

Article

Bioelectrosynthetic Conversion of CO₂ Using Different Redox Mediators: Electron and Carbon Balances in a Bioelectrochemical System

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Abstract: Microbial electrosynthesis (MES) systems can convert CO₂ to acetate and other value-added chemicals using electricity as the reducing power. Several electrochemically active redox mediators can enhance interfacial electron transport between bacteria and the electrode in MES systems. In this study, different redox mediators, such as neutral red (NR), 2-hydroxy-1,4-naphthoquinone (HNQ), and hydroquinone (HQ), were compared to facilitate an MES-based CO₂ reduction reaction on the cathode. The mediators, NR and HNQ, improved acetate production from CO₂ (165 mM and 161 mM, respectively) compared to the control (without a mediator = 149 mM), whereas HQ showed lower acetate production (115 mM). On the other hand, when mediators were used, the electron and carbon recovery efficiency decreased because of the presence of bioelectrochemical reduction pathways other than acetate production. Cyclic voltammetry of an MES with such mediators revealed CO₂ reduction to acetate on the cathode surface. These results suggest that the addition of mediators to MES can improve CO₂ conversion to acetate with further optimization in an operating strategy of electrosynthesis processes.

Keywords: carbon dioxide; microbial electrosynthesis (MES); redox mediator; carbon and electron balance

1. Introduction

Global greenhouse gas emissions were estimated to be 3.38×10^4 million tons and increased by 2.0% in 2018 [1]. These emissions are expected to grow rapidly due to population and economic expansion in developing countries [2]. Efforts to reduce emissions have been made in industry and academia. Beyond emission reductions, the capture and reutilization of CO₂ have attracted considerable attention in the international research community. In nature, plants, algae, and photosynthetic microorganisms convert CO₂ to carbohydrates via photosynthesis using sunlight as the reducing power [3,4]. In addition to photosynthesis, chemoautotrophic and heterotrophic microorganisms use hydrogen, iron, and sulfur compounds as reducing reagents to metabolize and transform CO₂ to organic carbon and the building blocks of cells [4–6]. Yet these chemical reagents are expensive and unsustainable, and may not apply

to real processes. Therefore, a suitable strategy for providing the reducing power for the biological conversion of gaseous CO₂ is in strong demand [4,7].

Electrochemical reduction using noble metal catalysts [8,9] and carbon-based materials [10] has been examined as a means of converting CO₂ to value-added chemicals. Compared to electrochemical catalysts, biocatalysts have the advantages of cost-effectiveness, high specificity, and operation at room temperature and pressure. The whole cell-based microbial electrosynthesis (MES) system, which converts CO₂ to volatile fatty acids using electricity as the reducing power, has been highlighted [11–13]. The production of value-added chemicals from CO₂ through microorganisms has been studied extensively in many bioelectrochemical systems [14–18]. Electrosynthesis also provides a platform to utilize renewable electricity in bioprocesses and biotechnology and is an interesting topic for interdisciplinary research [19]. Compared to conventional chemical catalyst-based conversion, MES uses self-replicating bacteria as a catalyst at room temperature and pressure, allowing for a more cost-effective and environmentally friendly process. Rapidly developing synthetic biology and metabolic engineering can improve the electrochemically active strain further. Therefore, BES-based bioconversion is more cost-effective, allowing the efficient re-utilization and valorization of inorganic atmospheric carbon.

In MES, microorganisms exchange electrons directly or indirectly via electron shuttle molecules to metabolize CO₂ [14,20]. Several studies have reported that electrochemically active strains can obtain electrons directly from the electrode via a bacterial electron-transfer mechanism, and simultaneously produce methane, formate, and acetate from CO₂ [21,22]. Su et al. reported increased acetate production in an autotrophic mixed culture when a methanogen inhibitor was used in electrosynthesis [23]. In addition to simple organic acids, such as acetate, multi-carbon organics and longer chain fatty acids, can be produced through chain elongation [11–13]. Electrosynthesis processes were also used to produce ethanol, butanol, butyrate, and isopropanol from CO₂ [16,24].

On the other hand, electron-shuttle-based electron transfer in microbial fuel cells (MFCs) has been studied extensively to enhance electron transport to the electrode for electricity generation. Recently, a range of redox mediators, such as neutral red, methyl viologen, 2-hydroxy-1,4-naphthoquinone, and anthraquinone-2,6-disulfonate (AQDS), have been examined in MES to improve the efficiency and yield of CO₂ conversion [11,13,25–30]. The mechanisms and efficiencies of such mediators, however, have not been studied extensively in MESs. Moreover, previous studies on MESs were carried out in continuous CO₂ feeding mode to prevent negative pressures resulting from the conversion of gaseous CO₂ in the headspace to aqueous acetate, which might result in the undesirable introduction of oxygen into the reactor [24]. The ion exchange membrane-implemented BES (bioelectrochemical system) is particularly weak to such pressure drop processes in laboratory-scale reactors. While the conversion efficiency of CO₂ and electron/carbon balances in electrosynthesis could not be evaluated properly in a continuous operating mode, it is important information for further improving MES systems.

