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Electrofermentation and redox mediators enhance glucose conversion into butyric acid with mixed microbial cultures

Paola Paiano, Miriam Menini, Marco Zeppilli, Mauro Majone, Marianna Villano*

Department of Chemistry, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome (Italy)

*Corresponding Author: Marianna Villano

(E-mail: marianna.villano@uniroma1.it; Tel: +30 06 49913651)

ABSTRACT

Electrofermentation (EF) is an emerging and promising technology consisting in the use of a polarized electrode to control the spectrum of products deriving from anaerobic bioprocesses. Here, the effect of electrode polarization on the fermentation of glucose has been studied with two mixed microbial cultures (MMC) both in absence and in presence of exogenous redox mediators. In unmediated experiments, the main effect of EF was an increase in the yield of butyric acid production, with respect to open circuit control experiments, provided that glucose was consumed along with its own fermentation products (i.e. acetic acid and ethanol). This effect occurred with both MMC, even though to a different extent, when the cathode was polarized at -700 mV *vs.* the Standard Hydrogen Electrode (SHE) and the maximum obtained yield accounted for 0.60 mol mol^{-1}. Mediated experiments were performed with Neutral Red or AQDS at a concentration of 500 μM both in absence and in presence of the electrode polarized at -700 mV or -300 mV *vs*. SHE, respectively. Importantly, with mediators butyric acid production also occurred in absence of externally available glucose fermentation products suggesting that both Neutral Red and AQDS were able to replace glucose in supplying microbial cells with the reducing power needed to drive the condensation of acetate and ethanol into butyric acid. Overall, mediators showed a high selectivity towards the generation of n-butyric acid isomer, whose production yields resulted higher with AQDS than with Neutral Red.

KEYWORDS:

Electrofermentation; Mixed-microbial culture; Butyric acid production; Biomass conversion; Redox mediators

1. INTRODUCTION

Acidogenic fermentation (AF) is an anaerobic biotechnological process in which a consortium of microorganisms cooperatively transforms complex organic substrates, including waste materials (e.g., agro-industrial wastes), into a mixture of simpler molecules called volatile fatty acids (VFA), which include acetic, propionic, butyric, valeric and hexanoic acids [1]. These molecules have a substantial commercial interest since they are excellent chemical precursors for the production of higher-value chemicals such as polyhydroxyalkanoates, biofuels, bio-based solvents, as well as medium chain fatty acids [2]. Over the last years, the ever-increasing attention towards the implementation of a bio-based economy has rapidly spurred the scientific interest towards the development of sustainable bioprocesses targeting the production of VFA from renewable resources [3,4]. However, in spite of the substantial economical value of VFA, acidogenic fermentation is not yet developed as a stand-alone technology at the industrial scale, mostly due to difficulties in implementing long-term operational strategies which allow tuning and regulating the product spectrum (i.e., the composition of produced VFA), particularly when waste organic substrates of undefined composition are used as feedstock materials and mixed-microbial cultures (MMC) are employed as biocatalysts [5,6]. Indeed, to date, acidogenic fermentation is mainly exploited as one of the key steps of the anaerobic digestion (AD), a well-established bioprocess, widely implemented at the industrial scale, whereby biogas (i.e., a gaseous mixture rich in methane and carbon dioxide) is ultimately generated from the treatment of an organic waste by means of at least four interdependent phases (hydrolysis, acidogenesis, acetogenesis and methanogenesis) [7].

In the context of AD, VFA are intermediate metabolites whose carbon is subsequently converted into the most oxidized (CO_2) and reduced (CH_4) forms. To allow for VFA accumulation, which have a higher economic value compared to biogas, methanogenesis needs to be impaired. To this aim, a number of strategies have been proposed. These

include, as an example, operating the system at low pH values (e.g., 4,5 – 7), at low temperatures (e.g., 5 - 20°C), at low solids retention times (SRT) (e.g., < 8 days) and/or at high organic loading rates (OLR) (e.g., >10 gCOD Ld^{-1}), with all of these strategies typically favoring the accumulation of VFA over methane [8-10]. Besides preventing VFA conversion into methane, MMC-based acidogenic fermentation of organic wastes is also challenged by the lack of practical strategies which allow predicting and manipulating, in a reproducible manner, the distribution of VFA produced, an aspect which is critical to the performance and sustainability of downstream applications. This mainly occurs due to a lack of understanding on how operational factors ultimately affect the microbial community/functionality, and hence the product spectrum [11]. By far, pH is the most widely investigated operational process parameter to regulate product distribution during fermentations, both using pure and mixed microbial cultures, although, highly controversial results have been typically reported in the literature [12-14]. Additionally, in the case of MMC-fermentations a factor which further complicates predicting the effect of pH, is the fact that this parameter may not only affect the biochemical pathway of fermentation but also the composition of the microbial community [15].

In recent studies, a novel bioelectrochemical approach, termed "electro-fermentation" has been proposed to manipulate product distribution in MMC-based fermentations [16-20]. In brief, electro-fermentation relies on the possibility to influence metabolic patterns by either supplying electrons to the culture, which are in turn used as co-reducing agents during fermentative conversion of organic substrates, or by modifying the oxidation–reduction potential (ORP) of the reaction medium with a polarized electrode, so as to affect the intracellular reduced/oxidized NAD balance which, in turn, affects the fermentation pathways triggering towards specific end-products [21,22]. In a previous electro-fermentation study [23], a polarized (i.e., -700 mV *vs.* Standard Hydrogen Electrode, SHE) electrode substantially (i.e., 20-fold) enhanced the yield of (iso)butyric acid production

during the MMC-based anaerobic fermentation of a ternary mixture of glucose, ethanol, and acetic acid. In another study [24], the yield of 1,3-propanediol production from (waste) glycerol using an MMC-process was also enhanced by the presence of a polarized (i.e., in the range from -600 mV to -900 mV *vs.* SHE) electrode. Similar results were also reported in a more recent investigation [25], whereby the spectrum of glucose fermentation products by a mixed culture could be successfully tuned by introducing a polarized (i.e., from -800 mV to 0 mV *vs.* SHE) electrode within the fermentation reactor.

Despite these promising results, however, very limited information is still available on the factors and conditions ultimately affecting the efficacy of electro-fermentation processes. Particularly, it is still unknown to what extent the effect of electrode polarization depends on the composition of the substrate and of the source microbial culture. Furthermore, it is unclear whether the observed alteration of the fermentation pathway is exclusively due to the ORP change triggered by the polarized electrode, or whether the latter may serve directly or indirectly (e.g., via diffusible redox mediators) as an additional source of reducing power to microorganisms, or also whether both mechanisms simultaneously occur.

In this context, the aim of the present study was twofold: (i.) analyze the impact of electro-fermentation on the distribution of glucose fermentation products using mixed microbial cultures; (ii.) verify whether the fermentative metabolism by the used cultures could be influenced by the presence of soluble redox mediators such as AQDS (anthraquinone-2,6-disulfonate) and Neutral Red (3-amino-7- dimethylamino-2-methyllphenazine).

