GC system, Agilent Technologies, US). The micro GC was equipped with two columns, a CP-molesive 5 A for methane (CH_4), carbon monoxide (CO), H_2, oxygen (O_2), and nitrogen (N_2) analysis; and a CP-Poraplot U for CO_2 analysis. Both columns were connected to a thermal conductivity detector (TCD). VFAs and alcohols of pre-filtered samples were analyzed using a gas chromatograph (Agilent 7890 A, Agilent Technologies, EUA) equipped with a DB-FFAP column and flame ionization detector (FID). pH and optical density (OD) were measured by means of a pH meter (pH meter Basic 20 +, Crison Instruments, Spain) and a spectrophotometer (CECIL 1021, CECIL INSTRUMENTS, Cambridge, UK) respectively.

The total hydrogen production was calculated as a summation between the hydrogen present in the headspace calculated through the partial pressure (pH_2) and gas composition, the concentration of dissolved H_2 and the H_2 equivalents, corresponding to the organic compounds detected in the liquid phase.

Dissolved H_2 and CO_2 concentrations in the liquid were calculated employing Henry's law at 25 ºC (Eq. 1), where C_i is the concentration of the component i in the media (mol L^{-1}), $K_{H,I}$ is Henry's constant (mol L^{-1} atm^{-1}) of the component i at working temperature (0.0008 for H_2 and 0.0337 for CO_2) and $_{Pi}$ (atm) is the partial pressure of i in the gas phase.

$$C_i = K_{H,i} * P_i \tag{1}$$

Production rates over dry cell weight (DCW) were calculated (Eq. (2)) using Lai et al. (2016) equality, where the Pr_i is the production rate of component i in the fermenter test X (mmol L^{-1} d^{-1}), DCW_{Ai} is the average dry cell weight of the fermenter test X (g DCW_{Ai} L^{-1}). Given that chain elongation is a cyclic process where each produced organic can be employed as a substrate for achieving a more reduced compound, production rates of intermediate organic compounds are unseen. Hence, net productions were calculated according to measured concentrations.

$$P_\chi = \frac{Pr_i}{DCW_{Ai}} \tag{2}$$

Coulombic efficiency (CE) was calculated according to Patil et al. (2015) to determine the consumed energy to produce organic compounds (Eq. (3)) [25]. Where F is the faradaic constant, M_i number of moles of product i, the number of consumed electrons per mole of product, and is the integral of the current density supplied along the period.

$$CE\,(\%) = \frac{F \cdot \Sigma M_i \cdot \Delta e_i}{\int \mathrm{I}\,\mathrm{dt}} \tag{3}$$

Moreover, energy consumption – given by the electricity consumed along the bioelectrochemical system step – and its average were calculated by Eq. (4) where E_{cons} is the energy consumption (W h), P is the power (W) consumed along the period. Previously, the power was estimated through Eq. (5) where CD is current density (A) and E_{cell} is the cell voltage (V). Later on, the consumed energy was determined per mg of product (Eq. (6)). Where, ΔE_{cons} is the energy consumed during the interval and $\Delta Product$ is the amount of product formed in milligrams along the experimental period.

$$E_{cons} = \quad \Sigma \quad P \quad dt \tag{4}$$

$$P = CD * E_{cell} \tag{5}$$

$$E_{cons} = \frac{\Delta E_{cons}}{\Delta Product} \tag{6}$$

It should be stressed that neither the energy consumption of peripheral devices (e.g. peristaltic pumps, potentiostat, data loggers and computer) nor the energy required for producing the added hydrogen used in the fermentation stage were included in the energy consumption calculations.

3. Results and discussion

The integrated process consisted of a microbial electrochemical technology system stage coupled to a fermentation unit. In the first step, CO_2 was bioelectrochemically reduced into ethanol and acetic acid, with the aim of achieving a desired proportion to exploit the effluent as a substrate for the coupled chain elongation reactor. In the second step, these reduced compounds were therefore elongated to acids under the required operational conditions (Figure S2).

3.1. Bio-electro CO_2 recycling into acetic acid and ethanol

Fig. 2 shows the behavior of both METs, operated as replicates. After five days of operation, organic compounds production started. In R1 acetic acid (HAc) was the first compound to be produced, followed by ethanol (EtOH) formation. Differently, in R2 acetate and ethanol production were concomitant. The total organics concentration corresponds to the sum of acetic acid and ethanol, together with other minor carbon-based compounds detected in the systems (i.e. butyric and isobutyric acid and butanol). Those differences might be explained by slight variations in the reactor setup which could consequently change the pH. Both replicates reported parallel production slopes (1.1 for HAc and 1.2 for EtOH) in R1, calculated from the day the compound production began (HAc: day 7.79, EtOH: day 9.79). In R2, production slopes were calculated for both compounds with data from day 6.84 of operation until the end of the experiment, were nearly parallel between days 7–12 of operation yet ethanol production rate increased from that day on. The similar production slopes matched in time when pH conditions were between 6.0 and 6.5. Production rates of organic compounds changed in both reactors (from day 27 in R1 and day 13 in R2) due to a pH drop (pH < 6.0), which promoted ethanol production. The decreasing of pH was related to acetic acid accumulation and the successive CO_2 feedings.

