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Abstract

Microbial electrosynthesis is a bioprocess where microbes reduce CO₂ into multicarbon chemicals with electrons derived from the cathode of a bioelectrochemical reactor. Developing a highly productive microbial electrosynthesis reactor requires excellent electrical connection between the electrochemical setup, the cathode, and the microbes. Copper is a highly conductive cathode material widely employed in electrochemical apparatuses. However, the antimicrobial properties of copper limit its usage for bioelectrochemistry. Here, biocompatible reduced graphene oxide coated on copper foam is synthesized as a cathode material for the microbial electrosynthesis of acetate from CO₂. Dense and electroactive *Sporomusa ovata* biofilms form on the surface of reduced graphene oxide-coated copper foam electrodes while only scattered and damaged cells cover uncoated copper electrodes. Besides the formation of metabolically-active biofilms, acetate production rate from CO₂ is 21.3 and 43.5-fold higher with this novel composite cathode compared with an uncoated copper foam cathode and a reversed cathode made of reduced graphene oxide foam coated with copper, respectively. The results demonstrate that reduced graphene oxide can be employed as a biocompatible and conductive buffer between microbes and bactericidal electrode materials with excellent electrochemical property to enable highly performant microbial electrosynthesis.

Keywords:

Microbial electrosynthesis; copper foam; reduced graphene oxide; cathode; biocatalyst

1. Introduction

Microbial electrosynthesis (MES) is an emerging biotechnology, where electrons coming from the cathode of a bioelectrochemical reactor are used by a microbial catalyst for the reduction of CO₂ into multicarbon compounds or methane [1,2]. In MES, electrons and protons driving biological reduction reactions at the cathode are coming from chemical or biological oxidation reactions occurring at the anode [3-5]. The electricity required to drive MES processes can be generated from multiple renewable energy sources including wind turbine and sunlight [6,7]. Under these circumstances, MES is a technology developed for the storage of electricity from intermittent energy sources into the chemical bonds of easy-to-store molecules [8].

In recent years, research efforts have been carried out to increase the productivity of MES systems for potential scaling up. Microbial catalysts, growth medium, and cathodes are all components of MES reactors that have been studied and optimized [9-18]. Electron transfer from the cathode to microbes is at the core of MES and multiple strategies have pursued the development of novel electrode materials and spatial arrangements to improve it. The utilization of cathodes with better electrical conductivity, larger surface area, higher catalytic activity, low charge-transfer resistance, and good biocompatibility have led to the development of MES reactors with enhanced current density and CO₂ reduction rates [19,20]. For instance, modifying carbonaceous cathodes with biopolymers, carbon nanotubes, gold, palladium or nickel improved MES performance because of increased surface area-to-volume ratio, superior catalytic properties and stronger electrostatic interactions with microbes [15,21,22].

Copper, which has high electrical conductivity and a relatively low price, could be an interesting alternative for the fabrication of high-performance MES cathodes. In bioelectrochemical systems aiming at the generation of an electrical current from the microbial oxidation of organic carbon molecules, copper anode has been shown to have comparable performance to benchmark graphite anode [23]. For this application, fabrication of copper anode could reduce the system cost because the higher conductivity of copper allows the utilization of smaller electrodes for the same productivity. In many MES systems, electrons transferred from the cathode to the microbes are reported to be shuttled *via* H₂ [24,25]. Cathodes made of metallic copper, copper alloys or comprising copper oxides have been used for the electrochemical or photoelectrochemical evolution of H₂ [26,27]. However, copper surfaces are known to inhibit microbial cell growth, which could be incompatible with MES applications [28,29].

Reduced graphene oxide (rGO) is a honeycombed one-atom-thick carbon material with many of the qualities required for the development of high-performance electrodes for bioelectrochemical applications such as biocompatibility, high electrical conductivity and low production cost [30-33]. In the case of MES, composite non-metallic carbonaceous cathodes comprising rGO or its derivative have been shown to increase significantly acetate production rates and current density [19,20]. Because of its large specific surface area and superior electron mobility, graphene-based materials have also been used in the fabrication of cathode for the photoelectrochemical or electrochemical H₂ evolution [34-37]. Furthermore, when graphene-based materials were combined with copper-based materials for the fabrication of inexpensive electrochemical electrodes, a synergistic effect improving H₂ evolution was observed [38,39].

During MES, electron transfer rate from the cathode to the microbe can be an important bottleneck restraining productivity. One of the purposes of this study was to develop a MES system where the flux of reducing equivalents under the form of H₂ would be sufficient to drive the biological

reduction of CO₂ without being a limitation. To achieve this goal, we investigated the performance and bioelectrochemical behavior of *Sporomusa ovata*-driven MES reactors equipped with cathodes poised at low potential and made of the four following materials: copper foam, rGO foam, copper foam coated with rGO (rGO-CuF) and rGO foam electroplated with copper (epCu-rGO). Biofilm formation at the surface of the different cathodes was also studied via confocal laser microscopy (CLSM) and scanning electron microscopy (SEM).

2. Materials and Methods

2.1. The microbial catalyst *Sporomusa ovata* DSM 2662

Wild-type *S. ovata* DSM-2662 was acquired from the Deutsche Sammlung Mikroorganismen und Zellkulturen (DSMZ) [40]. Bacterial cultures were routinely maintained anaerobically in 311 medium with 40 mM betaine as carbon source and electron donor under N₂:CO₂ (80:20). For autotrophic cultivation with H₂ as the electron source, no betaine was added to 311 medium and the atmosphere was H₂:CO₂ (80:20) or N₂:CO₂:H₂ (83:10:7) [41]. Casitone, sodium sulfide, yeast extract, and resazurin were omitted from 311 medium under all growth conditions. Cysteine was also omitted from 311 medium for MES experiments.