This study examines the MES process for acetate production from CO₂ with different redox mediators, such as 2-hydroxy-1,4-naphthoquinone (HNQ), neutral red (NR), and hydroquinone (HQ), which have different standard reduction potentials and chemical properties. The carbon and electron balance of acetate production and cell growth during electrosynthesis were examined in the batch mode operation of an MES system with periodic replenishment of headspace CO₂. The electrochemical characteristics of CO₂ reduction reactions on the cathode electrode were investigated using cyclic voltammetry.

2. Materials and Methods

2.1. Microbial Electrosynthesis Reactor Configuration and Operation

Two-chamber H-type microbial electrosynthesis reactors were constructed with a customized glass bottle, as described previously [13]. The anode and cathode chambers (both 350 mL and a working volume of 250 mL) were joined with a glass tube and separated using a cation exchange

membrane (Fumasep FKB-PK-130, Fumatech BWT GmbH, Germany). The cathode electrode was a 4 cm × 5 cm piece of carbon felt (Cera Materials, Amherst, NY, USA) connected by an electrode holder (Sanshe Semiconductor Equipment Co, Ltd., Shanghai, China). The cathode chamber was connected to four syringes to balance the internal pressure change to make up the positive/negative pressure caused by CO₂ electrosynthesis (see Figure S1). The internal pressure of the headspace was maintained at approximately 1 atm. The headspace of the cathode chamber was fully replenished every two days during the experiment. Graphite granules (30 g) were added to the anode chamber, and a 5 cm graphite rod connected to titanium wire was used as a current collector from the graphite granules. The reference electrode, Ag/AgCl (3M KCl), was placed in the cathode chamber.

The culture medium had the following composition (g/L): 3 g KH₂PO₄, 5.8 g K₂HPO₄, 4 g NaHCO₃, 0.5 g NH₄Cl, 0.09 g MgCl₂·6H₂O, 0.0225 g CaCl₂·2H₂O, 20 mL trace metal solution, and 20 mL vitamin solution (medium 141-DSMZ). The yeast extract (0.5 g) was implemented into the medium to enhance the acetogenic and electrosynthesis activity [31]. In addition, 5 mM of sodium 2-bromoethanesulfonate were added to inactivate the methanogenic activity.

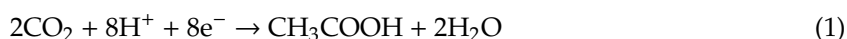
Then 10 mL of anaerobic digester sludge (Suyoung Wastewater Treatment Plant, Busan, Korea) were inoculated into 240 mL of media. Initially, the inoculated BES reactors were pre-operated over one week with only CO₂ to remove the organic matter in the anaerobic digester sludge. The cathode electrode was poised with −1.11 V vs. Ag/AgCl (3M KCl) using a potentiostat (WonAtech WMPG1000, Seoul, Korea). Ultrapure CO₂ (purity > 99.999%) was introduced into the cathode chamber as the sole carbon source. The experiments were carried out under anaerobic conditions in batch mode. The three redox mediators (neutral red, NR; 2-hydroxy-1,4-naphthoquinone, HNQ; hydroquinone, HQ) were added to the cathode chamber to facilitate electron transfer from the electrode to the bacteria. The batch process proceeded for eight cycles with periodic headspace gas replenishment every two days (total of 16 days). All experiments were carried out at 26 ± 2 °C.

2.2. Analysis of the Acetate Concentration and Gas Composition

Acetate production was analyzed using the following method. A liquid sample (1 mL) was collected from the cathode chamber and filtered through a syringe filter (0.22 µm, Shanghai Instrument Consumables Co., Shanghai, China). The filtered catholytes were analyzed by high-performance liquid chromatography (HPLC, Agilent 1100 series Agilent Technologies, Santa Clara, CA, USA) equipped with a 300 × 7.8 mm Aminex HPX-87H (Bio-Rad, Santa Clara, CA, USA) column at 65 °C, and a refractive index (RI) and photodiode array (PDA) detector. The mobile phase was 2.5 mM H₂SO₄ (flow rate = 0.5 mL/s). The cell concentration (OD₆₀₀) was measured using a UV–Vis spectrophotometer (Optizen POP QX, Mecasys Co., Ltd., Daejeon, Korea). Gas samples were taken with a 250 µL syringe and analyzed by gas chromatography (6500GC Agilent Technologies, Young Lin Instrument Co. Anyang, Korea) using a molecular sieve/porapak N column at an oven temperature of 48 °C. Detection was achieved using a flame ionization detector (FID) and a thermal conductivity detector (TCD). The injector, FID, and TCD temperatures were 150 °C, 250 °C, and 100 °C, respectively. The flow rate of the carrier gas (Ar) was 14 mL/min. All the samples were analyzed in triplicate.

2.3. Estimation of Electron Recovery and Carbon Balance and Electrochemical Analyses

Electron recovery was estimated from the ratio of coulombs consumed by the electrode, and the coulombs recovered into the acetate according to the following equations:



$$\text{Coulombs consumption} = \int_0^t I \, dt \quad (2)$$

$$\text{Coulombs recovered in the products} = n \times F \times m \quad (3)$$

where I , t , n , F , and m are the current consumption monitored by a potentiostat, time, moles of recovered electrons in the products, Faraday's constant (96,485 C/mole of e^-), and concentration of acetate (mol/L) produced during operation from 0 to t , respectively. The carbon balance was estimated by the ratio of CO_2 consumption at the cathode chamber and the carbon recovered in the products according to the ideal gas law.

$$PV = nRT \quad (4)$$

where n , P , V , R , and T are the moles (amount) of the gas, gas pressure of the cathode chamber, gas volume, ideal gas constant ($0.0821 \text{ atm L mol}^{-1} \text{ K}^{-1}$), and absolute temperature for the experiments, respectively. The carbon balance of the first cycle (i.e., first two days) was not estimated because the initially contained buffer component, $NaHCO_3$ (4 g/L), might have been counted in the carbon balance. This effect might decrease and become negligible through gas replenishment in the subsequent cycles.