Organic compounds generation occurred on accounts of in-situ hydrogen generation, which is linked with the current density demand (Fig. 2). The current density was different during non-producing periods compared to the periods where organics were detected, as reported in

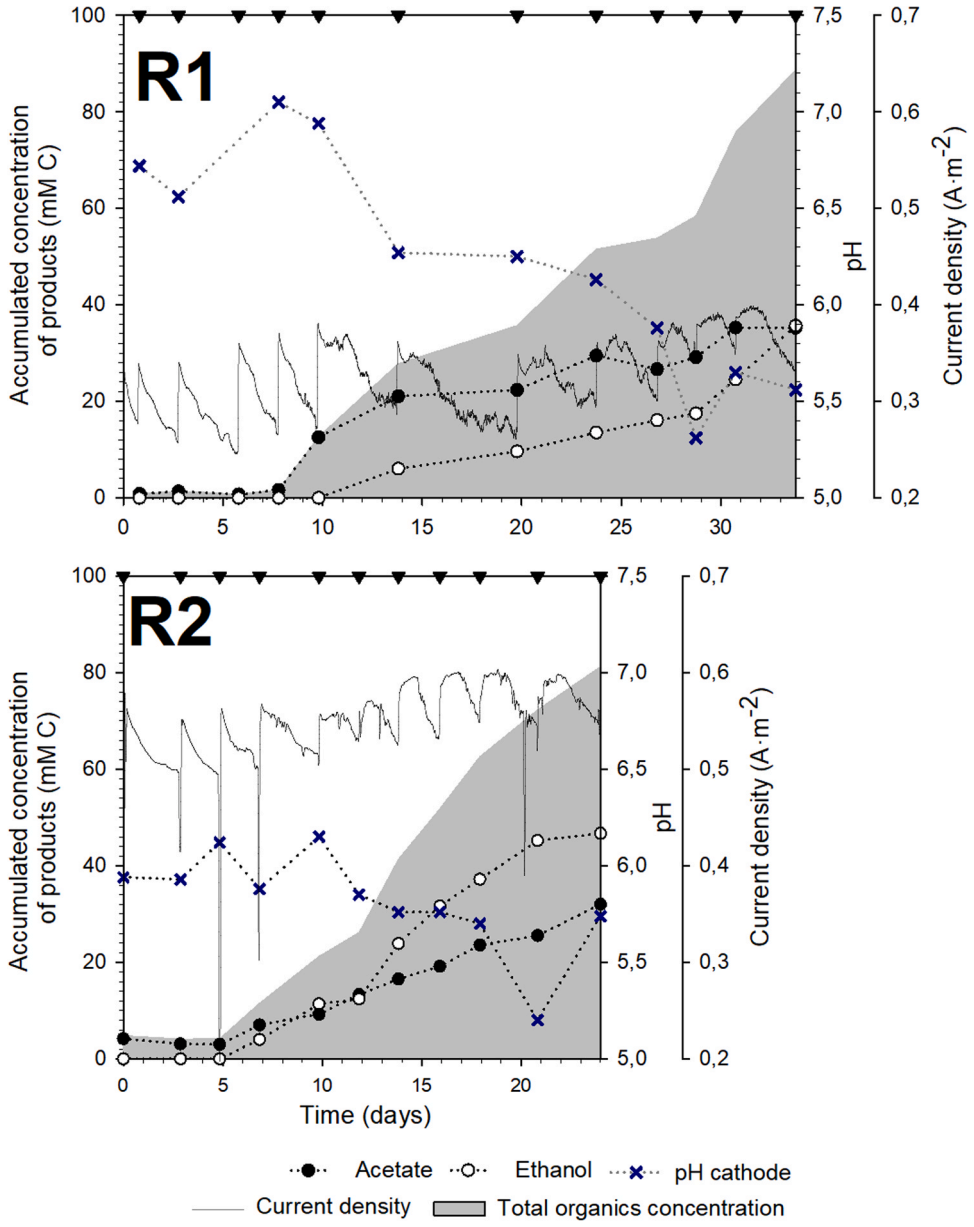

Fig. 2. Accumulated concentration of organic compounds in METs, pH and current density in both reactors. Black inverted triangles on the top of the figure indicate CO_2 flushing and sampling.

previous studies [14,19]. In non-producing cycles, current density was lower, increasing after the gas feeding due to the drop of pH, and step-wise decreased until the next feeding event. In contrast, along the producing cycles, current density signal increased just after feeding with CO_2 reaching a plateau ending up with a progressive diminish (Blasco-Gómez et al., 2019).