2.2. Synthesis of graphene oxide

Graphene oxide (GO) was prepared via a modified version of the Hummers' method [42]. 0.5 g of graphite (325 mesh, Alfa Aesar) was loaded into a round bottom flask. 24 ml concentrated H₂SO₄ (95-98%, Sigma-Aldrich) was added, and the reaction mixture was cooled on ice with stirring. After 10 minutes, 3 g of KMnO₄ was added in small portions over 30 minutes and the reaction was maintained on ice for an additional 1.5 hour. The mixture was then transferred to an oil bath heated to 35 °C and incubated for 16 hours. 80 ml Milli-Q water was added slowly into the reaction mixture and the temperature was subsequently raised to 90 °C for 15 minutes. The reaction mixture was allowed to cool down to room temperature and 100 ml Milli-Q water was added followed by 8 ml H₂O₂ (33%, Sigma-Aldrich). The obtained GO was then washed five times with Milli-Q water by centrifugation [43]. GO flake exfoliation was obtained subsequently using bath sonication for 30 minutes. Large aggregates were removed and further refining to mono- and few-layer GO was achieved by 5 times of centrifugation at 620 x g. Finally, the GO solution was centrifuged two times at 9800 x g for 20 minutes to remove oxidative debris.

2.3. Coating of copper foam with reduced graphene oxide

Handling of the copper foam (Goodfellow, USA) was performed under a nitrogen atmosphere to avoid extensive copper oxidation. Initially, the copper foam scaffold was washed in isopropanol, ethanol and Milli-Q water for 15 minutes, respectively, prior to GO coating. Coating of the copper foam was carried out at 120 °C in a round bottom flask under a continuous flow of nitrogen. A 0.5 mg mL⁻¹ GO solution was dropped on both sides of the copper foam, and the GO solution was allowed to dry. This step was repeated three times until full coverage of the copper foam was achieved. GO was subsequently reduced to rGO with 60 ml ascorbic acid solution (0.5 mg mL⁻¹) for 2 hours. Finally, the rGO-CuF electrode was washed extensively with Milli-Q water to remove residual ascorbic acid and loosely-bound rGO flakes.

2.4. Fabrication of reduced graphene oxide foam

2 ml of 30 mg mL⁻¹ GO solution in Milli-Q water was poured into a cylindrical mold and freeze-dried overnight. The resulting GO foam was heated at a heating rate of 1 °C min⁻¹ until reaching 300 °C where it was maintained for 10 minutes. After heating, the GO foam was soaked in ascorbic acid for reduction and then washed extensively with Milli-Q water.

2.5. Fabrication of reduced oxide graphene foam electroplated with copper

rGO foam was coated with copper by electroplating. The electroplating apparatus comprises a copper plate anode separated by five centimeters from the rGO foam substrate, which served as the cathode in this setup. The electrolyte consists of 50 g l^{-1} $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ into a 150 g l^{-1} H_2SO_4 solution. For electroplating, the voltage was set at 1 volt for 10 minutes. The obtained epCu-rGO foam was washed with Milli-Q water until complete removal of residual acid and loosely-bound copper before being dried in a vacuum drying box.

2.6. Microbial electrosynthesis and cyclic voltammetry

The four different cathodes were tested in triplicate for MES experiments in dual chambered, three-electrode bioelectrochemical reactors. The MES experiments were conducted at $25\text{ }^\circ\text{C}$ with *S. ovata* DSM-2662 for the reduction of CO_2 into acetate as previously described [14,44]. Copper foam, rGO foam, rGO-CuF or epCu-rGO cathodes and graphite stick anode (36 cm^2) were immersed in 250 ml 311 medium in two chambers separated by a Nafion 115 ion-exchange membrane (Ion Power, Inc., New Castle, DE, USA). An Ag/AgCl electrode model ET072 (eDAQ, Denmark) was used as a reference electrode and the cathode was poised at a potential of -990 mV versus standard hydrogen electrode (SHE) with a CH Instrument potentiostat (CH Instruments, Inc, USA). *S. ovata* cultures of 100 ml grown on $\text{H}_2:\text{CO}_2$ at an optical density (545 nm) of ca. 0.1 were used to inoculate the cathode chamber. *S. ovata* cultures were established in the MES cathode chamber by bubbling with $\text{N}_2:\text{CO}_2:\text{H}_2$ and by swapping the medium two times. Each medium swap was performed after the cultures reached an OD_{545} of ca. 0.1. After the second medium swap, the gas mix was switched to $\text{N}_2:\text{CO}_2$ at which point data start being collected. The abiotic anode compartment was also bubbled with $\text{N}_2:\text{CO}_2$ during the MES experiment. During the cyclic voltammetry (CV) experiments, the tested electrodes were scanned at a rate of 1 mV s^{-1} in a potential window of 0 to -1300 mV versus Ag/AgCl. Further electrochemical analyses of the data generated during MES and CV experiments were done with the EC-Lab® software v.10.40 (BioLogic, France). Instability in the current density reported here was likely caused by experimental variation.

2.7. Gas chromatography and High-pressure liquid chromatography

For H_2 detection, gas samples from the headspace of MES reactors were collected in N_2 -flushed serum bottles and concentration was measured by gas chromatography with a Trace 1300 gas chromatograph (ThermoFisher Scientific, Denmark). Argon was used as a carrier gas with a HP-PLOT Molesieve column (Agilent) and H_2 was detected with a thermal conductivity detector (TCD). The oven temperature of the gas chromatograph was maintained at $130\text{ }^\circ\text{C}$. High-pressure liquid chromatography (HPLC) was used for the quantification of acetate. The HPLC apparatus was equipped with an HPX-87H anion exchange column (Bio-Rad Laboratories Inc., California, USA) set at a temperature of $30\text{ }^\circ\text{C}$. $5\text{ mM H}_2\text{SO}_4$ was the mobile phase at a flow rate of 0.6 ml min^{-1} . Acetate was detected with a refractive index (RI) detector and data were analyzed with the Chromeleon software (ThermoFisher Scientific). As described previously [2,14,22,44], acetate production rate and current density were normalized to the geometric surface area of copper foam or rGO-CuF cathodes, which were rectangular prisms, according to equation 1:

$$A = 2(wl + hl + hw) \quad (1)$$

The geometric surface area of copper foam and rGO-CuF (A) was 5.2 cm^2 with a width (w) of 0.2 cm, a length (l) of 2 cm and a height (h) of 1 cm. The geometric surface area of the rGO foam and epCu-rGO, which had a cylindric shape, was calculated according to equation 2:

$$A = 2\pi r^2 + 2\pi rh \quad (2)$$

The geometric surface area of rGO foam and epCu-rGO (A) was 8.12 cm² with a radius (r) of 0.55 cm and a height (h) of 1.8 cm.