2.4. Electrochemical and Microscopic Analyses of Electrosynthesis

Cyclic voltammetry (CV) analysis of electrosynthesis was carried out using a potentiostat (WMPG1000, WonA Tech, Seoul, Korea) with a range of -1.11 V to $+0.1 \text{ V}$ (vs. $Ag/AgCl$ 3M KCl) at a scan rate of 10 mV/s . The electrochemical characteristics of the electrosynthetic biocathode with and without a mediator were compared using CV. The morphological changes to the electrode surface by electrosynthesis were investigated by scanning electron microscopy (SEM, Vega II, TESCAN, Waltham, MA, USA). For protein analysis of the biomass attached to the electrode, the cathode was sliced into $1 \text{ cm} \times 1 \text{ cm}$ samples and rinsed gently three times with cold phosphate-buffered saline (PBS). The sampled electrode was placed into a 25 mL test tube with PBS and sonicated for 20 min (Power sonic 405, Hwashin Technology, Seoul, Korea) in a water bath at 4°C . All tubes containing the lysing matrix and above liquid were prechilled at 4°C . The tubes were then mixed and shaken in a lysis cell with a FastPrep-24™ 5G instrument at 6.0 m/s for 80 s . The supernatant was transferred to a new tube and centrifuged at $14,000 \text{ rpm}$ for 15 min at 4°C to remove any insoluble material remaining. The amount of protein in the carbon felt and the suspension was analyzed using the Bradford method [32].

3. Results and Discussion

3.1. Production of Acetate in MES with Different Redox Mediators

Figure 1 presents the level of acetate production under different redox mediators (0.1 mM). Acetate was produced continuously over eight cycles (two-day operation per cycle) of batch operation with full replenishment of the headspace gas every two days. The MES with the NR and HNQ mediators showed the highest level of acetate accumulation in comparison with the control without mediators, but the difference was insignificant, whereas the MES with HQ produced a relatively low acetate level. The total amounts of acetate produced during the eight cycles over 16 days with NR, HNQ, HQ, and without mediator were 165 mM (9.9 g/L), 161 mM (9.7 g/L), 115 mM (7.0 g/L), and 149 mM (8.9 g/L), respectively. The presence of redox mediators, such as NR and HNQ, appeared to facilitate electron transfer and simultaneously increase acetate production in the MES system. In contrast, HQ had a negative effect on electrosynthesis. Compared to the other mediators, HQ had a reduction potential ($+0.09 \text{ V}$ vs. Standard hydrogen electrode (SHE) at pH 7) that was lower than that of the conversion of CO_2 to acetate (-0.29 V vs. SHE pH 7) (Table 1).