Concerning hydrogen production, pH_2 increased up to 1.03 ± 0.02 in both replicates at the end of each feeding cycle (Figure S3). Once hydrogen was available and the culture was adapted to the working conditions, both reactors reported an increasing current density, which was considered a change in the pattern. Furthermore, the current demand increased just after the CO_2 feeding (Fig. 2) and decreased once CO_2 was depleted (data not shown) and H_2 accumulated in the headspace (Figure S3). In agreement with the stated by Blasco-Gómez and colleagues [7], during the producing cycles, the current demand trend suggests the reaction was occurring in the system. When the current density increases, acetic acid (C_2) is likely being produced, whereas a drop of current signal is considered an indicative of a shift towards ethanol production.

Table 2 summarizes the mean values of pH, production rates, current

Table 2
Average production rates of organic compounds, remaining non-converted H_2, total H_2, ethanol-to-acetic acid ratio (EtOH:HAc ratio) average, the maximum concentration of acetic acid ([Hac]) and ethanol ([EtOH]), current density, coulombic efficiency (CE) and average pH during the production cycles.

	R1	R2	Units
Organics production rate	4.70 ± 4.79	6.86 ± 4.15	**(mmol C m^{-2} d^{-1})**
Remaining H_2	5.22 ± 0.19	6.70 ± 1.32	**(mmol m^{-2} d^{-1})**
Total H_2	19.84 ± 14.90	27.53 ± 13.79	**(mmol m^{-2} d^{-1})**
EtOH: HAc ratio	1.01	1.62	
Maximum [HAc]	35.30	28.98	**(mM C)**
Maximum [Et]	35.60	46.88	**(mM C)**
Current density	0.33 ± 0.04	0.55 ± 0.04	**(A m^{-2})**
CE	14 ± 7	12 ± 10	**%**
pH	6.1 ± 0.4	5.6 ± 0.6	

density, ethanol-to-acetic acid ratio, and maximum concentrations achieved in both reactors of the productive period. *In-situ* generated H_2 produced at the applied cathode potential was comparable in both reactors. This hydrogen is likely to be produced both, biotically and abiotically [26]. Total H_2 production (consumed plus accumulated) was 19.84 ± 14.90 at R1 and 27.53 ± 13.79 mmol m^{-2} d^{-1} at R2. The accumulated amount (in form of H_2) was 5.22 ± 0.19 and 6.70 ± 1.32 mmol m^{-2} d^{-1} at R1 and R2, respectively, whereas the rest was consumed for the formation of mainly acetic acid and ethanol. Therefore, although ethanol production required H_2, it was detected a surplus of this gas available, which could be circumvented as the electron donor in a second stage.

Hydrogen was exploited as reducing power for the production of organic compounds. Acetic acid production rates were similar in the two systems whereas ethanol differed between them. Total production rates were 4.70 ± 4.79 vs. 6.86 ± 4.15 mmol C m^{-2} d^{-1} in R1 and R2, respectively. However, maximum acetic acid concentration was higher in R1 while ethanol concentration was higher in R2, achieving an ethanol-to-acetic acid ratio of 1.62 vs. 1.01 in R1. The different ratios may have resulted from the differences between pH evolution in each reactor. Nevertheless, the obtained ratios were higher compared to the previously reported in the literature [4,7,35]. Blasco-Gomez et al., [7] provided specific working conditions key operational parameters such as pH, pH_2 and dissolved CO_2 to enhance solventogenesis over acetogenesis and monitored those parameters values during the study. Hence, they were able to boost ethanol formation by achieving enough acetate concentration, high pH_2 (above 1 atm), low pH (below 5.4), and low CO_2 availability (below 100 mg L^{-1}). The achievement of these ratios allowed the follow-up chain elongation process to take place. However, they could be improved by applying new working strategies, for example, to keep all produced H_2 available along the time [8].

Along with all producing cycles, the average pH was 6.1 ± 0.4 in R1 and 5.6 ± 0.6 in R2, depending on whether acid was being produced or consumed for alcohol formation (Table 2). Acidic pH (pH < 6.0) is required to trigger solventogenesis, although it must be kept above the acid crash point [14]. In addition, avoiding the undissociated organic acids toxicity stress-induced effect (acetic acid pK_a at 25 ºC is 4.76) must be a priority [18].

3.2. Chain elongation using the broth from the bio-electro CO_2 recycling METs

Eight fermenters were filled with the outlet broth from R2 (previously analyzed and modified to achieve desired ratios as explained in Section 2.2) to study the influence of different operational conditions on product spectrum and selectivity (Table 1). From the beginning of the tests, selective process conditions of pH, feeding gas and initial substrate ratio were set. pH was maintained along the operational period and set at two different values, according to the literature (5.5 and 7.0) to reinforce the chain elongation process [34]. All fermenters were fed twice a week. All tests were provided with CO_2, while T1, T3, T5 and T7 were supplied with H_2 (CO_2: H_2 20: 80% *v/v*) as well. Thereby, the effect of H_2 and CO_2 partial pressures triggering elongated organic acid compounds production can be assessed. These parameters were reported as key factors to avoid the oxidation of produced carboxylic acids and alcohols [3,10].