2.8. Scanning electron microscopy and confocal laser scanning microscopy

For SEM images, abiotic cathode and biocathode samples from electrochemical reactors were collected at the end of MES experiments after ten days of operation and fixed with 0.1M buffer solution at pH 7.0 containing 2.5% glutaraldehyde for 5 hours at room temperature. Cathode samples were then washed with the buffer solution without glutaraldehyde before being immersed successively in acetonitrile and ethanol as described previously [22]. Cathodes were dried under nitrogen and images were taken with a Quanta 200 FEG scanning electron microscope (FEI) at an accelerating voltage of 10 V under high vacuum condition.

For CLSM images, biocathodes were taken from the MES reactor after ten days and stained with the LIVE/DEAD® BacLight™ Bacterial Viability Kit (Thermo Fisher Scientific) as described previously [20]. With this kit, cells staining in red have a compromised membrane and are considered dead or dying whereas cells staining in green have an intact membrane and are considered alive. CLSM images were taken with a Zeiss LSM 5 Pascal microscope, and the images were analyzed with the ZEN imaging software (Zeiss, Germany).

2.9. Analytical methods

Energy-dispersive X-ray spectroscopy (EDS) data were collected with an Oxford IE250 energy-dispersive X-ray spectrometer (Oxford Instruments, UK). Raman spectra of the four cathodes were obtained with a DXR confocal Raman microscope (Thermo Fisher Scientific) at an excitation wavelength of 455 nm with 5 mW of laser power. The Brunauer–Emmett–Teller (BET) method was used as previously described to determine the specific surface area of cathodes [45]. Inductively coupled plasma-optical emission spectrometry (ICP-OES) used to measure Cu²⁺ in electrolytes was conducted with an iCAP 7400 ICP-OES analyzer (Thermo Fisher Scientific).

3. Results and discussion

3.1. Cathodes characterization

Four cathode materials were investigated in this study: commercially-available copper foam, rGO foam, epCu-rGO and rGO-CuF. The latter was fabricated by coating copper foam with GO before proceeding to its reduction with ascorbic acid (Fig 1A-B). SEM images of the rGO-CuF cathode showed a three-dimensional globular structure (Fig. 1C-E). After the coating process, a crumpled paper-like film made of rGO was covering the copper foam surface (Fig. 1D-F). Raman spectroscopy was employed to confirm the successful modification of the copper foam substrate with rGO (Fig. S1). Before modification, the copper foam material exhibited two Raman peaks between 500 and 700 cm⁻¹, which are characteristic of copper oxides found at the surface of metallic copper [46]. After modification, only a single weaker peak at ca. 620 cm⁻¹ remained in this region of the Raman spectrum. More importantly, Raman peaks D (1360 cm⁻¹), G (1570 cm⁻¹) and 2D (2700 cm⁻¹) that are characteristic of rGO were present on the Raman spectrum, confirming the successful coating of the copper foam [47]. Besides changing the cathode surface structure, coating rGO on the copper foam substrate resulted in a 161-fold increase of the specific surface area to 1.13 m² g⁻¹.

The epCu-rGO cathode was synthesized by electroplating of copper on the rGO foam (Fig. 2A-B). Both the rGO foam and the epCu-rGO cathodes had the characteristic crumpled paper-like structure of rGO (Fig. 2C-D-E-F). EDS showed the presence of copper on the surface of the epCu-rGO cathode (Fig. 2F inset). The presence of the D, G and 2D peaks on the Raman spectrum confirmed the successful synthesis of rGO foam (Fig. S2). The epCu-rGO cathode had less intense D and G peaks and no 2D peak. A large peak between 500 and 700 cm^{-1} was observed due to the presence of copper at the surface of the epCu-rGO cathode. The rGO foam and the epCu-rGO cathodes had a specific surface area of 0.60 $\text{m}^2 \text{g}^{-1}$ and 14.64 $\text{m}^2 \text{g}^{-1}$, respectively.

3.2. Microbial electrosynthesis with the copper foam cathode

A MES system equipped with a copper foam cathode poised at -990 mV vs. SHE and *S. ovata* DSM-2662 as the microbial catalyst had an acetate production rate of $79.6 \pm 24.4 \text{ mmol m}^{-2} \text{d}^{-1}$ and a current density of $-7.1 \pm 2.5 \text{ A m}^{-2}$ (Fig. 3, Table 1). As expected, the abiotic MES system equipped with a copper foam cathode did not generate any acetate while H_2 accumulated in the headspace of the reactor at a rate of $995.0 \pm 63.4 \text{ mmol m}^{-2} \text{d}^{-1}$. In the copper foam-MES reactor populated with *S. ovata*, H_2 accumulated in the gas phase at a higher rate of $2500.9 \pm 461.4 \text{ mmol m}^{-2} \text{d}^{-1}$. Assuming that in the MES system described here most of the electrons used for the biological reduction of CO_2 into acetate are transferred to the microbe from the cathode under the form of H_2 , the electrosynthesis of one mole of acetate requires four moles of H_2 according to equation 3.

This means that to maintain the acetate production rate observed with the copper foam-MES system, bioelectrochemically-evolved H_2 needs to be oxidized at a rate of $318.4 \text{ mmol m}^{-2} \text{d}^{-1}$. Actual accumulation of H_2 in the gas phase was 7.9 fold faster than the calculated H_2 oxidation rate, which is probably the main reason behind the low coulombic efficiency of only $10.0 \pm 6.5 \%$ of the electrons from the cathode used for the biological synthesis of acetate from CO_2 . Furthermore, results presented here suggest that the presence of bacterial cells accelerated H_2 evolution reaction (HER) by the copper foam cathode. This phenomenon has been observed before with other types of cathode materials and it has been proposed that the microbial deposition of redox enzymes or biometals on the MES cathode had a beneficial effect on HER [25,48,49]. Reduction of H_2 concentration near the cathode surface due to quick biological oxidation could also be a factor stimulating HER [50].