2. EXPERIMENTAL SECTION

2.1 Inocula

An anaerobic sludge deriving from a mesophilic pilot-scale anaerobic digester placed in Treviso (Italy) and fed with the organic fraction of solid municipal wastes was used as inoculum of all batch tests. Particularly, the anaerobic sludge was collected from the digester twice, approximately in correspondence of spring and winter, and these two inocula are differentiated throughout the manuscript and referred to as MMC-1 and MMC-2. Once collected, the anaerobic sludge was settled to remove most of the residual organic substrates contained in the supernatant which was replaced with the anaerobic mineral medium, whose composition is reported elsewhere [23]. In order to avoid the growth of methanogens, competitors for investigated substrates, the medium pH was buffered at 5,5. Prior to being used, the microbial cultures were kept in borosilcate glass bottles (with a liquid volume of 0,5 L) thermostated at 25°C under anaerobic conditions which were guaranteed by flushing the liquid and gaseous phase with a N_2/CO_2 (70:30 v/v) gas mixture. MMCs were characterized in terms of biomass concentration, measured as volatile suspended solid (VSS), which accounted for about 5 g L^{-1}.

2.2 Reactoristic configuration

Two- chamber (H-type) cells were used to perform all the tests herein described. The cells consisted of two gastight borosilicate glass bottles (each with a total volume of about 270 mL) representative of the anodic and the cathodic compartment, respectively; and separated by a Nafion® 117 proton exchange membrane (PEM) with a 3cm^2 cross-sectional area. The PEM was previously treated by means of boiling in H_2O_2 (3% v/v) for 2 h, followed by boiling in deionized water for 2 h, successively in 0,5M H_2SO_4 for 2 h, and finally again in deionized water for 2h. A graphite rod electrode (10cm length, 5mm

diameter, Sigma-Aldrich, Italy) equipped with a titanium wire (0,81mm, Sigma-Aldrich, Milano, Italy) was placed in each compartment of the cells, functioning as the working (cathode) or the counter (anode) electrode. A KCl saturated Ag/AgCl reference electrode (+199mV *vs.* the Standard Hydrogen Electrode, SHE) was also placed in the cathode compartment. The titanium wire allowed electrodes connections to a potentiostat (Ivium Technologies, Eindhoven, The Netherlands) for the electrochemical measurements.

Throughout the manuscript, all voltages are reported with respect to SHE.

2.3 (Electro)-Fermentation batch experiments

Mixed culture glucose fermentation has been herein studied both in presence and in absence of electrode polarization. To accomplish this objective, a set of batch experiments was performed with the cathode (working electrode) compartment of each cell inoculated with anaerobic sludge to a final biomass concentration of 0,2 g L^{-1} (measured as volatile suspended solids). Glucose was used as sole carbon source and it was twice supplied at the cathode compartment, initially (day 0) at a theoretical concentration of 5 mmol L^{-1} and subsequently (day 11) at a theoretical concentration of 2,5 mmol L^{-1}. Along with glucose, anaerobic mineral medium [23] was also added at the cells cathode whereas the anode compartment was only filled with mineral medium. The liquid phase of each compartment was magnetically stirred and accounted for 180 mL. Prior to start each experiment, anaerobic conditions were guaranteed by flushing both the liquid and the gaseous phase with a N_2/CO_2 (70:30 v/v) gas mixture. Unmediated electrofermentation (EF) tests were performed with the cathode electrode controlled at -700 mV by means of a potentiostat (Ivium Technologies, Eindhoven, The Netherlands). Unmediated open circuit (OC) tests were performed as control experiments in absence of electrode polarization and a digital multimeter (Keithley Instruments, Cleveland, OH) was used to periodically measure the value of the cathode potential.

Mediated batch experiments were carried out in presence of two exogenous redox mediators in order to evaluate their effect on MMC-glucose fermentation both in presence and in absence of electrode polarization. Particularly, the used mediators were anthraquinone-2,6-disulfonate (AQDS, $E^{o'}$= -0,185 V) and 3-amino-7- dimethylamino-2-methyllphenazine (Neutral Red, $E^{o'}$ = -0,325 V). Mediators were added at the cathode compartment of the cells at a final concentration of 500 μM. Mediated EF experiments were performed in presence of cathode polarization and AQDS or NR as redox mediator, whereas mediated OC experiments were carried out in absence of polarization but in presence of the redox mediator. Based on cyclic voltammetry tests, the potential of the cathode electrode was controlled at -700 mV when Neutral Red was used as mediator and at -300 mV when AQDS was used as mediator. In the mediated EF experiments, the cathode electrode was polarized overnight prior to inoculate the cathode compartment of the cells, in order to ensure mediators reduction. In both EF- and OC- mediated tests glucose was once added at the beginning of the experiments at a theoretical concentration of 5mmol L^{-1}.

Throughout the study, for each tested condition two parallel experiments (i.e. EF and OC tests) were simultaneously started.

2.4 Analytical methods

The concentration of microorganisms, reported as VSS, was determined according to the standard method APHA [26]. Daily measurements of liquid filtered (0,22μm porosity) samples were carried out for substrate and fermentation products determination. More in detail, glucose concentration was analysed according to the Sulfuric Acid - UV method [27] while VFAs (acetic, propionic, isobutyric and butyric acids) and ethanol were analysed by injecting 1μL of aqueous sample into a Dani Master (Milan, Italy) gas-chromatograph (2m x 2mm glass column packed with Carbopack, He carrier gas at 25mL min^{-1}, oven

temperature of 175°C; flame ionization detector (FID) temperature of 200°C). Gas-phase of each cell was sampled with a gas-tight Hamilton syringe for hydrogen determination by injecting 500 μL of the gaseous sample into a Dani Master (Milan, Italy) gas-chromatograph (stainless-steel column packed with molecular sieve, Supelco; N_2 carrier gas at 60,9 mL min^{-1}; oven temperature at 70°C; thermal conductivity detector (TCD) temperature 150°C). Methane was also analysed by injecting 100μL of sample headspace (with a gas-tight Hamilton syringe) into a Varian (Lake Forest, CA, USA) 3400 gas chromatograph (GC; 2m x 2mm glass column packed with 60/80 mesh Carbopack B/ 1% SP-1000, Supelco; N_2 carrier gas at 18mL min^{-1}; oven temperature at 50°C; FID temperature 260°C) [28, 29]. Headspace concentrations were converted to aqueous-phase concentrations using tabulated Henry's law constants [30].

Cyclic voltammetry tests were carried out at the beginning and at the end of each experiment, in a potential range from -1,1 to +0,2 V with a scan rate of 5 mV s^{-1}, in order to determine possible interactions between microorganisms and the electrode as well as to identify the value of the potential to apply to the cathode for the mediated experiments.