Tests 1–4 were initiated at pH 5.5 (Fig. 3) and showed an adaptation period of 10 days where average optical density (OD_A) remained low (<0.05). After those days, despite the low OD_A, biological activity was ongoing as some compounds were produced. Ethanol production along

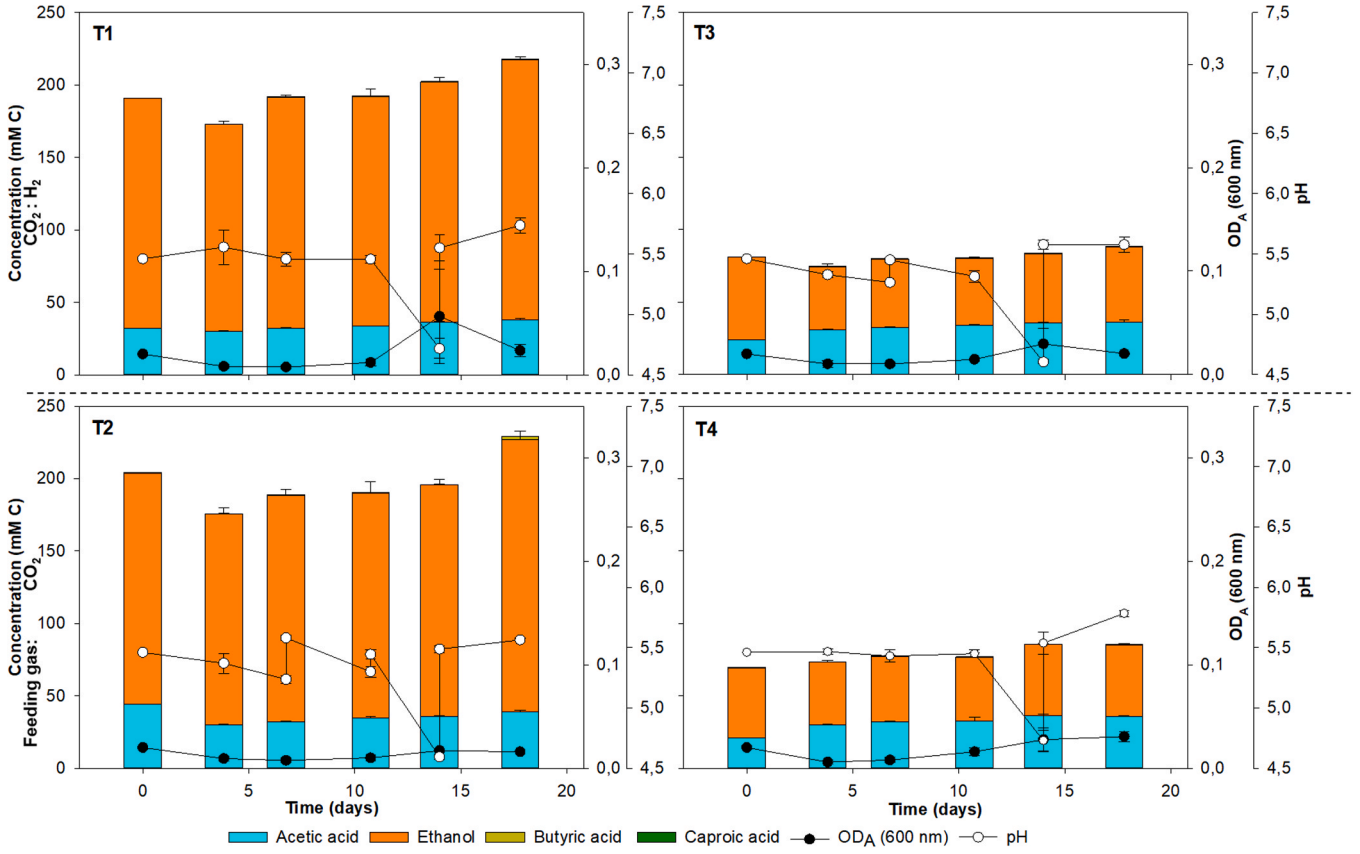

Fig. 3. Evolution of pH, OD, and concentration of organic compounds over time in tests at pH 5.5 (T1–4). For each test, all data corresponds to the average and standard deviation of the three replicates. T1 and T3 were fed with a CO_2: H_2 mixture while the other feeding gas was CO_2. Concerning the initial substrate ratio (EtOH : HAc), T1 and T2 hold a 3:1 while the rest were been provided with a 1:1.

the test was measured at T1 (28.60 ± 2.31 mmol L^{-1}) and T2 (21.19 ± 1.83 mmol L^{-1}), which triggered gas fermentative processes. However, lower ethanol productions were detected in T3 and T4. With respect to the tests where hydrogen was not provided, ethanol might have been produced from the degradation of some intracellular not-measured compounds [29]. Particularly, small amounts of butyric (2.37 ± 3.45 mmol L^{-1}) were detected in T2, denoting that chain elongation reactions could take place.