3.3. Microbial electrosynthesis with the rGO-CuF cathode

When copper foam was coated with rGO and used as the cathode for *S. ovata*-driven MES, acetate production rates, as well as current density were significantly increased to $1697.6 \pm 298.1 \text{ mmol m}^{-2} \text{d}^{-1}$ and $-21.6 \pm 2.1 \text{ A m}^{-2}$, respectively (Fig. 4, Table 1). The acetate production rate per m^2 of electrode surface observed with this composite cathode is comparable with one of the high-performance cathodes previously developed for MES. Jourdin et al. reported an acetate production rate of $11407 \text{ mmol m}^{-2} \text{d}^{-1}$ with a MES system populated with a mixed community and equipped with a reticulated vitreous carbon (RVC) cathode coated with multiwalled carbon nanotubes (MWCN) [15]. This outstanding result was normalized to the projected area or footprint of the base of the electrode that was 1.36 cm^2 [13,15,51,52]. If the RVC-MWCN cathode surface area is instead calculated based on the geometric surface, the acetate production rate becomes $1983.8 \text{ mmol m}^{-2} \text{d}^{-1}$. This is comparable to the results presented here since the geometric surface area of the RVC-MWCN cathode was ca. 7.82 cm^2 when including the six faces of the rectangular prism. The

specific surface area was not used for normalization here because bacterial cells are too large to interact everywhere on the cathode surface when there are small pores present.

One important characteristic of the rGO-CuF cathode compared to RVC-MWCN is its lower cost of fabrication. For instance, RVC with a porosity between 10 to 100 pores per inch has a price tag of 1292 USD per m² [53]. MWCNT price is 55 USD per gram [54]. In comparison, copper foam price is 130 USD per m² [55]. Graphene usually has a low-price tag since it is fabricated from graphite. For example, Xie et al reported that the capital cost of a pure 2 mm-thick graphene sponge electrode was 4 USD per m² [56]. The electrode developed by this group was fabricated with 2 grams of graphene per m².

Coulombic efficiency in the MES system with the rGO-CuF cathode was 70.2 ± 14.1 % (Table 1). In a similar way as the copper foam cathode, a large quantity of H₂ was not oxidized by the microbial catalyst and accumulated in the gas phase of the MES reactor. This was the main reason that a fraction of the electrons from the cathode has not been used for the biological reduction of CO₂ into acetate. Still, coulombic efficiency with the rGO-CuF cathode was significantly better than the one observed with the copper foam cathode.

H₂ accumulation rate in the gas phase of the abiotic rGO-CuF MES reactor was 2161.6 ± 223.5 mmol m⁻² d⁻¹, which is 2.2 fold higher than with the abiotic copper foam MES reactor. Besides the intrinsic properties of rGO, the larger specific area of the rGO-CuF cathode compared to uncoated copper foam cathode, which increases the quantity of cathode-electrolyte junctions, probably explains the faster HER observed.

In the *S. ovata*-driven rGO-CuF MES system, H₂ must be oxidized at a rate of 6790.4 mmol m⁻² d⁻¹ to sustain the observed acetate production rate. Adding this H₂ oxidation rate to the detected H₂ accumulation rate in the gas phase resulted in an overall HER of 9389.6 mmol m⁻² d⁻¹. This was 4.3 fold higher than what was observed in the abiotic rGO-CuF MES system. As with the copper foam cathode, the presence of biomass could accelerate HER.

Investigating the bioelectrochemical behavior of the *S. ovata*-driven rGO-CuF MES system with cyclic voltammetry showed a higher cathodic current response than the abiotic controls as well as the *S. ovata* copper foam MES system (Fig. S3). This result was consistent with the higher current density and the faster HER observed with the MES reactor equipped with the rGO-CuF composite cathode. Furthermore, the abiotic rGO-CuF MES system exhibited a higher cathodic current response than the abiotic copper foam MES system, a result also consistent with the observed current densities, HER rates as well as with the increased specific surface area.

3.4. Performance of the rGO foam and epCu-rGO cathodes

Two other cathodes made of rGO foam and of epCu-rGO were tested in the MES system. When compared with rGO-CuF, the rGO foam had an acetate production rate and a current density 7.6 and 8.3 times lower, respectively (Table 1, Fig. 5). The coulombic efficiency was 76.4 ± 18.6 %. The core of the rGO-CuF is made of copper, which has an electrical conductivity three orders of magnitude higher than rGO [57]. This is probably the main reason why rGO-CuF, which combined highly conductive copper covered with a layer of biocompatible rGO, is more performant for MES than pure rGO foam. Another possible reason for the higher MES performance of the rGO-CuF cathode is that its specific surface area is 1.9 times higher than the pure rGO foam cathode, which is

likely to augment the number of active sites for microbe-cathode interactions and the rate of reducing equivalents exchange.

The epCu-rGO cathode was much less significantly performant than the rGO-CuF cathode for MES with an acetate production rate and a current density 43.5 and 5.0 times lower, respectively (Table 1, Fig. 6). The coulombic efficiency for acetate production with the epCu-rGO cathode was only $8.1 \pm 4.1\%$. Most of the electrons derived from the cathode accumulated as H_2 in the gas phase of the MES reactor. The poor MES performance of epCu-rGO could be attributed to direct contact between microbes and the bactericidal copper. Cyclic voltammetry of the rGO foam and epCu-rGO cathodes confirmed the superiority for MES of the rGO-CuF cathode, which had a higher cathodic current response (Fig. S3-S4).

Total HER including electrons ending up into acetate for both the rGO foam and the epCu-rGO cathodes was accelerated by the presence of biomass (Table 1). Under sterile conditions, rGO foam was the less performant cathode for HER, while the epCu-rGO cathode evolved H_2 at a rate comparable to copper foam but 2.1 times lower than rGO-CuF. Slower HER by epCu-rGO, which had a 13-fold higher specific surface area than rGO-CuF, may be related to the lower conductivity of the rGO foam core compared to the copper foam core.

3.5. Cell viability at the surface of the cathodes

The low acetate production rate and coulombic efficiency observed with both the copper foam and the epCu-rGO can be explained by direct exposure of bacterial cells to copper, which is widely considered as antimicrobial with an oligodynamic effect [23,29]. SEM images taken after 10 days of operation of MES reactors showed the presence of *S. ovata* cells attached at the surface of the copper foam and the epCu-rGO cathodes (Fig. 3C-D, Fig. 6C-D). CLSM images of the copper foam and epCu-rGO biocathodes revealed that a large fraction of *S. ovata* cells stained in red, which indicated that they had a compromised membrane and were either dead or dying (Fig. 7A-B). In this study, the inhibiting impact of copper on *S. ovata* was evident under the MES operational conditions tested here.