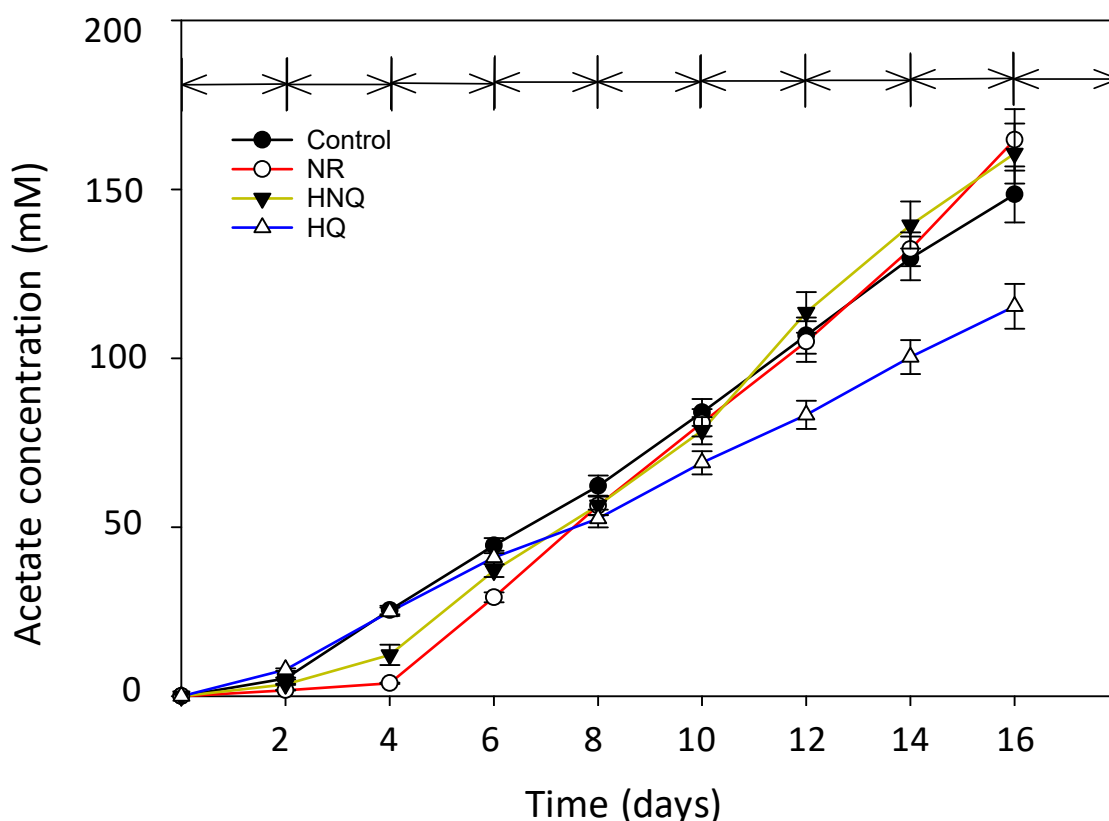


Figure 1. Acetate production during eight cycles of electrosynthesis using 0.1 mM of each redox mediator. The arrow indicates replenishment of the headspace gas in a batch operation. The potential (−1.11 V vs. Ag/AgCl (3M KCl)) was applied continuously to the cathode electrode using a potentiostat. NR: neutral red; HNQ: 2-hydroxy-1,4-naphthoquinone; HQ: hydroquinone.

Table 1. Standard reduction potential of possible cathode reaction and redox mediators at pH 7 [11,27,28,33–37].

Standard Reduction Potential	E° (V) vs. SHE	E° (V) vs. Ag/AgCl (3M KCl)
$2\text{CO}_2 + 8\text{H}^+ + 8\text{e}^- \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O}$	−0.29	−0.5
$\text{CO}_2 + 4\text{H}^+ + 4\text{e}^- \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	−0.24	−0.45
$2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$	−0.42	−0.63
NADH/NAD ⁺	−0.32	−0.53
neutral red ox/red	−0.33	−0.54
methylene blue ox/red	0.011	−0.2
anthraquinone-2,6-disulfonate ox/red	−0.18	−0.39
methyl viologen ox/red	−0.44	−0.65
2-hydroxy-1,4-naphthoquinone ox/red	−0.14	−0.35
hydroquinone ox/red	0.09	−0.12

When the mediator concentration was increased further to 0.5 and 1 mM, NR and HNQ exhibited slightly less acetate production than the operation with 0.1 mM: 99 mM (5.9 g/L) and 113 mM (6.8 g/L) with 0.5 and 1 mM NR, respectively, and 157 mM (9.4 g/L) and 138 mM (8.3 g/L) with 0.5 and 1 mM HNQ, respectively (Figure S2). HQ also produced less acetate (95 and 104 mM, respectively). The lower acetate production with increasing mediator concentration might be due to their toxicity and inhibitory effects on the microbial metabolism. Interestingly, the control without mediators presented comparable or even higher acetate production than those with mediators. With further increases in the mediator to 7 mM, acetate production by HQ was similar to the control (53 mM with HQ, and 51 mM with the control under four cycles) which was also similar to 0.1, 0.5, and 1 mM of the redox mediator.

In contrast, NR and HNQ showed significantly less acetate production (Figure S5A). With higher mediator concentrations, formate production was obtained with 7 mM of NR and HNQ (formate: 45 mM with NR, and 46 mM with HNQ) for eight cycles of operation (Figure S5B). Hydrogen gas production also increased significantly in the headspace of MES with 7 mM of NR and HNQ but was not quantified precisely due to the high volume of gas production (more than 200 mL per day). In abiotic control with mediators, the levels of acetate and hydrogen production were negligible, which indicates that the mediators do not transfer electrons to protons or CO₂ directly without bacteria (data not shown).

The molecular size of the mediators and redox potential, as well as the chemical characteristics, may influence the electron delivery efficiency significantly through the cellular membrane. Different mediators have been studied extensively in many microbial fuel cell systems [29,38–42], whereas less attention has been paid to microbial electrosynthesis for CO₂ conversion. A higher concentration of such mediators and concomitantly larger concentration gradient across the cell membrane may damage the cellular structure when they are transported through the membrane [43,44]. Compared to NR (molecular weight, 288.77) and HNQ (MW 174.15), the molecular weight of HQ is relatively small (MW 110.11). The penetration of HQ into the periplasmic space is easy, but it also acts as a respiratory inhibitor (i.e., uncoupler for the electron transport chain) for natural cell metabolism.

3.2. Estimation of the Electron and Carbon Recovery of Electrosynthesis

Figure 2 shows the electron recoveries estimated by the ratio of the number of electrons captured in acetate (Equation (3)), and the electron flow from the electrode (Equation (2)) of each cycle. Although the initial electron recoveries were low (14% to 59%), they increased continuously to between 72% and 84% with mediators. Without mediators, a relatively high efficiency of 81% was obtained (except HQ) (Figure 2). On the other hand, the cumulative amount of coulombs to acetate (i.e., acetate production in electrosynthesis) was higher with the mediators (except for HQ) than that without a mediator (control). Figure S3 shows the average electron recovery during eight cycles.