3. RESULTS AND DISCUSSION

3.1 Unmediated (electro)fermentation batch experiments

The alteration in the metabolic pathway of two mixed microbial cultures (MMC), here referred to as MMC-1 and MMC-2, has been evaluated by potentiostatically controlling the cathode potential of a two-chamber BES at -700 mV during glucose fermentation. Interestingly, the two mixed microbial cultures displayed a very similar glucose fermentation pattern, with no significant differences being observed in terms of glucose utilization and accumulation of its main fermentation products, namely acetate, ethanol,

and hydrogen. Accordingly, Figure 1 shows the time course of the above-mentioned parameters, in both electro-fermentation (EF) and open circuit (OC) tests, as the mean value of the two different cultures, which were thus regarded to as replicates. During the experiments, two successive glucose pulses were carried out, the first one (on day 0) of about 5 mmol L^{-1} and the second one (on day 11) of about 2,5 mmol L^{-1}. As shown in Figure 1A, following the first pulse, glucose was rapidly and completely consumed, without any initial lag phase, in approximately 3 days, resulting in the production of acetic acid (nearly 2 mmol L^{-1}), ethanol (nearly 2,8 mmol L^{-1}), and hydrogen (nearly 3 mmol L^{-1}). Subsequently, the concentration of these fermentation products remained almost stable until day 11, when a second pulse of glucose was added to the cathodic compartment of the cells. Apparently, during the first phase of the experiment (days 0-11), no appreciable differences were observed between the OC and the EF tests. The second pulse of glucose, resulted in a further production of acetate, as well as in the consumption of ethanol and hydrogen. Specifically, by the end of the experiment, slightly more acetic acid (Figure 1B) was ultimately produced in the OC control compared to the EF test (9,8 ± 0,57 vs. 8,3 ± 1,1 mmol L^{-1}). As far as ethanol is concerned (Figure 1C), a more extensive consumption was observed in the EF experiment compared to the OC test. By contrast, no differences were noticed for hydrogen, which was almost completely consumed by the end of the test both in the OC and the EF experiments.

Regardless of the electrode polarization, a negligible formation of methane was observed in all experiments, most probably as a consequence of the pH of the mineral medium which was buffered at 5,6, a value which is known to inhibit the growth and metabolism of methanogens.

FIGURE 1

Besides the fermentation products previously described, also i-butyric and n-butyric acids were formed during glucose fermentation, with trends, however, which differed for the two mixed microbial cultures. For this reason, the concentrations of C4 fermentation products are separately reported in Figure 2 for tests conducted using MMC-1 and MMC-2. Specifically, as for MMC-1, the production of both i-butyric and n-butyric acids started only after the second pulse of glucose, possibly indicating that butyric acid (i- and n- isomers) was not produced directly from glucose rather derived from acetic acid and ethanol via reverse β-oxidation. By the end of the experiment, the production of i-butyric acid accounted for around 0,30 mmol L^{-1}, with no significant differences between the EF and OC treatments (Figure 2A). Interestingly, n-butyric acid also started to accumulate after the second glucose pulse and its production was found to be remarkably enhanced by the presence of the electrode polarization, resulting in a 4-fold higher concentration in the EF test relative to the OC test by the end of the experiment (0,8 vs. 0,2 mmol L^{-1}, respectively).

Qualitatively, very similar trends for C4 acids were obtained also with MMC-2, the only difference with MMC-1 being the relative concentrations. Indeed, also in this case, i-butyric acid was produced only after the second pulse of glucose, though it accumulated at lower concentration levels (up to about 0,09 mmol L^{-1}) with respect to MMC-1 (Figure 2C), whereas the production of n-butyric acid was substantially higher in MMC-2 (Figure 2D). Interestingly, in the latter case, n-butyric acid was already detected from day 9 with a slightly higher accumulation in the EF test compared to the control experiment (2,8 vs. 2,1 mmol L^{-1}, respectively). Furthermore, in correspondence to the second pulse of glucose, the concentration of n-butyric acid remained nearly constant until the end of the experiment in absence of polarization, whereas it significantly increased in the EF test. By the end of the test, the amount of n-butyric acid produced in the EF test was around 1,5-fold higher than that measured in the OC test (4,5 vs. 2,8 mmol L^{-1}, respectively), hence

providing a further confirmation of the positive effect of electrode polarization on the production of n-butyric acid with both MMC-1 and MMC-2.

FIGURE 2

At the end of the fermentation experiments, a mass balance (in terms of COD, chemical oxygen demand) indicated that, in the case of MMC-2, all the removed glucose was nearly stoichiometrically recovered into the measured fermentation products both in the EF (93%) and OC (90%) tests. However, in the case of MMC-1, approximately 83% and 73% of the glucose supplied in the EF and OC tests was recovered into fermentation products, respectively; possibly indicating a higher percentage of newly formed biomass and /or that other yet unidentified (e.g., lactic acid) products were formed during glucose fermentation. Indeed, due to the several microbial pathways involved, other compounds were probably formed and, among them, lactic acid has been reported as one of the most common products deriving from MMC- driven glucose conversion [31] and it probably represents the highest fraction of unidentified products. The obtained differences in terms of mass recovery and distribution of fermentation products are most probably related to a different microbial composition. Indeed, as reported by Sravan and colleagues [32], the presence of a polarized electrode during the EF of food waste induced changes in the microbial culture composition, creating an enrichment of fermentative bacteria, mostly belonging to genus Clostridia, known not only for their high VFA production but also as electroactive bacteria [33].

Even though Figure 2 clearly shows that the profiles of C4 products for the two mixed microbial cultures are not coinciding, the observed trends clearly point to an enhanced production of butyric acid (primarily the n-isomer) in the presence of electrode polarization, especially when glucose is present simultaneously with its main fermentation products

(i.e., acetic acid and ethanol). This latter finding is consistent with the fact that the condensation of acetate and ethanol to produce n-butyric acid via reverse β-oxidation involves NADH-dependent steps (e.g., the reduction of Acetoacetyl CoA to Hydroxybutyryl CoA and reduction of Crotonyl CoA to Butyryl CoA) that are typically fuelled by glucose, and that, in principle, can be promoted by altering the intracellular $NADH/NAD^+$ ratio during the fermentation process. Overall, these findings are consistent with the results reported in our recent paper [23], whereby it was found that a polarized cathode triggered a nearly 20-fold increase (relative to open circuit controls) in the yield of butyrate production during the anaerobic fermentation of a ternary mixture of substrates, consisting of glucose, acetic acid and ethanol.

Throughout the EF and OC tests, the potential of the working electrode was measured on a daily basis (Figure 3A). As expected, the cathode potential of the EF tests remained stable at the set value of -700 mV, while during the OC control tests, it gradually decreased from 0 to -200 mV. Figure 3B shows the time course of the electric current flowing in the circuit of the EF tests, as the average value of the two mixed cultures. The (cathodic) current rapidly increased from approximately -21 ± 4 μA to nearly -5 ± 2 μA during the first day of the test and then remained nearly constant around this value throughout the remainder of the experiments.

FIGURE 3

3.2 Mediated (electro)fermentation batch experiments

Bioelectrochemical batch experiments were carried out with MMC-1 and MMC-2 in order to explore the viability of using soluble redox mediators (i.e., NR and AQDS), in

combination with an electron supply through a polarized cathode, to enhance the production of C4 during glucose fermentation in MMC-1 and MMC-2.

Prior to the start of the experiments, cyclic voltammetry tests were carried out in order to characterize the redox behavior of the two mediators under actual fermentation conditions. These cyclic voltammograms were recorded in the presence of the microbial culture, but in the absence of the substrate. The voltammogram recorded in the presence of NR (Figure 4A) displayed two main reduction peaks, centered at around -500 mV and -760 mV, in agreement with the previously reported multiple redox states of NR. Along this line, a previous study [34] has pointed out that the NR redox state, which is capable to reduce NAD^+ to NADH, is generated at the most reducing potential. Hence, for this reason a potential of -700 mV was chosen and applied to the cathode for all the electrofermentation tests involving the use of NR. This potential would be sufficiently negative to allow the reduction of NR to the desired redox state, without resulting in the abiotic (purely electrochemical) generation of molecular hydrogen. As for AQDS, the voltammogram (Figure 4B) displayed, as expected, one single redox process, linked to the reduction of quinone to hydroquinone, centered at around -300 mV. Accordingly, a potential of -300 mV was applied to the working electrode, throughout the electrofermentation tests employing this redox mediator.