SEM images of rGO foam and rGO-CuF biocathodes showed the presence of *S. ovata* cells on the rGO surface (Fig. 4C-D, Fig. 5C-D). The biocompatibility of rGO was evident since almost all the *S. ovata* cells stained in green on both cathodes, suggesting that most cells were alive with intact membranes (Fig. 7C-D). This was expected since Aryal et al. have shown previously that the acetogen *S. ovata* remained viable on rGO [10,20].

CLSM results indicated that coating copper with rGO mitigated the toxic effects of copper on microbes. To confirm this observation, the presence of deleterious copper ions (Cu^{2+}) in the electrolytes of an abiotic MES system equipped with a rGO-CuF cathode was measured over a period of 10 days by ICP-OES (Fig. S5). Cu^{2+} ions concentration was negligible from day 0 to day 10. In comparison, ca. $0.8 \text{ mg l}^{-1} Cu^{2+}$ was detected in the electrolytes at day 0 when the MES reactor was equipped with an epCu-rGO cathode. Interestingly, Cu^{2+} ion became barely detectable after day 3 within this MES system. These results indicated that there was no copper leakage in the proximity of living cells with the rGO-CuF cathode during the MES experiment. The significant diminution of Cu^{2+} ion concentration after day 0 in the electrolyte of the epCu-rGO cathode could be attributed to the reduction of Cu^{2+} ions to elemental copper on the cathode surface.

4. Conclusion

Here, a MES reactor with a rGO-CuF cathode was developed to provide a non-limiting flux of reducing equivalents for microbial CO₂ reduction. Copper is a highly conductive and inexpensive material, and using it to fabricate electrodes could reduce the cost of bioelectrochemical applications. As expected, the cathodes with a copper surface are unsuitable for MES due to its antimicrobial properties. However, when biocompatible rGO was coated on copper cathode, acetate production rate was increased to a level comparable to highly-performant MES reactors. This indicates that rGO-CuF cathodes have features that are beneficial for MES and for other bioelectrochemical applications.

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Appendix A. Supplementary figures

References

- [1] D.R. Lovley, K.P. Nevin, Electrobiocommodities: powering microbial production of fuels and commodity chemicals from carbon dioxide with electricity, *Curr. Opin. Biotechnol.* 24 (2013) 385–390. doi:10.1016/j.copbio.2013.02.012.
- [2] K.P. Nevin, T.L. Woodard, A.E. Franks, Z.M. Summers, D.R. Lovley, Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds, *MBio.* 1 (2010) e00103-10. doi:10.1128/mBio.00103-10.
- [3] K. Rabaey, R.A. Rozendal, Microbial electrosynthesis - revisiting the electrical route for microbial production, *Nat. Rev. Microbiol.* 8 (2010) 706–716. doi:10.1038/nrmicro2422.
- [4] M. Zeppilli, M. Villano, F. Aulenta, S. Lampis, G. Vallini, M. Majone, Effect of the anode feeding composition on the performance of a continuous-flow methane-producing microbial electrolysis cell, *Environ. Sci. Pollut. Res. Int.* 22 (2015) 7349–7360. doi:10.1007/s11356-014-3158-3.
- [5] Y. Gong, A. Ebrahim, A.M. Feist, M. Embree, T. Zhang, D. Lovley, K. Zengler, Sulfide-driven microbial electrosynthesis, *Environ. Sci. Technol.* 47 (2013) 568–573. doi:10.1021/es303837j.
- [6] P.-L. Tremblay, T. Zhang, Electrifying microbes for the production of chemicals, *Front. Microbiol.* 6 (2015) 201. doi:10.3389/fmicb.2015.00201.
- [7] T. Zhang, More efficient together, *Science.* 350 (2015) 738–739. doi:10.1126/science.aad6452.
- [8] D.R. Lovley, K.P. Nevin, A shift in the current: new applications and concepts for microbe-electrode electron exchange, *Curr. Opin. Biotechnol.* 22 (2011) 441–448. doi:10.1016/j.copbio.2011.01.009.
- [9] N. Aryal, F. Ammam, S.A. Patil, D. Pant, An overview of cathode materials for microbial electrosynthesis of chemicals from carbon dioxide, *Green Chem.* 19 (2017) 5748–5760. doi:10.1039/C7GC01801K.
- [10] N. Aryal, A. Halder, M. Zhang, P.R. Whelan, P.-L. Tremblay, Q. Chi, T. Zhang, Freestanding and flexible graphene papers as bioelectrochemical cathode for selective and efficient CO₂ conversion, *Sci. Rep.* 7 (2017) 9107. doi:10.1038/s41598-017-09841-7.