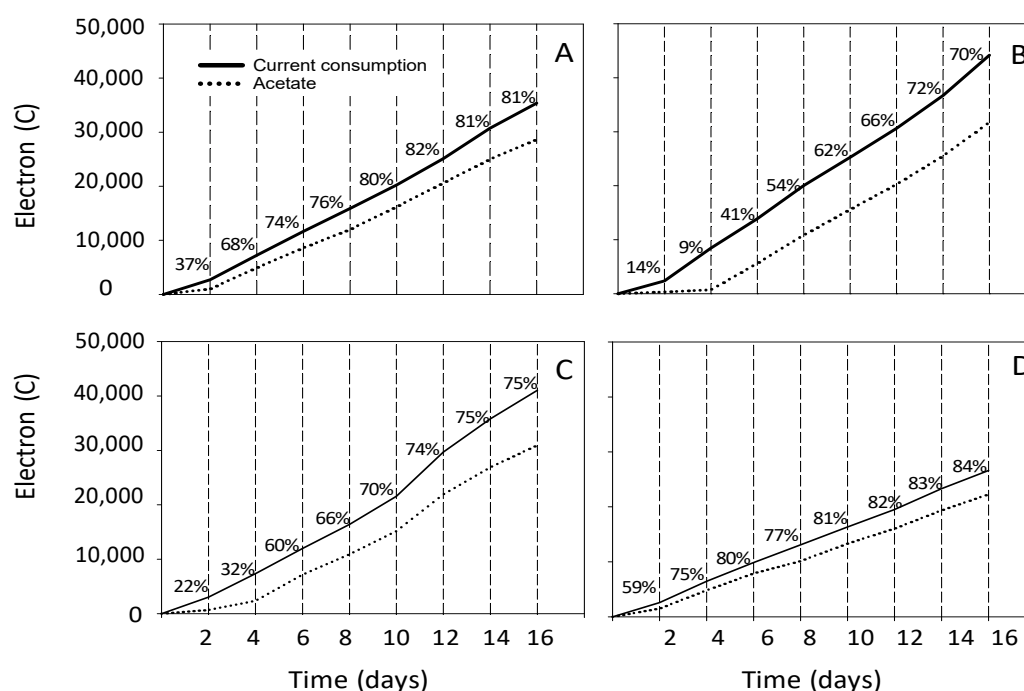


Figure 2. Electron recovery into acetate at 0.1 mM redox mediators. (A) Control, (B) NR, (C) HNQ, (D) HQ. Dotted line represents each cycle.

Figure 3 presents the carbon recovery of electrosynthetic CO₂ conversion, which shows the headspace CO₂ consumption vs. acetate production in each cycle. The headspace CO₂ was always maintained at approximately 1 atm using the installed syringes containing 100% CO₂. The headspace CO₂ was also balanced with the dissolved HCO₃[−] and CO₃^{2−} at neutral pH. When bacteria consume the inorganic carbon in solution, the headspace CO₂ immediately dissolves into the solution to maintain equilibrium. Therefore, the amount of headspace CO₂ dissolution is the same as the consumption of carbonate species in the solution by microbial electrosynthesis. The carbon balance was estimated by the decrease in headspace volume measured by syringes. The first cycles were not considered for the averaged estimation to eliminate the initial effects of NaHCO₃, which is contained as a carbonate buffer.

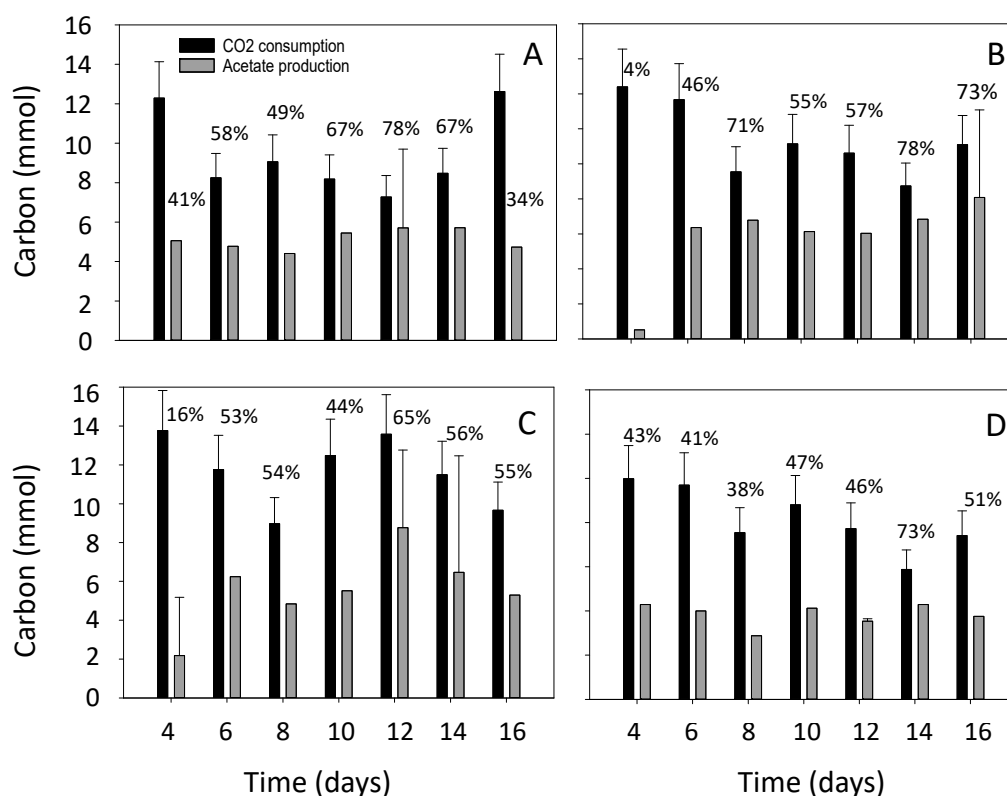


Figure 3. Carbon recovery to acetate at 0.1 mM redox mediators. The recovery was estimated from the second to eighth cycles of each replenishment. (A) Control, (B) NR, (C) HNQ, and (D) HQ. The carbon balance of the first cycle (i.e., first two days) was not included to remove the initial effects of bicarbonate in the medium.