FIGURE 4

Figure 5 shows the time course of glucose fermentation with NR, in the presence and in the absence of the electrode polarization, as an average value of results obtained with MMC-1 and MMC-2. Analogously to the unmediated experiments, the concentration profiles of i- and n-butyric acids are presented separately for MMC-1 and MMC-2 in Figure 6. Both in the presence and in the absence of polarization, glucose was almost completely

depleted in around 5 days. Similarly, to what observed during unmediated experiments (Figure 1), glucose consumption (5 mmol L^{-1}) was linked to the formation of acetate (up to a maximum concentration of approximately 4 mmol L^{-1}), ethanol (up to a maximum concentration of approximately 3 mmol L^{-1}), and hydrogen (up to a maximum concentration of approximately 3,6 mmol L^{-1}). As for these fermentation products, no statistically relevant differences were noticed between EF and OC tests. The most striking difference with unmediated experiments was, however, observed for n-butyric acid (no i-butyric acid was formed in mediated experiments), as shown in Figure 6. To start with, in the presence of NR, n-butyrate was produced from the condensation of glucose fermentation products (e.g., acetate, ethanol), also in the absence of externally available glucose. This likely suggests that electrically-reduced NR could replace glucose in supplying cells with the reducing power needed to fuel the condensation of acetate and ethanol into butyric acid. Furthermore, also in the presence of NR, n-butyric acid production was greatly enhanced by the presence of the electrode polarization, relative to the OC experiments. As an example, on day 10, the concentration of n-butyric acid in the EF experiment was nearly 8-times higher (1,93 mmol L^{-1} vs. 0,25 mmol L^{-1}, as mean values of both cultures) than in the OC experiment. Interestingly, also in the OC experiment n-butyric acid gradually accumulated over the course of the experiment. This finding possibly indicates that, even in the absence of electrode polarization, NR was biologically or chemically reduced by the microorganisms and/or by the reduced fermentation products (e.g., H_2), though probably to a lower extent and rate than in the presence of electrode polarization. Finally, it is also worth noting that in all the NR-mediated experiments negligible production of the i-isomer was noticed. These results are consistent with what reported in other studies [35], whereby the electrofermentation of sucrose by a pure culture of *Clostridium tyrobutyricum* showed an increased butyrate concentration (from 5 to 8,8 g L^{-1}) and yield (from 0,33 up to 0,44 g g^{-1}) with respect to an

open circuit control test. The authors hypothesized that electrically-reduced Neutral Red may have served as an additional source of reducing power for the production of the extra butyrate.

FIGURE 5

FIGURE 6

Figure 7 shows the time course of glucose fermentation with AQDS, in the presence and in the absence of the electrode polarization, as an average value of results obtained with MMC-1 and MMC-2. The concentration profiles of i-, and n-butyric acids are presented separately for MMC-1 and MMC-2 in Figure 8. Glucose fermentation pattern in EF and OC experiments was very similar (Figure 7), most likely due to the fact that AQDS can be biologically or chemically reduced during glucose fermentation, even in the absence of electrode polarization. Analogously to NR, also in the presence of AQDS, glucose fermentation was accompanied by a substantial production of n-butyric acid, in marked contrast with what observed in unmediated experiments whereby butyric acid production occurred primarily when glucose was spiked in the presence of its own fermentation products. Particularly, with MMC-1 the positive effect of AQDS on butyric acid production (Figure 8) was boosted by the presence of the electrode polarization.

As for the previous experiments, in all mediated tests (with NR and AQDS) negligible methane production was observed.

FIGURE 7

FIGURE 8

3.3 Yields of butyric acid production in unmediated and mediated EF and OC tests

Figure 9 summarizes the effect of electrofermentation and of soluble redox mediators on the yield of (i- + n-)butyric acid production from glucose for MMC-1 and MMC-2. In the case of unmediated experiments, the cumulative amount of glucose consumed (upon the first and second spike) was accounted for. Even though, as mentioned previously, the two mixed cultures displayed slightly different (i- and n-)butyric acid production capabilities, it is apparent that under electrofermentative conditions the yield of butyric acid formation was remarkably enhanced. The highest yield of butyric acid production (0,6 mol mol^{-1}) was observed with MMC-2 in the absence of soluble redox mediators, but when glucose was supplied along with its own fermentation products. A very similar production yield (0,59 mol mol^{-1}) was observed with MMC-2 in the presence of AQDS, whereby, however, butyric acid production via condensation of acetate and ethanol proceeded also in the absence of externally available glucose.

Interestingly, for both cultures AQDS enhanced the yield of butyric acid production relative to the corresponding unamended treatment more than Neutral Red, in spite of its substantially higher standard redox potential. This possibly indicates that the two mediators have different modes of action and/or different specificities with respect to their capacity to interfere with the metabolism and/or redox state of microorganisms. The different effect of the two redox mediators could also relate to their chemical structure and in turn with their capability to interact with specific cellular components. Indeed, although they are both water-soluble, Neutral Red has a substantially higher octanol/water partition coefficient and therefore a higher tendency to interact with lipidic components of the cell membrane.

It is also worth of noting that,

FIGURE 9

4. CONCLUSIONS

Here, glucose electrofermentation has been studied with two mixed microbial cultures both in the absence and in the presence of redox mediators. Unmediated experiments showed an about 2-fold increase in the yield of butyric acid production in EF experiments with respect to OC experiments with both cultures (maximum obtained yield accounting for 0.60 mol mol^{-1} with MMC-2), provided that glucose was consumed in the presence of its own fermentation products (i.e., acetic acid and ethanol). However, in mediated experiments butyric acid production also occurred in absence of externally available glucose fermentation products suggesting that both Neutral Red and AQDS can replace glucose in supplying microbial cells with the reducing power needed to drive the condensation of acetate and ethanol into butyric acid. Importantly, while in absence of mediators both the i- and n- isomers of butyric acid were produced, in presence of mediators only the n-isomer was produced indicating a high selectivity of the used exogenous mediators towards specific compounds. The combined effect of mediators and electrode polarization was more evident in the presence of Neutral Red than of AQDS, even though the latter allowed gaining a higher yield of production which was substantially higher than that obtained with MMC-1 in unmediated electrofermentation test but with a double glucose spike (0,49 mol mol^{-1} vs. 0,15 mol mol^{-1}, respectively) and fully comparable to that obtained with MMC-2 in unmediated test (0,59 mol mol^{-1} vs. 0,60 mol mol^{-1}, respectively).

Overall, by also taking into account the variability of mixed culture fermentation processes, whose metabolism depends on so many factors, this study clearly highlights the possibility to use electrofermentation as a simple tool to control products distribution also in

combination with the use of exogenous redox mediators. However, the real effect of mediators both in presence and in absence of electrode polarization, needs to be further elucidated, also using more complex feedstoks.