Tests 5–8 (T5–8) ran under pH 7.0 showed higher production rates than tests ran under pH 5.5 (Fig. 4). The adaptation period lasted 10 days, in which neither organic acids production nor OD_A increased. The observed lag phase may ows to the need nfor the microbial community to adapt to different operational conditions. Other studies reported similar adaptation periods [14,17,34] when no yeast extract was added. This probably occurs because the community growth and the activation of target reactions require time. OD_A only began to increase when feeding with CO_2:H_2 (T5 and T7), which reached up to 0.31 and 0.16 AU (Absorbance Units), respectively. T5 and T7 were the only ones that showed a substantially high activity and relevant productions of butyric and caproic acid of 20- and 40- mM C, respectively. The product spectrum and the evolution of different compounds in both tests were comparable along the study. However, they differed in the production yields, since T5 final OD_A was more than 2-fold higher compared to T7, and so were the concentrations of elongated compounds. In fact, both tests (T5 and T7) revealed caproic acid production (Fig. 4). On the other hand, the only discrepancy between T5 and T6 working conditions was the absence of hydrogen available in the last one. Furthermore, butyric and caproic acid productions were recorded in T6, at the end of the operational period. Achieved concentrations were below 15- and 2-mM C, rather low values probably due to the poor OD_A obtained due to unfavourable working conditions. Neither VFA nor alcohol production was recorded in T8. The obtained results indicated hydrogen availability should be considered a critical parameter to drive chain elongation processes. The fact that other studies did not require hydrogen to enhance the elongation process [12] might be explained by the differences between operation length and/or culture employed. However, presented results agreed with the ones stated by Leng et al., [22] which highlighted the need of hydrogen availability to perform chain elongation.

These results agreed with those exposed by Raes and co-workers (2017), who claimed that high ethanol to acetic acid ratios favored caproic acid production. On the other hand, low ethanol-to-acetic acid ratios favored butyric acid production, probably due to the lower amounts of available reducing equivalents, since butyric and caproic acid formation requires 20 and 32 electrons, respectively [28].

In addition, pH must be also considered a key operational parameter since fermenters with hydrogen availability (in addition to ethanol one) at pH 7.0 were capable to efficiently carry out chain elongation processes, whereas pH 5.5 was proven to be inadequate for the employed mixed culture to drive these reactions. Results differed from the ones reported in previous studies [33,34,40], which examined the production of elongated compounds under different conditions. Besides, the employed microbial community could play a key role. On the other hand, recent studies reached high concentrations and production rates of elongated compounds under pH around 7.0 [15,30,42]. The product spectrum obtained in T5 and T7 were similar, despite of different obtained concentrations. However, they had a different available amount of reducing power in form of ethanol (130 vs. 43 mM C, respectively).

In our experiments, butyric acid was produced from acetic acid and ethanol (Eq. (7)). Those substrates were subsequently involved in