Similar to electron recovery, the carbon recovery was also low in the initial period. As the headspace gas replenishment proceeded, however, it generally increased to more than 70% with NR, while the carbon recovery with HNQ and HQ was generally lower than the control and NR. These results suggest that the use of mediators may divert the carbon flux to metabolites other than acetate, which is in agreement with the results obtained from the electron balances, as shown in Figure 2. Based on eight cycles of operation, with increasing concentration of the redox mediator to 0.5 and 1 mM, the average carbon recovery showed a decreasing trend in efficiency (Figure S4). The leakage of electron delivery from the electrode to the inner membrane protein might also reduce the carbon and electron flux to acetate production [45].

On the other hand, MES involves the conversion of gaseous CO₂ to aqueous acetate. Therefore, an accurate estimation of the balance is generally difficult because of the difficulty in controlling the headspace gas pressure as well as the inexact sampling and analytical error, as shown in the enlarged error range in Figure 3. The presence of redox mediators appeared to produce more

gaseous hydrogen than the control (Figure 4). The average electrons accumulated by hydrogen was estimated: without a mediator (1.8%), NR (11%), HNQ (9.6%), and HQ (0.5%). This suggests that the presence of NR and HNQ resulted in a more diverse electrosynthetic pathway, including hydrogen formation. The cell growth with different mediators was relatively insignificant during 16 days of electrosynthesis (see Figure S6). The estimated carbon and electron recovered into the cell mass in microbial electrosynthesis were also significantly lower (approximately 2–4%) than those in acetate production (Tables S1 and S2) [46,47].

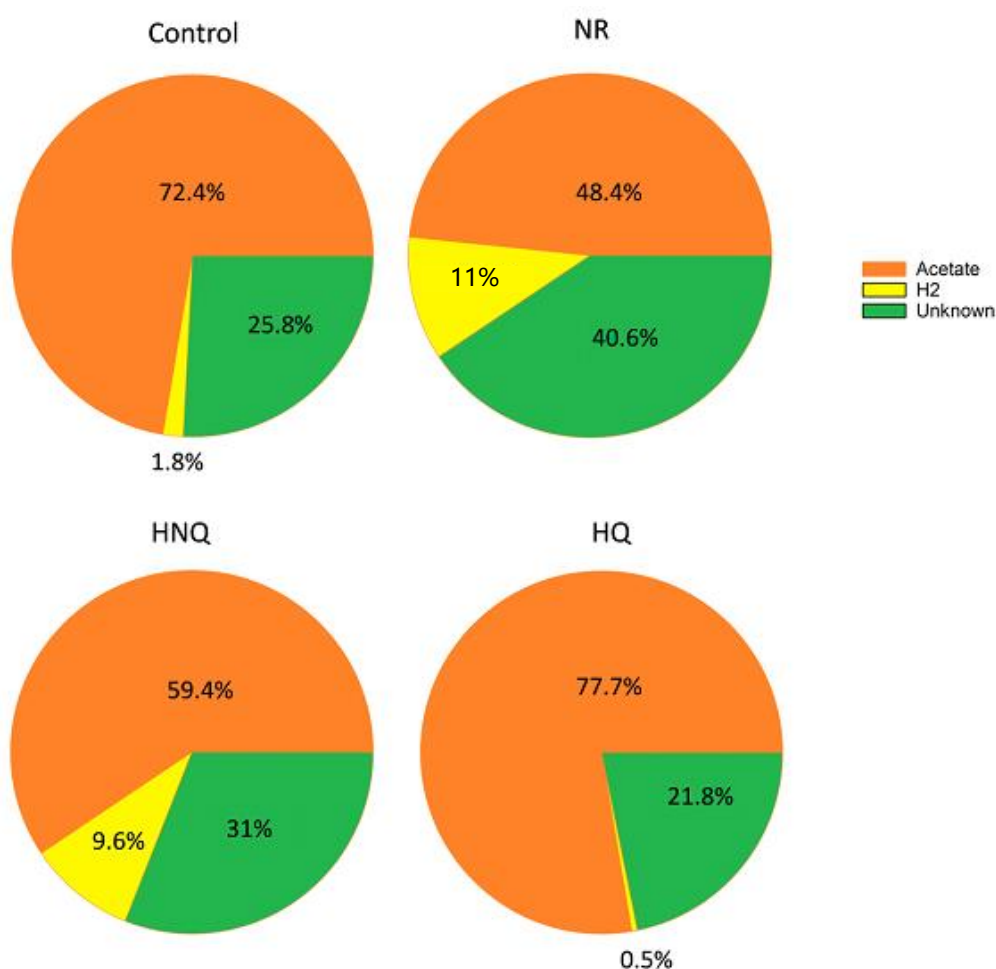


Figure 4. Average electrons accumulated in acetate and hydrogen at 0.1 mM redox mediators from the second to the eighth cycle.