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FIGURE LEGEND

Figure 1. Time course of profiles of glucose (A) and its main fermentation products, namely acetic acid (B), ethanol (C), and hydrogen (D) recorded during both electrofermentation and open circuit experiments. Black arrows indicate glucose addition. Error bars represent the standard deviation of batch experiments conducted with two different mixed microbial cultures

Figure 2. Time course of i-butyric and n-butyric acid profiles in both the electrofermentation and open circuit experiments performed with MMC-1 and MMC- 2. Black arrows indicate glucose addition

Figure 3. Time course of the profile of the working electrode potential during electrofermentation and open circuit tests (A) and current in the EF tests (B). Error bars represent the standard deviation of batch experiments conducted with the two different mixed microbial cultures

Figure 4. Profiles of a typical cyclic voltammetry (scan rate of 5mV s^{-1}) recorded at the beginning of the tests performed in presence of Neutral Red (A) or AQDS (B) as redox mediator

Figure 5. Time course of profiles of glucose (A) and its main fermentation products, namely acetic acid (B), ethanol (C), and hydrogen (D) recorded during both electrofermentation and open circuit experiments under Neutral Red-mediated conditions. Error bars represent the standard deviation of batch experiments conducted with two different mixed microbial cultures

Figure 6. Time courses of n-butyric acid profiles in both electrofermentation and open circuit experiments performed in presence of Neutral Red as an electron shuttle. Concentration trends for MMC-1 (A) and MMC- 2 (B) are reported separately

Figure 7. Time courses of profiles of glucose (A) and its main fermentation products, namely acetic acid (B), ethanol (C), and hydrogen (D) recorded during both electrofermentation and open circuit experiments under AQDS-mediated conditions. Error bars represent the standard deviation of batch experiments conducted with two different mixed microbial cultures

Figure 8. Time courses of n-butyric acid profiles in both electrofermentation and open circuit experiments performed in presence of AQDS as an electron shuttle. Concentration trends for MMC-1 (A) and MMC- 2 (B) are reported separately

Figure 9. Effect of electrofermentative conditions and redox mediators on the yield of (i- + n-)butyric acid production from cumulatively consumed glucose

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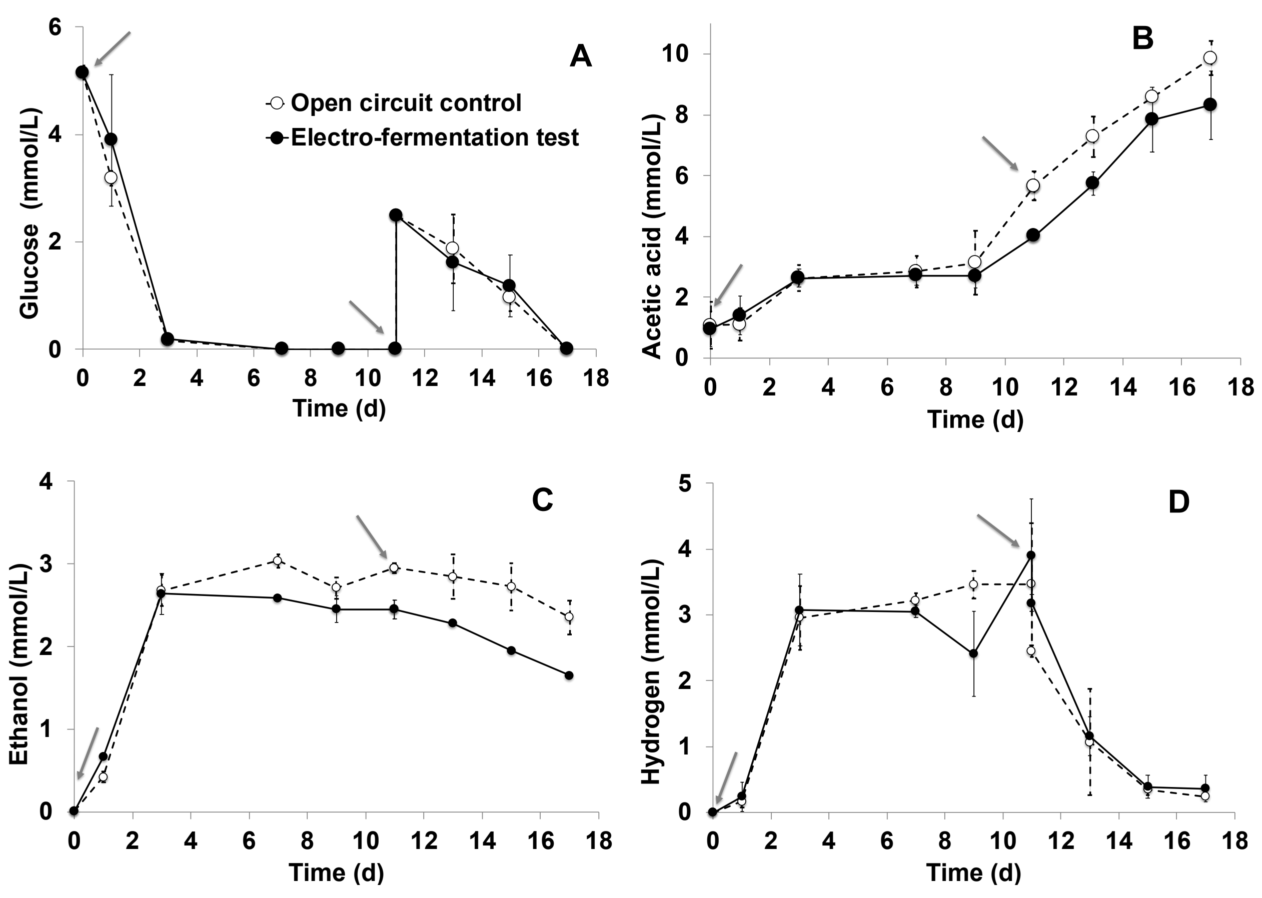

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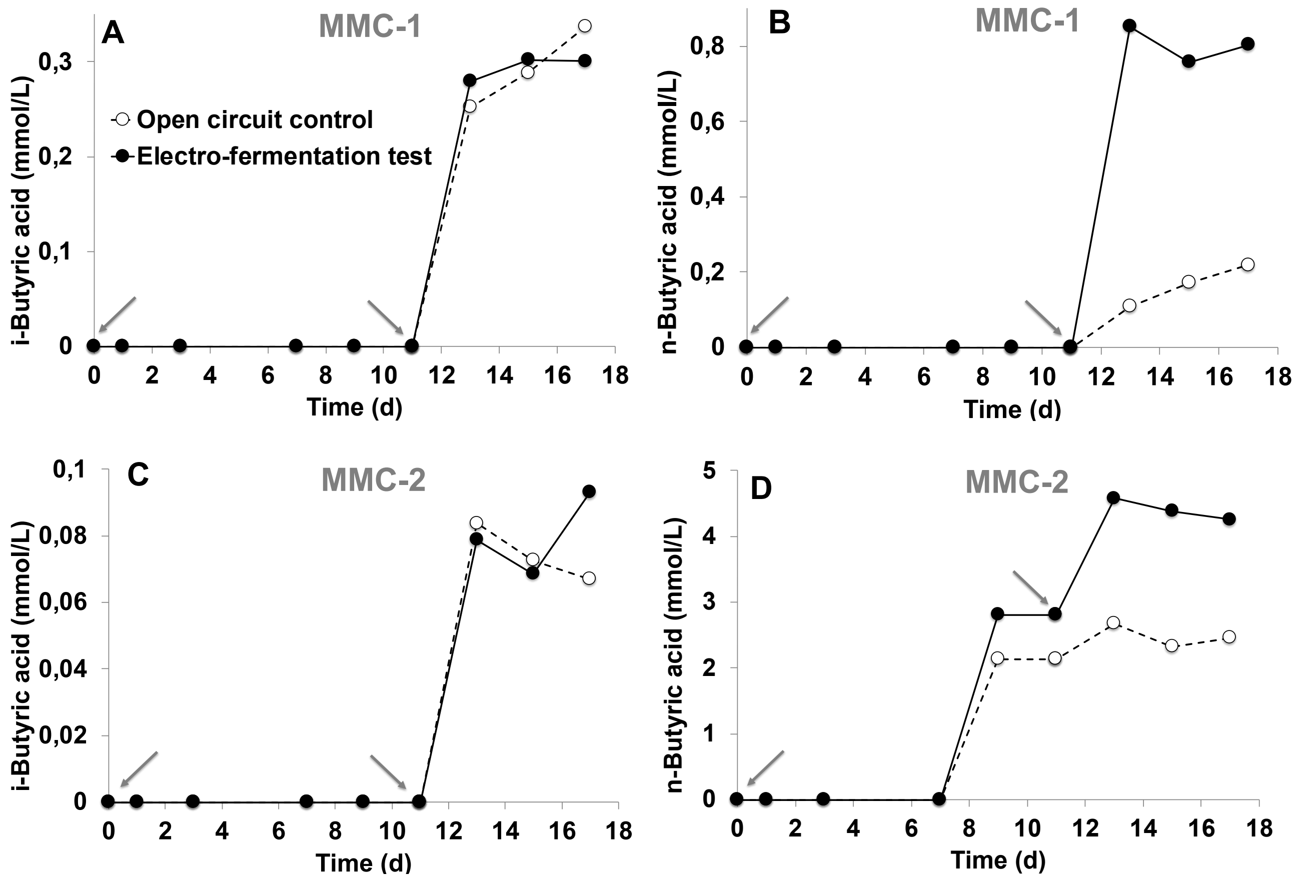