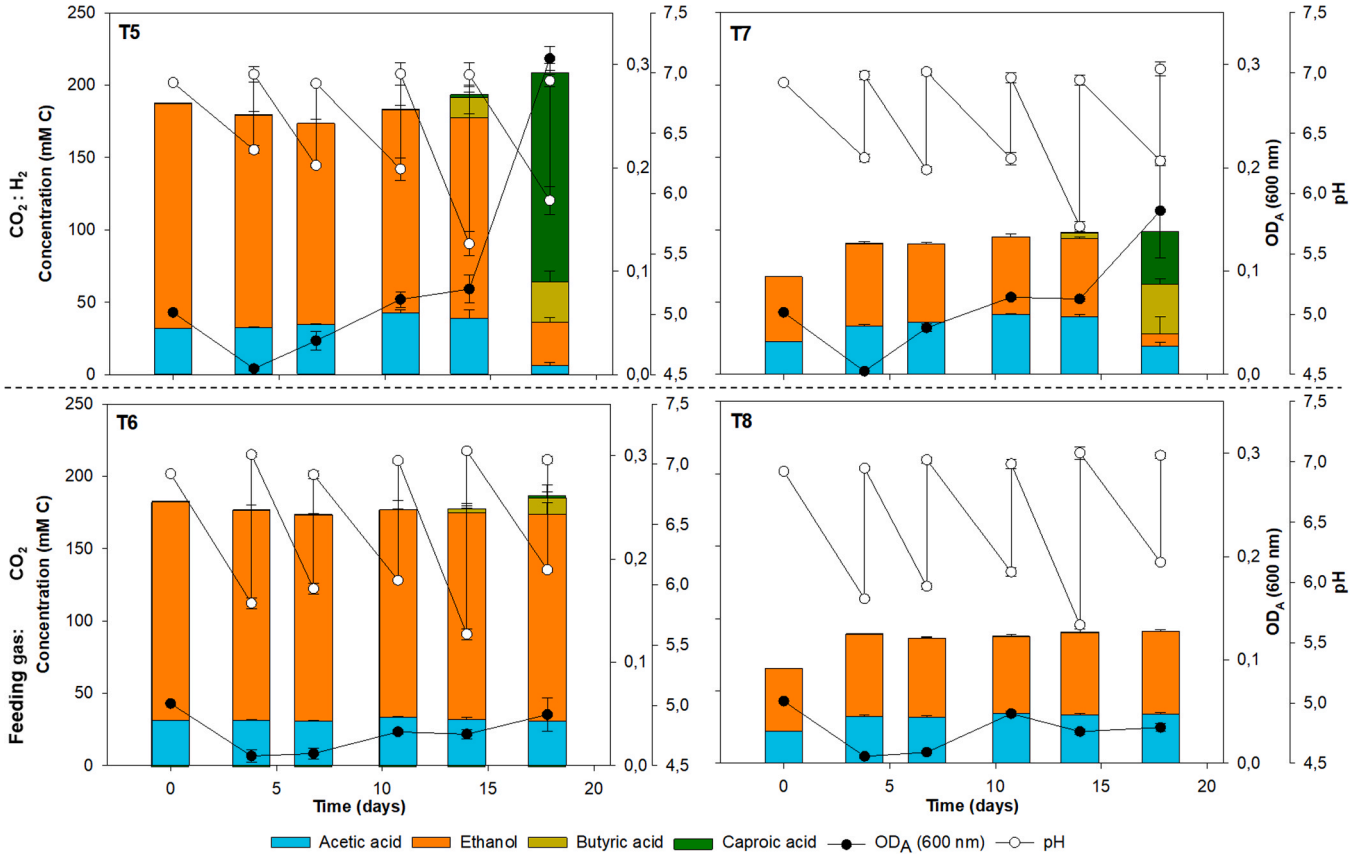

Fig. 4. Progression of pH, OD and VFAs and ethanol in tests 5–8 (T5–8) operated at pH 7.0, along the whole operation period. All test data corresponds to the average and standard deviation of the results of the three operated replicates. T5 and T7 were fed with a mixture of CO_2: H_2 while the rest had CO_2 as feeding gas. In respect of the initial substrate ratio (EtOH : HAc), T1 and T2 hold a 3:1 while the rest were been provided with a 1:1.

caproic acid formation as well (Eq. (8)) [10]. Therefore, only accumulated butyric that was not used for further elongation reactions was quantified.

T5 showed butyric and caproic acid production, which resulted in concentrations of 27.85 ± 7.53 and 144.94 ± 6.41 mM C, respectively. The correspondent specific production rates were 34.39 and 353.66 mmol C·g DCW_{Ai}^{-1}·d^{-1}, respectively. Concentrations of 33.88 ± 4.02 and 44.99 ± 0.41 mM C butyric and caproic acid were detected in T7. Their analogous specific production rates were 193.99 and 289.52 mmol C g DCW_{Ai} $^{-1}$ d^{-1}, respectively. Butyric production rate was 6-fold higher in T7 compared to T5 while caproic acid production rates were similar. These results suggested a limitation of reducing power in the form of ethanol in T7, to boost caproic acid production [32]. In addition, conditions in T5 promoted the growth of microbes performing chain elongation instead of their activity. Moreover, visual differences between tests were reported (Figure S4). A reddish coloration was noticeable in fermenters where caproic acid was produced (T5 and T7), in agreement with Angenent and co-workers [3]. In case of T5, higher caproic acid production was linked to the higher ethanol availability as substrate.

Meanwhile, assuming the chain elongation takes place according to Eq. (7) and Eq. (8), the total butyric acid produced can be calculated based on stoichiometry. These calculations resulted in butyric acid production rates of 388.06 and 483.48 mmol C·g DCW_{Ai} $^{-1}$ d^{-1} for T5 and T7, respectively. However, other feasible metabolic routes involved in butyric and caproic acid production, such as the direct ethanol-elongation to caproic acid might explain its consumption [10].

$$CH_3CH_2OH + CH_3COO^- \rightarrow CH_3(CH_2)_2COO^- + H_2O \quad \Delta G^o_{25} - 38{\cdot}62 \text{ kJ mol}^{-1} \tag{7}$$

$$CH_3CH_2OH + CH_3(CH_2)_2COO^- \rightarrow CH_3(CH_2)_4COO^- + H_2O \quad \Delta G^o_{25} - 38{\cdot}74 \text{ kJ mol}^{-1} \tag{8}$$

Taking into account all the presented results, the reason for the lack of chain elongation in the bioelectrochemical step due to the absence of key enzymes, was discarded since it showed up in the fermentation step. A possible explanation for this was that the reverse β-oxidation metabolic pathway was suppressed due to the working METs conditions were not suitable for the culture, as proved by tests T1-T4. Albeit, the culture itself was able to carry it out under more favorable conditions as suggested T5-T8.

3.3. Evaluation of the overall process

An evaluation of CO_2 conversion efficiency into organic compounds was performed in an overall combined approach. The first step of the process suggested favorable conditions to trigger acetogenesis and solventogenesis. It was noticed that low pH (< 6.0) enhanced solventogenesis over acid production. Nonetheless, slight changes in the pH during operation are likely to improve production ratios. Besides, high hydrogen partial pressure was another key parameter to carry out these target reactions in METs. Despite of all, systems were able to obtain an equimolar concentration of acetic acid and ethanol (C_2 compounds).