- [11] F. Ammam, P.-L. Tremblay, D.M. Lizak, T. Zhang, Effect of tungstate on acetate and ethanol production by the electrosynthetic bacterium *Sporomusa ovata*, *Biotechnol. Biofuels.* 9 (2016) 163. doi:10.1186/s13068-016-0576-0.
- [12] S. Bajracharya, A. ter Heijne, X. Dominguez Benetton, K. Vanbroekhoven, C.J.N. Buisman, D.P.B.T.B. Strik, D. Pant, Carbon dioxide reduction by mixed and pure cultures in microbial electrosynthesis using an assembly of graphite felt and stainless steel as a cathode, *Bioresour. Technol.* 195 (2015) 14–24. doi:10.1016/j.biortech.2015.05.081.
- [13] L. Jourdin, S. Freguia, B.C. Donose, J. Chen, G.G. Wallace, J. Keller, V. Flexer, A novel carbon nanotube modified scaffold as an efficient biocathode material for improved microbial electrosynthesis, *J. Mater. Chem. A.* 2 (2014) 13093–13102. doi:10.1039/C4TA03101F.
- [14] P.-L. Tremblay, D. Höglund, A. Koza, I. Bonde, T. Zhang, Adaptation of the autotrophic acetogen *Sporomusa ovata* to methanol accelerates the conversion of CO₂ to organic products, *Sci. Rep.* 5 (2015) 16168. doi:10.1038/srep16168.
- [15] L. Jourdin, T. Grieger, J. Monetti, V. Flexer, S. Freguia, Y. Lu, J. Chen, M. Romano, G.G. Wallace, J. Keller, High Acetic Acid Production Rate Obtained by Microbial Electrosynthesis from Carbon Dioxide, *Environ. Sci. Technol.* 49 (2015) 13566–13574. doi:10.1021/acs.est.5b03821.
- [16] N. Aryal, P.-L. Tremblay, M. Xu, A.E. Daugaard, T. Zhang, Highly Conductive Poly(3,4-ethylenedioxythiophene) Polystyrene Sulfonate Polymer Coated Cathode for the Microbial Electrosynthesis of Acetate From Carbon Dioxide, *Front. Energy Res.* 6 (2018) 72. doi:10.3389/fenrg.2018.00072.
- [17] L. Jourdin, S.M.T. Raes, C.J.N. Buisman, D.P.B.T.B. Strik, Critical Biofilm Growth throughout Unmodified Carbon Felts Allows Continuous Bioelectrochemical Chain Elongation from CO₂ up to Caproate at High Current Density, *Front. Energy Res.* 6 (2018) 7. doi:10.3389/fenrg.2018.00007.
- [18] B. Bian, M.F. Alqahtani, K.P. Katuri, D. Liu, S. Bajracharya, Z. Lai, K. Rabaey, P.E. Saikaly, Porous nickel hollow fiber cathodes coated with CNTs for efficient microbial electrosynthesis of acetate from CO₂ using *Sporomusa ovata*, *J. Mater. Chem. A.* 6 (2018) 17201–17211. doi:10.1039/C8TA05322G.
- [19] L. Chen, P.-L. Tremblay, S. Mohanty, K. Xu, T. Zhang, Electrosynthesis of acetate from CO₂ by a highly structured biofilm assembled with reduced graphene oxide–tetraethylene pentamine, *J. Mater. Chem. A.* 4 (2016) 8395–8401. doi:10.1039/C6TA02036D.
- [20] N. Aryal, A. Halder, P.-L. Tremblay, Q. Chi, T. Zhang, Enhanced microbial electrosynthesis with three-dimensional graphene functionalized cathodes fabricated *via* solvothermal synthesis, *Electrochim. Acta.* 217 (2016) 117–122. doi:10.1016/j.electacta.2016.09.063.
- [21] H. Nie, T. Zhang, M. Cui, H. Lu, D.R. Lovley, T.P. Russell, Improved cathode for high efficient microbial-catalyzed reduction in microbial electrosynthesis cells, *Phys. Chem. Chem. Phys.* 15 (2013) 14290–14294. doi:10.1039/c3cp52697f.
- [22] T. Zhang, H. Nie, T.S. Bain, H. Lu, M. Cui, O.L. Snoeyenbos-West, A.E. Franks, K.P. Nevin, T.P. Russell, D.R. Lovley, Improved cathode materials for microbial electrosynthesis, *Energy Environ. Sci.* 6 (2013) 217–224. doi:10.1039/C2EE23350A.
- [23] A. Baudler, I. Schmidt, M. Langner, A. Greiner, U. Schröder, Does it have to be carbon? Metal anodes in microbial fuel cells and related bioelectrochemical systems, *Energy Environ. Sci.* 8 (2015) 2048–2055. doi:10.1039/C5EE00866B.
- [24] H.D. May, P.J. Evans, E.V. LaBelle, The bioelectrosynthesis of acetate, *Curr. Opin. Biotechnol.* 42 (2016) 225–233. doi:10.1016/j.copbio.2016.09.004.

- [25] P.-L. Tremblay, L.T. Angenent, T. Zhang, Extracellular Electron Uptake: Among Autotrophs and Mediated by Surfaces, *Trends Biotechnol.* 35 (2017) 360–371. doi:10.1016/j.tibtech.2016.10.004.
- [26] K. Ngamlerdpokin, N. Tantavichet, Electrodeposition of nickel–copper alloys to use as a cathode for hydrogen evolution in an alkaline media, *Int. J. Hydrog. Energy.* 39 (2014) 2505–2515. doi:10.1016/j.ijhydene.2013.12.013.
- [27] A. Paracchino, V. Laporte, K. Sivula, M. Grätzel, E. Thimsen, Highly active oxide photocathode for photoelectrochemical water reduction, *Nat. Mater.* 10 (2011) 456–461. doi:10.1038/nmat3017.
- [28] J.A. Lemire, J.J. Harrison, R.J. Turner, Antimicrobial activity of metals: mechanisms, molecular targets and applications, *Nat. Rev. Microbiol.* 11 (2013) 371–384. doi:10.1038/nrmicro3028.
- [29] G. Grass, C. Rensing, M. Solioz, Metallic copper as an antimicrobial surface, *Appl. Environ. Microbiol.* 77 (2011) 1541–1547. doi:10.1128/AEM.02766-10.
- [30] B.G. Choi, M. Yang, W.H. Hong, J.W. Choi, Y.S. Huh, 3D Macroporous Graphene Frameworks for Supercapacitors with High Energy and Power Densities, *ACS Nano.* 6 (2012) 4020–4028. doi:10.1021/nm3003345.
- [31] J. Hou, Z. Liu, P. Zhang, A new method for fabrication of graphene/polyaniline nanocomplex modified microbial fuel cell anodes, *J. Power Sources.* 224 (2013) 139–144. doi:10.1016/j.jpowsour.2012.09.091.
- [32] M. Segal, Selling graphene by the ton, *Nat. Nanotechnol.* 4 (2009) 612–614. doi:10.1038/nnano.2009.279.
- [33] H. Yuan, Z. He, Graphene-modified electrodes for enhancing the performance of microbial fuel cells, *Nanoscale.* 7 (2015) 7022–7029. doi:10.1039/C4NR05637J.
- [34] U. Sim, T.-Y. Yang, J. Moon, J. An, J. Hwang, J.-H. Seo, J. Lee, K.Y. Kim, J. Lee, S. Han, B.H. Hong, K.T. Nam, N-doped monolayer graphene catalyst on silicon photocathode for hydrogen production, *Energy Environ. Sci.* 6 (2013) 3658–3664. doi:10.1039/C3EE42106F.
- [35] H. Fei, J. Dong, M.J. Arellano-Jiménez, G. Ye, N.D. Kim, E.L.G. Samuel, Z. Peng, Z. Zhu, F. Qin, J. Bao, M.J. Yacaman, P.M. Ajayan, D. Chen, J.M. Tour, Atomic cobalt on nitrogen-doped graphene for hydrogen generation, *Nat. Commun.* 6 (2015) 8668. doi:10.1038/ncomms9668.
- [36] Z. Huang, P. Zhong, C. Wang, X. Zhang, C. Zhang, Silicon Nanowires/Reduced Graphene Oxide Composites for Enhanced Photoelectrochemical Properties, *ACS Appl. Mater. Interfaces.* 5 (2013) 1961–1966. doi:10.1021/am3027458.
- [37] A. Xie, N. Xuan, K. Ba, Z. Sun, Pristine Graphene Electrode in Hydrogen Evolution Reaction, *ACS Appl. Mater. Interfaces.* 9 (2017) 4643–4648. doi:10.1021/acsami.6b14732.
- [38] X.-J. Lv, S.-X. Zhou, C. Zhang, H.-X. Chang, Y. Chen, W.-F. Fu, Synergetic effect of Cu and graphene as cocatalyst on TiO₂ for enhanced photocatalytic hydrogen evolution from solar water splitting, *J. Mater. Chem.* 22 (2012) 18542–18549. doi:10.1039/C2JM33325B.
- [39] S. Muralikrishna, T. n. Ravishankar, T. Ramakrishnappa, D. h. Nagaraju, R. Krishna Pai, Non-noble metal graphene oxide-copper (II) ions hybrid electrodes for electrocatalytic hydrogen evolution reaction, *Environ. Prog. Sustain. Energy.* 35 (2016) 565–573. doi:10.1002/ep.12238.
- [40] B. Möller, R. Oßmer, B.H. Howard, G. Gottschalk, H. Hippe, *Sporomusa*, a new genus of gram-negative anaerobic bacteria including *Sporomusa sphaeroides* spec. nov. and *Sporomusa ovata* spec. nov., *Arch. Microbiol.* 139 (1984) 388–396. doi:10.1007/BF00408385.
- [41] N. Aryal, P.-L. Tremblay, D.M. Lizak, T. Zhang, Performance of different *Sporomusa* species for the microbial electrosynthesis of acetate from carbon dioxide, *Bioresour. Technol.* 233 (2017) 184–190. doi:10.1016/j.biortech.2017.02.128.