FIGURE 2

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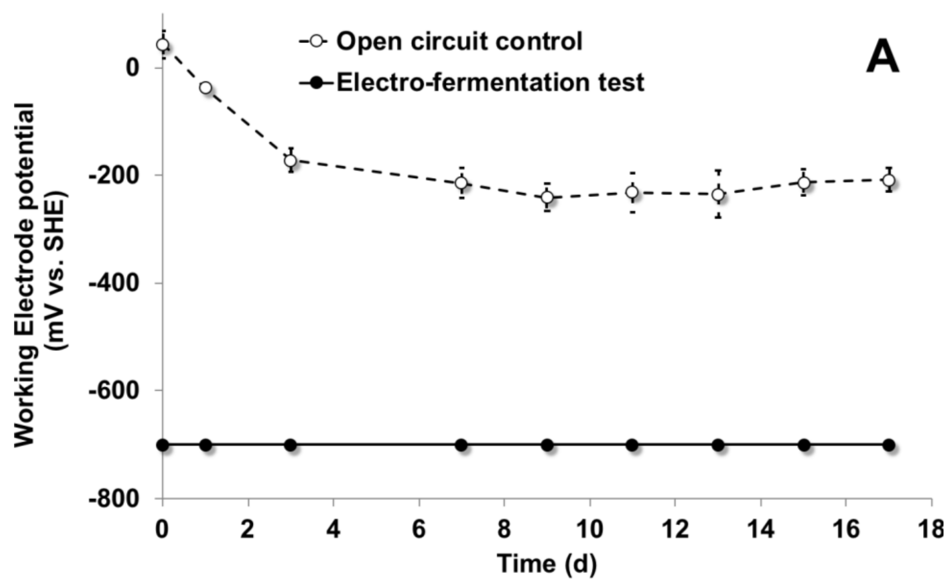

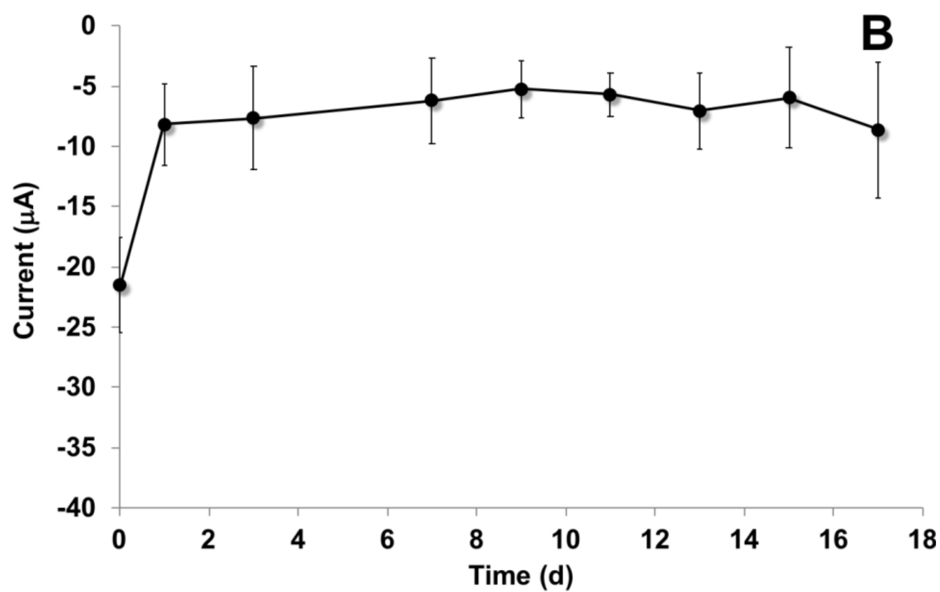

FIGURE 3

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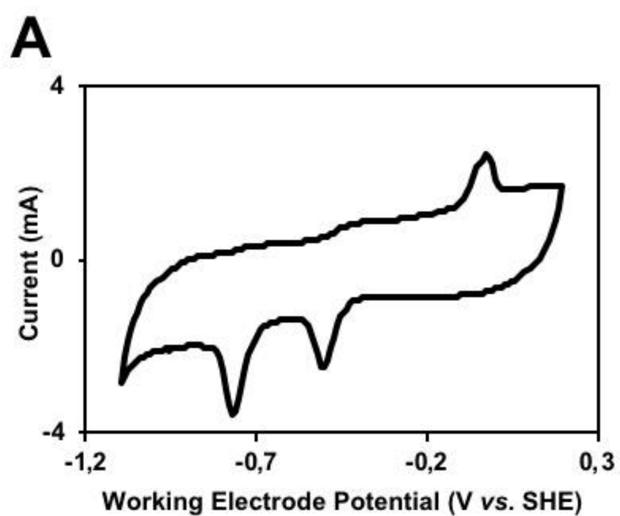