3.3. Electrochemical Characteristics of CO₂ Electrosynthesis and SEM Analysis

The initial and final electrochemical characteristics of MES with different mediators were compared using cyclic voltammetry (Figure 5). At day 0, all reactors showed a typical reduction peak at approximately -0.3 V in the reverse scan, indicating CO₂ reduction at that potential, as described elsewhere [41,48]. The peak current of HNQ and HQ was higher than the control. The reduction potentials of NR and HNQ were more negative than -0.3 V, which indicates that the electrons of the mediator are delivered to CO₂ reduction (see Table 1). NR, which has the highest theoretical reduction potential (-0.54 V vs. Ag/AgCl), did not show a clear reduction peak compared to the other mediators and control. NR has a similar reduction potential to the redox of Nicotinamide adenine dinucleotide (NADH/NAD⁺, -0.53 V vs. Ag/AgCl) and is adsorbed readily in the cell membrane. Thus, the electrochemical properties of NR at this concentration (0.1 mM final) might not be apparent in the CV trace. After 16 days of cultivation under MES, the oxidation peak in the forward scan

increased significantly, possibly due to the re-oxidation of acetate produced by electrosynthesis. The HNQ implemented electrosynthesis resulted in a significant increase in the oxidation peak at -0.4 V. The capacitance estimated by the area between forward and reverse scan increased during 16 days of cultivation, which suggests that biofilm formation altered the physical properties of the electrode surface. The CV results suggested that biological CO_2 conversion could be adapted by the poised potential and current, and was facilitated when a redox mediator is present.

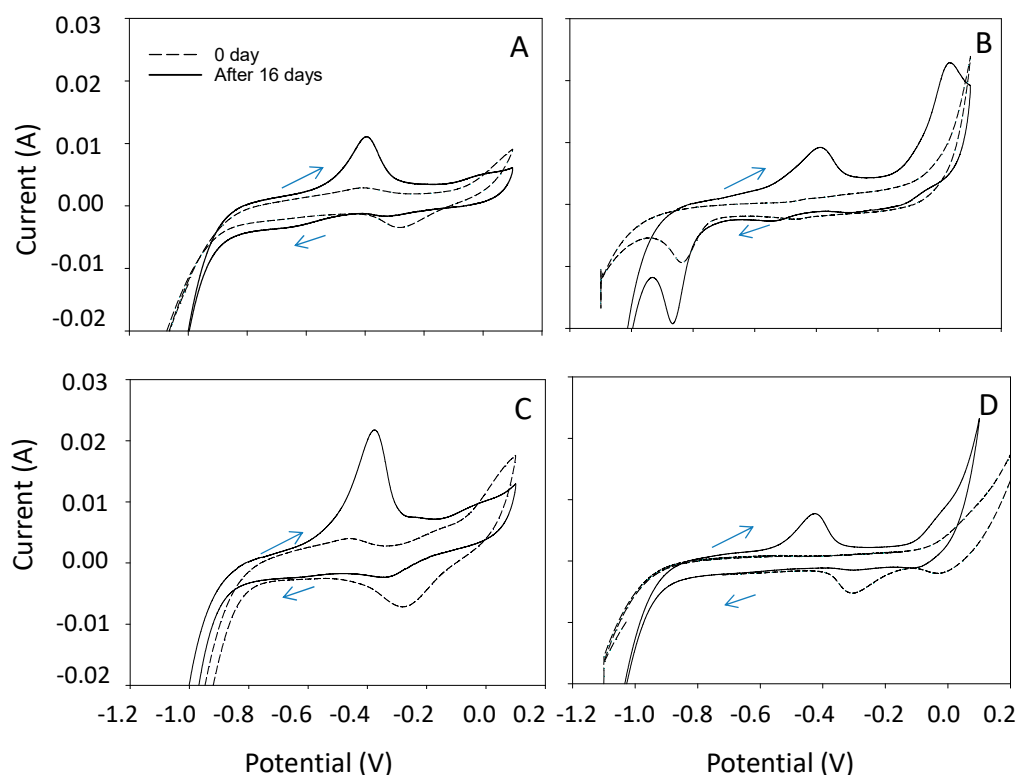


Figure 5. Cyclic voltammetry analysis with different redox mediators. (A) Control, (B) NR, (C) HNQ, (D) HQ. The dotted line indicates the initial cyclic voltammetry (CV) analysis before the cycles, and the solid line is CV analysis after the eighth cycle. Electrode area: 4 cm $\times$ 5 cm carbon felt. The scan range was between -1.11 and $+0.1$ V (vs. Ag/AgCl 3M KCl), and the scan rate was 10 mV/s.

The use of a mediator may also influence the attachment of electrochemically active bacteria on the electrode. SEM was carried out to examine the morphology of the cathode surface (Figure 6). The microscopic observations revealed the presence of microorganisms attached to the cathode electrode, both with and without mediators. In addition, the amount of protein on the electrode surface in the mediators and the control (without mediator) was determined (Table 2). Interestingly, the control without a mediator resulted in higher protein attachment to the electrode compared to those with mediators (approximately 20% higher). This suggests that a direct electron transport process might be a more dominant pathway for acetate production in the control MES (i.e., no mediators) compared to the other mediator-based MES reactors. In contrast, the presence of mediators resulted in a slightly higher planktonic protein than the control except for HQ (Table 2).