- [42] W.S. Hummers, R.E. Offeman, Preparation of Graphitic Oxide, *J. Am. Chem. Soc.* 80 (1958) 1339–1339. doi:10.1021/ja01539a017.
- [43] A. Naumov, F. Grote, M. Overgaard, A. Roth, C.E. Halbig, K. Nørgaard, D.M. Guldi, S. Eigler, Graphene Oxide: A One- versus Two-Component Material, *J. Am. Chem. Soc.* 138 (2016) 11445–11448. doi:10.1021/jacs.6b05928.
- [44] K.P. Nevin, S.A. Hensley, A.E. Franks, Z.M. Summers, J. Ou, T.L. Woodard, O.L. Snoeyenbos-West, D.R. Lovley, Electrosynthesis of Organic Compounds from Carbon Dioxide Is Catalyzed by a Diversity of Acetogenic Microorganisms, *Appl. Environ. Microbiol.* 77 (2011) 2882–2886. doi:10.1128/AEM.02642-10.
- [45] R. Poreddy, C. Engelbrekt, A. Riisager, Copper oxide as efficient catalyst for oxidative dehydrogenation of alcohols with air, *Catal. Sci. Technol.* 5 (2015) 2467–2477. doi:10.1039/C4CY01622J.
- [46] Y. Deng, A.D. Handoko, Y. Du, S. Xi, B.S. Yeo, *In Situ* Raman Spectroscopy of Copper and Copper Oxide Surfaces during Electrochemical Oxygen Evolution Reaction: Identification of Cu(II) Oxides as Catalytically Active Species, *ACS Catal.* 6 (2016) 2473–2481. doi:10.1021/acscatal.6b00205.
- [47] A.Y.W. Sham, S.M. Notley, A review of fundamental properties and applications of polymer–graphene hybrid materials, *Soft Matter*. 9 (2013) 6645–6653. doi:10.1039/C3SM00092C.
- [48] L. Jourdin, Y. Lu, V. Flexer, J. Keller, S. Freguia, Biologically Induced Hydrogen Production Drives High Rate/High Efficiency Microbial Electrosynthesis of Acetate from Carbon Dioxide, *ChemElectroChem*. 3 (2016) 581–591. doi:10.1002/celec.201500530.
- [49] J.S. Deutzmann, M. Sahin, A.M. Spormann, Extracellular Enzymes Facilitate Electron Uptake in Biocorrosion and Bioelectrosynthesis, *MBio*. 6 (2015) e00496-15. doi:10.1128/mBio.00496-15.
- [50] K.A. Vincent, A. Parkin, F.A. Armstrong, Investigating and exploiting the electrocatalytic properties of hydrogenases, *Chem. Rev.* 107 (2007) 4366–4413. doi:10.1021/cr050191u.
- [51] V. Flexer, J. Chen, B.C. Donose, P. Sherrell, G.G. Wallace, J. Keller, The nanostructure of three-dimensional scaffolds enhances the current density of microbial bioelectrochemical systems, *Energy Environ. Sci.* 6 (2013) 1291–1298. doi:10.1039/C3EE00052D.
- [52] S.-E. Oh, B.E. Logan, Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells, *Appl. Microbiol. Biotechnol.* 70 (2006) 162–169. doi:10.1007/s00253-005-0066-y.
- [53] Reticulated Vitreous Carbon, [www.Duocelfoam.Com](http://www.duocelfoam.com). (n.d.). <http://www.duocelfoam.com/product-p/r4x4.htm> (accessed October 30, 2018).
- [54] The Carbon Nanotube Specialist - Nanocyl, (n.d.). <http://www.nanocyl.com/> (accessed October 30, 2018).
- [55] China Lithium Ion Battery Copper Foam for Battery Cathode Substrate, Purity> 99.99% - China Copper Foam, Battery Copper Foam, (n.d.). <https://gelonlib.en.made-in-china.com/product/boXJRnrDgFYk/China-Lithium-Ion-Battery-Copper-Foam-for-Battery-Cathode-Substrate-Purity-99-99-.html> (accessed October 30, 2018).
- [56] X. Xie, G. Yu, N. Liu, Z. Bao, C.S. Criddle, Y. Cui, Graphene–sponges as high-performance low-cost anodes for microbial fuel cells, *Energy Environ. Sci.* 5 (2012) 6862–6866. doi:10.1039/C2EE03583A.
- [57] R.A. Matula, Electrical resistivity of copper, gold, palladium, and silver, *J. Phys. Chem. Ref. Data*. 8 (1979) 1147–1298. doi:10.1063/1.555614.