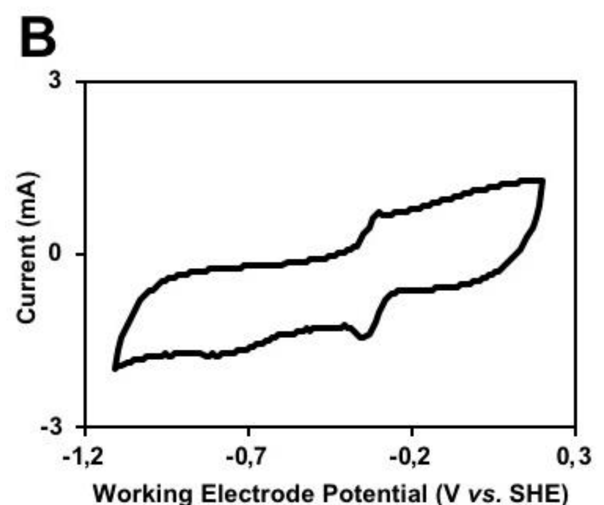

FIGURE 4

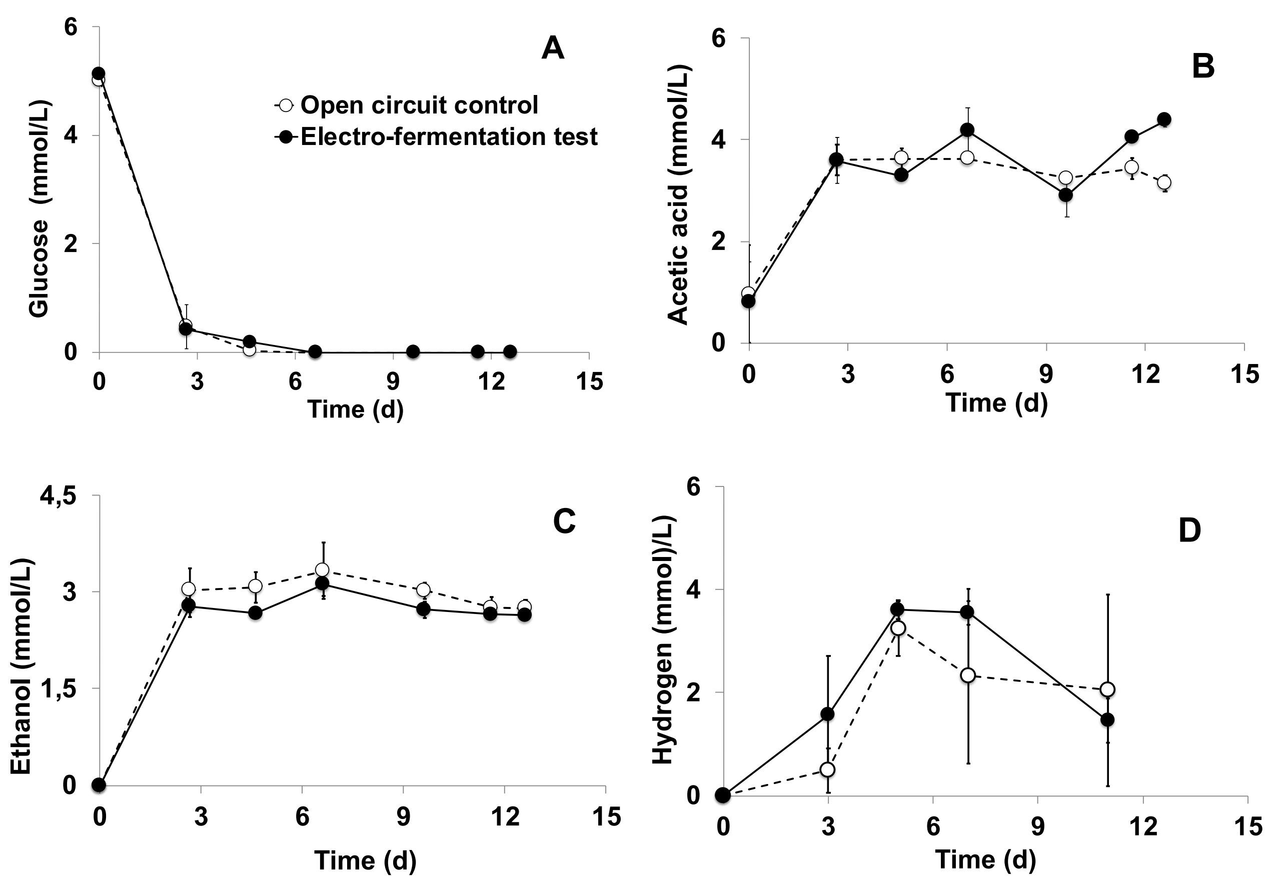

FIGURE 5

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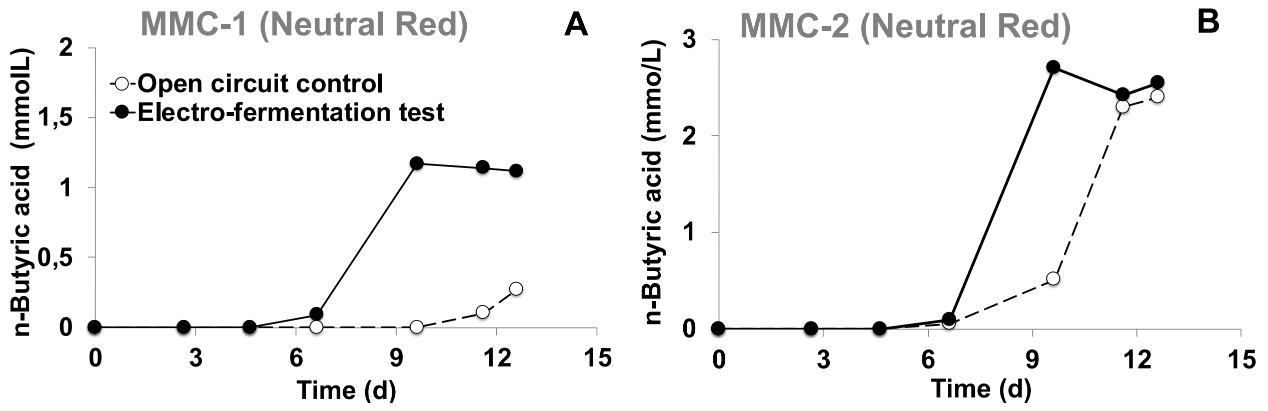