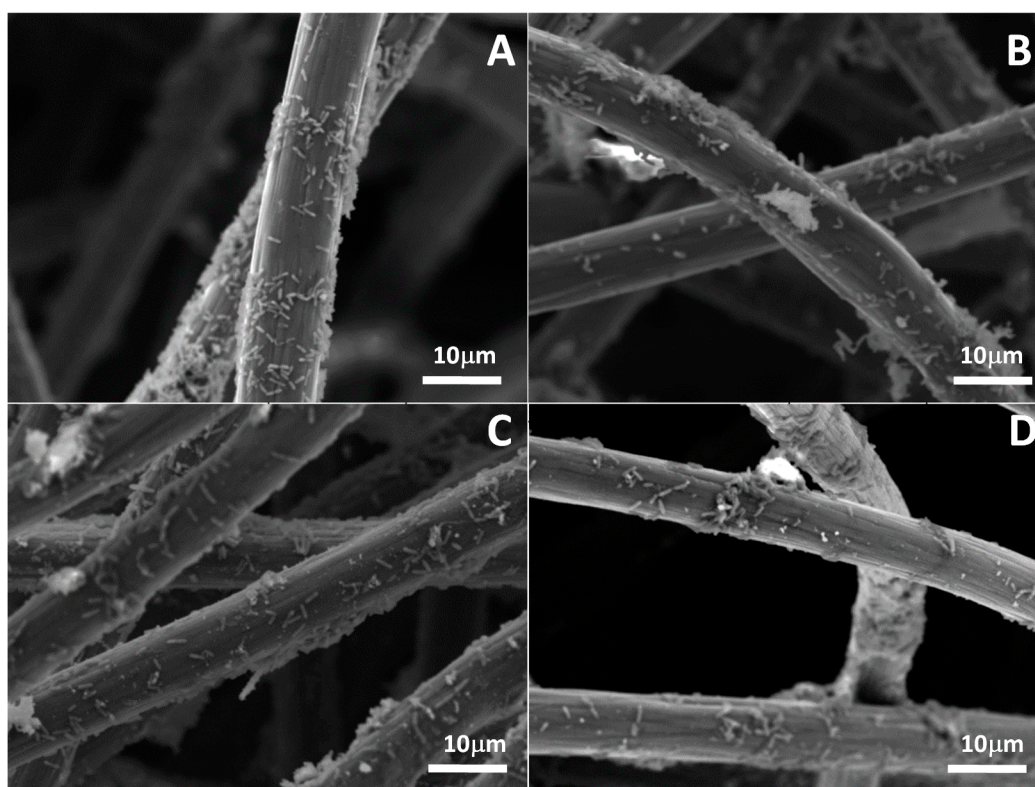


Figure 6. SEM image of the cathode electrode surface with different redox mediators. (A) Control, (B) NR, (C) HNQ, (D) HQ.

Table 2. Analysis of the protein attached to the cathode electrode surface and planktonic cells using different mediators.

Mediator	Electrode ($\mu\text{g}/\text{cm}^2$)	Suspension ($\mu\text{g}/\text{mL}$)
without mediator	1.02 ± 0.2	$1.40 \pm 0.04 \times 10^{-2}$
NR	0.83 ± 0.07	$1.50 \pm 0.07 \times 10^{-2}$
HNQ	0.88 ± 0.1	$1.82 \pm 0.05 \times 10^{-2}$
HQ	0.67 ± 0.3	$0.82 \pm 0.1 \times 10^{-2}$

4. Conclusions

This study examined the effects of different redox mediators, such as neutral red, 2-hydroxy-1, 4-naphthoquinone, and hydroquinone, on microbial electrosynthesis for acetate production from CO_2 . Periodic gas replenishment in batch mode was conducted to determine the electron and carbon balances in MES systems. The addition of mediators to the MES system increased the level of acetate production, but lower recovery efficiencies of the electrons and carbon were observed because of the undesired metabolic pathways. CV showed typical reduction peaks at approximately -0.3 V, which were close to the CO_2 reduction potential, indicating the occurrence of microbial electrosynthesis in the presence of mediators. Further optimization of the microbial electrosynthesis process, along with the selection and optimization of the mediator, can reduce CO_2 to a range of valuable chemicals, including acetate, through electrode-based electron transfer in bioelectrochemical systems.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1996-1073/13/10/2572/s1>, Table S1: Estimation of electron recovered into biomass according to each cycle of headspace gas replacement. Table S2: Estimation of carbon recovered into biomass according to each cycle of headspace gas replacement. Figure S1: Schematic diagram of microbial electrosynthesis reactor, Figure S2: Acetate production at 0.5 mM and 1 mM, Figure S3: Average electron recovery into acetate with 0.1 mM, 0.5 mM, and 1 mM of redox mediators during eight cycles. Figure S4: Average carbon recovery into acetate at 0.1 mM, 0.5 mM, and 1 mM of redox

mediators during eight cycles, Figure S5: Acetate production with 7 mM of redox mediators, Figure S6: Cell growth (OD600) profiles at 0.1 mM of redox mediators during electrosynthesis on every eight cycles for 16 days.

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Conflicts of Interest: The authors declare no conflicts of interest.

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