Figure legends

Figure 1. (A) Copper foam and (B) rGO-CuF cathode. SEM images at (C) low- and (E) high-magnification of copper foam cathode, and (D) low- and (F) high-magnification of rGO-CuF foam cathode.

Figure 2. A) rGO foam and (B) epCu-rGO cathode. SEM images at (C) low- and (E) high-magnification of rGO foam cathode, and (D) low- and (F) high-magnification of epCu-rGO cathode. Panel F inset is EDS map of copper distribution on epCu-rGO cathode.

Figure 3. MES with copper foam cathode. (A) Acetate production ($\blacktriangle$), current density j (—), and electron transferred ($\bullet$ —) during *S. ovata*-driven MES. Electron transferred curve corresponds to the acetate production in mmol m^{-2} if all the electrons transferred were converted to acetate. Acetate production curve in mmol m^{-2} corresponds to the real progression of acetate concentration in the MES reactor detected by HPLC. (B) Current density with an abiotic copper foam cathode. No acetate was produced with the abiotic copper foam cathode. SEM images at (C) low- and (D) high-magnification of a copper foam cathode after 10 days of *S. ovata*-driven MES. Acetate production and electron transferred curves are the mean and standard deviation of three replicates. Current density curve shown is from a representative example of three replicate. d=day.

Figure 4. MES with rGO-CuF cathode. (A) Acetate production ($\blacktriangle$), current density j (—), and electron transferred ($\bullet$ —) during *S. ovata*-driven MES. Electron transferred curve corresponds to the acetate production in mmol m^{-2} if all the electrons transferred were converted to acetate. Acetate production curve in mmol m^{-2} corresponds to the real progression of acetate concentration in the MES reactor detected by HPLC. (B) Current density with an abiotic rGO-CuF cathode. No acetate was produced with the abiotic rGO-CuF cathode. SEM images at (C) low- and (D) high-magnification of a rGO-CuF cathode after 10 days of *S. ovata*-driven MES. Acetate production and electron transferred curves are the mean and standard deviation of three replicates. Current density curve shown is from a representative example of three replicate. d=day.

Figure 5. MES with rGO foam cathode. (A) Acetate production ($\blacktriangle$), current density j (—), and electron transferred ($\bullet$ —) during *S. ovata*-driven MES. Electron transferred curve corresponds to the acetate production in mmol m^{-2} if all the electrons transferred were converted to acetate. Acetate production curve in mmol m^{-2} corresponds to the real progression of acetate concentration in the MES reactor detected by HPLC. (B) Current density with an abiotic rGO foam cathode. No acetate was produced with the abiotic rGO foam cathode. SEM images at (C) low- and (D) high-magnification of a rGO foam cathode after 10 days of *S. ovata*-driven MES. Acetate production and electron transferred curves are the mean and standard deviation of three replicates. Current density curve shown is from a representative example of three replicate. d=day.

Figure 6. MES with epCu-rGO cathode. (A) Acetate production ($\blacktriangle$), current density j (—), and electron transferred ($\bullet$ —) during *S. ovata*-driven MES. Electron transferred curve corresponds to the acetate production in mmol m^{-2} if all the electrons transferred were converted to acetate. Acetate production curve in mmol m^{-2} corresponds to the real progression of acetate concentration in the MES reactor detected by HPLC. (B) Current density with an abiotic epCu-rGO cathode. No acetate was produced with the abiotic epCu-rGO cathode. SEM images at (C) low- and (D) high-magnification of an epCu-rGO cathode after 10 days of *S. ovata*-driven MES. Acetate production and electron transferred curves are the mean and standard deviation of three replicates. Current density curve shown is from a representative example of three replicate. d=day.

Figure 7. CLSM images of (A) a copper foam biocathode, (B) an epCu-rGO biocathode, (C) a rGO foam biocathode and (D) a rGO-CuF biocathode from *S. ovata*-driven MES reactors. Results shown are from a representative example of three replicate.

Table 1. MES performance with copper foam, rGO-CuF, rGO foam and epCu-rGO cathodes.^a

Cathode	Microbial catalyst ^b	Acetate production rate (mmol m ⁻² d ⁻¹) ^c	Average Current density (A m ⁻²) ^c	Coulombic efficiency for acetate (%) ^c	Hydrogen accumulation rate (mmol m ⁻² d ⁻¹) ^{c,d}
Copper foam	Sterile	N.D. ^e	-2.3 ± 1.1	N.A. ^f	995.0 ± 63.4
Copper foam	<i>S. ovata</i>	79.6 ± 24.4	-7.1 ± 2.5	10.0 ± 6.5	2500.9 ± 461.4
rGO-CuF	Sterile	N.D.	-4.6 ± 0.0	N.A.	2161.6 ± 223.5
rGO-CuF	<i>S. ovata</i>	1697.6 ± 298.1	-21.6 ± 2.1	70.2 ± 14.1	2599.2 ± 800.7
rGO foam	Sterile	N.D.	-1.1 ± 0.3	N.A.	493.4 ± 134.6
rGO foam	<i>S. ovata</i>	222.4 ± 33.1	-2.6 ± 0.5	76.4 ± 18.6	301.1 ± 77.8
epCu-rGO	Sterile	N.D.	-2.3 ± 0.6	N.A.	1031.6 ± 269.1
epCu-rGO	<i>S. ovata</i>	39.0 ± 19.6	-4.3 ± 0.0	8.1 ± 4.1	1517.1 ± 340.1

^a Cathode potential set at -990 mV vs SHE.

^b *S. ovata* is the wild type strain DSM-2662.

^c Each value is the mean and standard deviation of three replicates.

^d H₂ accumulation rate in the gas phase of the MES reactor.

^e Not detected.

^f Not applicable.

Graphical abstract

Highlights

- A cathode made of copper foam coated with reduced graphene oxide was fabricated.
- The novel cathode had outstanding performance for the MES of acetate from CO₂.
- MES with the composite cathode had an acetate production rate of 1697.6 mmol m⁻² d⁻¹.
- Biofilm on the composite cathode after 2 weeks of MES was still electroactive.
- Cathodes with a copper surface were antibacterial with low MES activity.

A **B** **C** **D** **E** **F**

Figure 1

A **B** **C** **D** **E** **F**

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7