FIGURE 6

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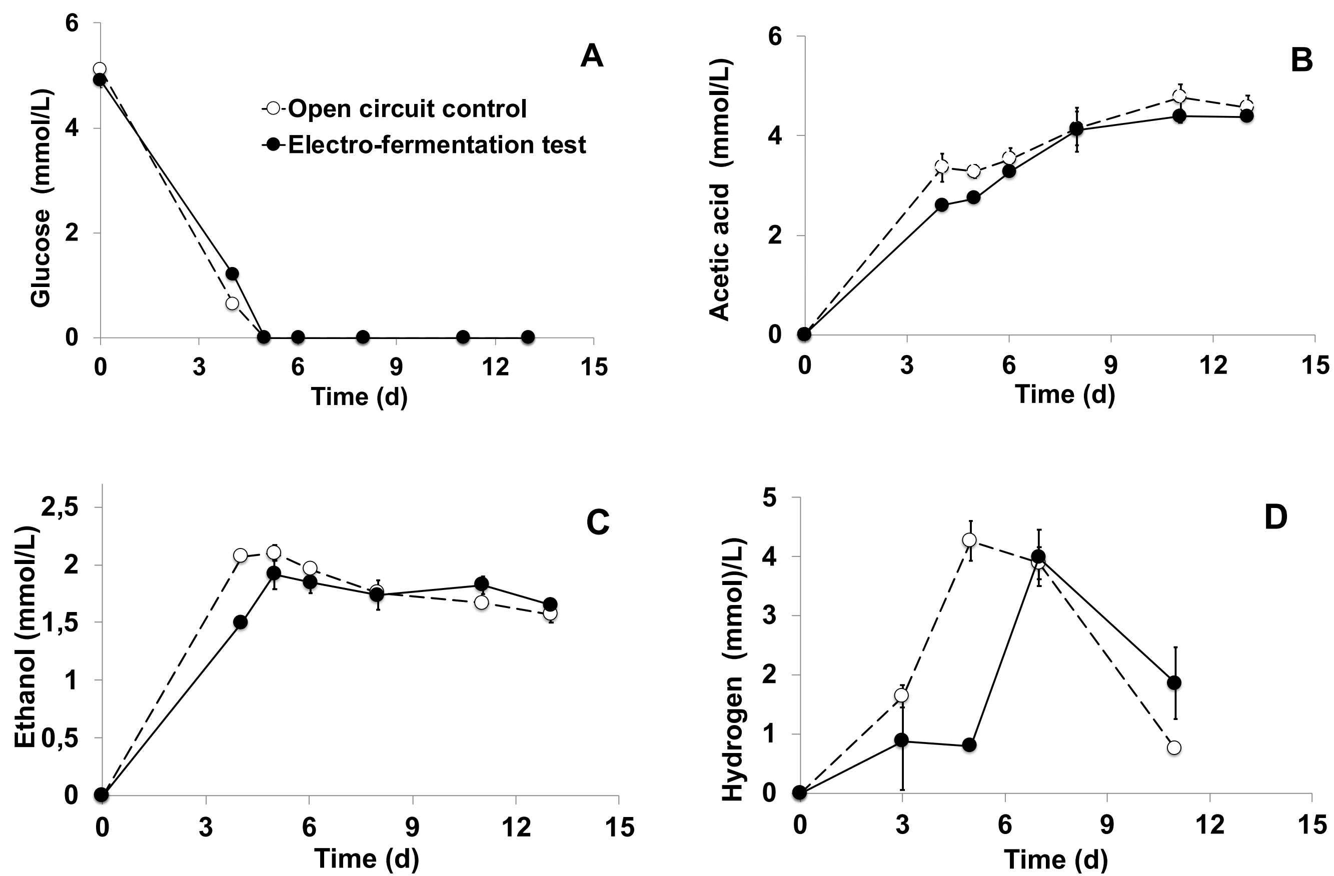

FIGURE 7

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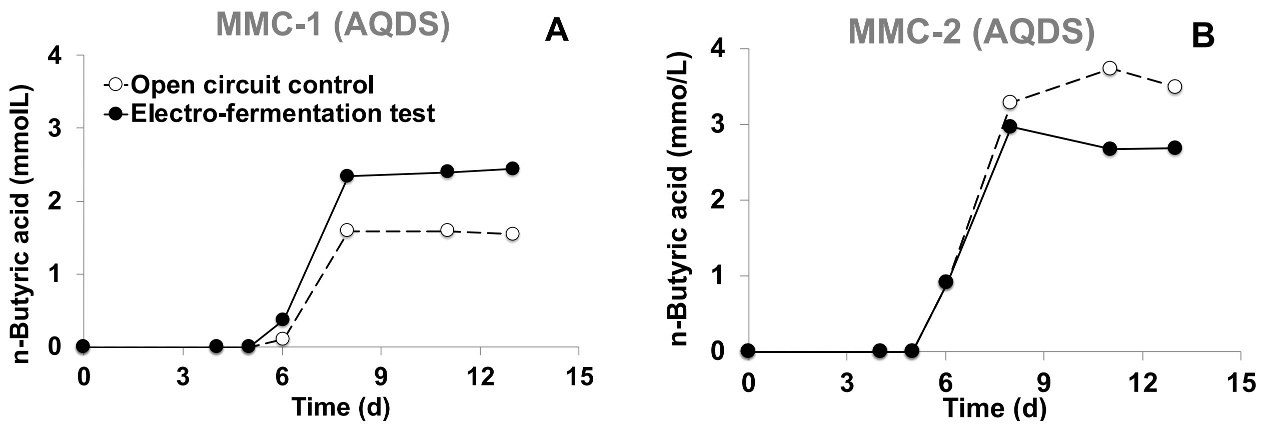

FIGURE 8

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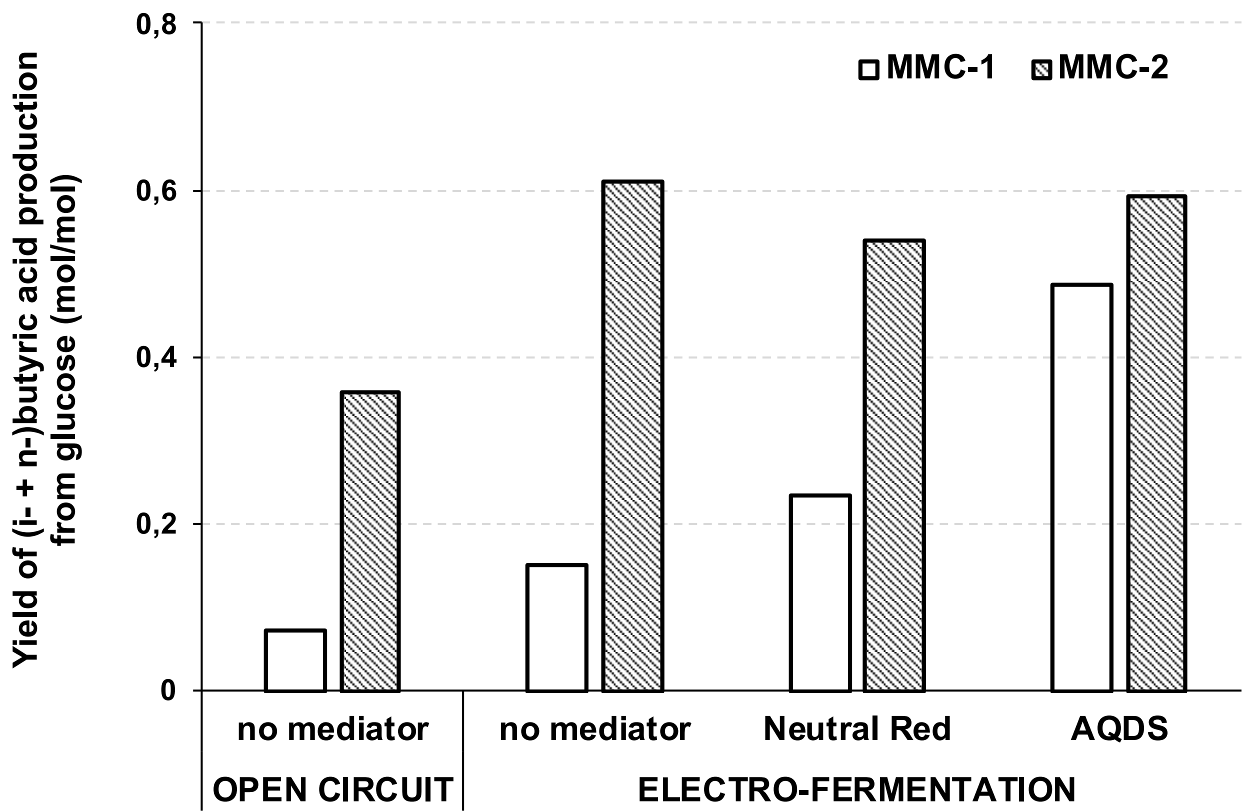

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