Table 3 summarizes added and produced carbon compounds (in milligrams of carbon; mg C) and the step conversion efficiency of the combined two processes. Since only R2 MET effluent was employed to carry out the fermentative stage, this reactor was the only considered for the calculations. Hence, considering only R2 MET, fed with CO_2 and operated with a pH around 5.6 the carbon conversion efficiency resulted in a 46 ± 6%, where 28 ± 4% corresponded to ethanol and 18 ± 2% to acetic acid. Acetic acid, ethanol and CO_2 sources are accounted for the added carbon calculations in the elongation step. Regarding stage 2 production, calculations were performed on basis of the carbon converted into products, excluding sources (remaining acetate, ethanol and CO_2). The second stage, at pH 7 and CO_2:H_2 feeding were tuned to perform chain elongation and obtain elongated acids (i.e. butyric and caproic acid) from the outlet of the previous step (bioelectrochemical). T5 showed an overall carbon conversion of 97 ± 2.9% (butyric 15.6 ± 0.4% and caproic acid 81.4 ± 2.5%). T7 converted 83 ± 0.3% of the total carbon fed, corresponding 33 ± 0.1% and 50 ± 0.2% to butyric and caproic acid, respectively. These differences might be explained by the higher available reducing power in T5 compared to T7.

Table 3
Added and produced carbon compounds (mg C) and CO_2 conversion efficiencies into products of each stage.

		Added (mg C)	Production (mg C)	Efficiency (%)
Stage 1 (MET)		300.2	138.1	46 ± 6
Stage 2 (Elongation)	T5	106.2	103.8	97 ± 2.9
	T7	54.14	44.90	83 ± 0.3

The overall CO_2 conversion efficiency into products was also calculated from T7 results since it used all the ethanol produced by METs (ratio 1:1). Here the total carbon conversion efficiency obtained was 38 ± 0.1%.

According to the data provided in Tables 3, 1 kg of CO_2 fed in the system resulted in the production of 0.38 kg of elongated acids (C_4-C_6). In other words, 1 m^3 of CO_2 (normal conditions) captured resulted in the production of 0.90 kg C_{C2} in the first place and 0.75 kg $C_{C4\text{-}C6}$ in the final step.

In terms of energy consumption of the whole process, considering the electricity consumed by the bioelectrochemical step with the average cell voltage were 6.28 ± 1.11 V and 9.82 ± 1.17 V in R1 and R2, respectively. R1 energy requirements were 0.70 ± 0.65 W h mg HAc^{-1}, 2.71 ± 1.54 W h mg $EtOH^{-1}$ and 20.94 ± 34.35 W h mmol H_2^{-1} while R2 energy requirements were 3.11 ± 1.53 W h mg HAc^{-1}, 6.45 ± 5.08 W h mg $EtOH^{-1}$ and 8.57 ± 3.07 W h mmol H_2^{-1}.

3.4. Implications

The production of elongated acids produced from a wide variety of feedstocks and by means of many different systems has been largely investigated. Its production in fermentation reactors fed by renewable carbon sources is therefore very interesting from the point of view of climate change mitigation. In case of caproic acid, it is industrially produced through various petrochemical and chemical based processes [39]. This C_6 carboxylate can be obtained through fractional distillation of coconut and palm oils, although the process is inefficient [31]. Table 4 summarizes the current state-of-the-art of this technology. Most studies employ organic matter as feedstock given that it can be directly oxidized to target compounds (such as butyric and caproic acid). Although all studies showed very promising yields, great importance should be given to the CO_2 capture capability in a context where decarbonized economy together with gas CO_2-streams usage is taking preeminence.

Related to the current context, further research has focused on the employment of streams containing CO_2 as feedstocks. Vasudevan and colleagues [37] studied syngas stream (synthesis gas, 65% carbon monoxide – CO –, 5% CO_2 and 30% H_2) and ethanol, playing the role of substrates, as potential feedstock with promising butyric and caproic acids productions. Steinbusch et al. [34] reported high caproic and caprylic acid production in anaerobic fermentation using granular sludge as inoculum and high initial concentrations of acetic acid and ethanol (as feedstocks) and H_2 (as reducing power). Later on, some two-step processes were proposed, thus reinforcing the proceedings by complementation. Two examples were exposed by Liew et al. [23] and Vasudevan and co-workers [37] who put forward an acidogenic step followed by an anaerobic filter. The upswing may be due to the addition of yeast extract and/or CO, able to act as electron donor which enhances product biosynthesis [37].

Later on, research has been geared towards the usage of inorganic carbon as feedstock, aiming at bio-electro CO_2 recycling. Some studies with similar approaches to reduce CO_2 into elongated compounds had

Table 4
Comparison between several anaerobic chain elongation studies using different technologies.

Substrate	System	Products (acids)	Maximum Production rate (g $L^{-1}d^{-1}$)	Maximum concentration (g L^{-1})	Initial pH	T°(°C)	Inoculum	Comments	Study
Unprocessed fermentation broth from brewery	ASBR and IE	Caproic	2.01*	2.1	5.5	30	Mixed culture	Liq-liq extraction	[1]
Sludge fermentation liquid and ethanol	Two stage process	Caproic	0.461*	0.845*	6.75	35 ± 1	Granular sludge from a WWTP	Yeast extract added	[38]
Leached of OFMSW and ethanol	Two stage process	Butyric Caproic Caprylic	n.r n.r n.r	3.2 12.6 0.4	6.5–7.0	30	Mixed culture	Continuous mode	[16]
Syngas, ethanol and bicarbonate	Two stage process	Butyric Caproic	20 1.7	n.r 1	5.5	30	Pre-adapted mixed culture, from [1]	> 50% *Clostridium* spp. Yeast extract added.	[37]
Acetic acid, ethanol and hydrogen	Fed-batch reactor	Caproic Caprylic	0.496* 0.053*	8.27 0.32	7.0	30	Granular sludge from brewery wastewater	Additional ethanol whenever was necessary	[34]
Electrons, CO_2	Continuous METs operation	Acetic Butyric Caproic	1.2 ± 0.3 0.6 ± 0.2 0.20 ± 0.09	16 ± 0.8 8.2 ± 0.8 2.4 ± 0.5	5.8	32	Natural environments and engineered anaerobically WWTP systems	Current density − 100.8 $A{\cdot}m^{-2}$MET, single chamber.	[21]
Electrons and ethanol	METs	Acetic Isobutyric Butyric Caproic	0.11* 0.04* 0.07* 0.04*	2.9 ± 0.2 1.6 3.1 1.2	Ac and CE: 6.9 So: 4.9	35	*Clostridium* spp.	2 separated cathodes, 1 anode	[36]
CO_2, electrons, H_2 from METs, Ethanol	Two stage process	Butyric Caproic	0.19 0.74	0.78 2.87	5.5 and 7.0	25	Mixed culture	MET followed by flask fermenters	This Study

* Calculated from reported results. ASBR: Anaerobic Sequential Batch Reactor, IE: *In-line* Extraction, OFMSW: Organic Fraction of Municipal Solid Waste; n.r: not reported; Ac: Acetogenesis; So: Solventogenesis; CE: Chain elongation; WWTP: WasteWater Treatment Plant. Two stage process means systems were divided into two separated steps.

been reported previously. Recently, Jourdin et al., [21] suggested coupling both processes in a single METs chamber. In addition, Vassilev and co-workers [36] proposed to carry out each reaction in two separated chambers. However, both reported low C_4 and C_6 production rates working at 32ºC and 35 ºC, respectively, apart from long start-up periods. Vassilev and colleagues [36] operated the system for 200 days at 35 ºC, reaching butyric and caproic acid concentrations of 3.1 and 1.2 g L^{-1} respectively. Their corresponding production rates were 0.07 and 0.04 g L^{-1} d^{-1}. The study also reports the production of other elongated compounds, so then the production selectivity was not considered. Jourdin and co-workers [21] evaluated operational parameters to enhance the conversion towards C_4 and C_6 achieving 39.40 ± 6.40 and 24.20 ± 3.60% C respectively. They work at 32 ºC, therefore they required energy to heat the system [21].

To the best of our knowledge, this was the first time the suggested configuration has been proposed. Furthermore, it is also postulated to reuse the unemployed H_2 produced over the first step in the second stage operation. In the present study, C_2 production was physically separated from C_4 and C_6 biosynthesis to complement and reinforce each step with a synergistic effect. Therefore, accumulated product selectivities in T5 (3-to-1 ethanol-to-acetic acid ratio) were 8.86% and 91.14%C in C_4 and C_6 respectively in T7 (1:1 C_2 ratio) were 40.12% and 59.88%C for C_4 and C_6 respectively. Similar approaches aiming at bio-electro CO_2 recycling into high-valued organic compounds had been carried out [21,24,35] although different drawbacks were pointed out.

4. Conclusions

The present study proposes a CO_2 recycling platform based on two steps, where the bio-electro reduction of CO_2 into acetic acid and ethanol at a 1-to-1 ratio is linked to a subsequent elongation step to produce valuable organic compounds (butyric and caproic). pH and employed feeding gas were exposed as key operational factors. Moreover, H_2 and ethanol availability were fundamental in chain elongation processes, boosting the elongation and permitting product selectivity into more reduced compounds. Setting a pH value of 7.0 and the use of CO_2:H_2 as feeding gas favoured the elongation processes and therefore more reduced compounds were obtained. In this sense, the bio-electro CO_2 recycling step was the limiting step. The integrated two step process was capable to produce 0.38 kg of elongated acids (C_4-C_6) from 1 kg of CO_2 fed in the system.

CRediT authorship contribution statement

The manuscript was written through the contributions of all authors. **M. Romans-Casas**: Conceptualization, Data curation, Investigation, Methodology, Writing - original draft; **R. Blasco-Gómez**: Investigation, Data curation, Writing – review & editing; **J. Colprim**: Writing – review & editing; **M.D. Balaguer**: Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing; **S. Puig**: Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing. All authors have given approval to the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2021.105909.

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