Porous Nickel Hollow Fiber Cathodes Coated with CNTs for Efficient Microbial Electrosynthesis of Acetate from CO₂ using *Sporomusa ovata*

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Microbial electrosynthesis (MES) allows recycling of CO₂ into value added products by coupling renewable energy to the microbial ability for complex product formation. To improve biofilm formation on the cathode and the rates of product generation, the design of cathodes possessing high specific surface area and enhanced electrode-microbe electron transfer is needed. This study aimed to demonstrate a novel cathode design that is made of porous nickel hollow fibers (Ni-PHF), for facilitating direct delivery of CO₂ to *Sporomusa ovata* in MES through the pores in the hollow fibers. Modification of the surface of Ni-PHF with carbon nanotubes (CNTs) resulted in an 11-fold increase in the CO₂ adsorption capability at atmospheric pressure, as well as 76.3% reduction of cathode electron transfer resistance. This cathode surface modification partially explained the higher acetate production rate of 247 ± 17 mM/d/m² from direct CO₂ delivery through the pores of the Ni-PHF/CNT cathode, compared to 145 ± 4 mM/d/m² for the Ni-PHF cathode. Higher electron recovery in the form of acetate (~83%) was also observed for the Ni-PHF/CNT cathode. As for the tests where CO₂ was sparged into the media, acetate production was 36% lower than tests with direct CO₂ delivery to *S. ovata* through the pores of Ni-PHF/CNT cathode. These results demonstrate that using PHF electrode design and modifying cathode morphology to enhance the microbe-electrode interactions and CO₂ availability for bacterial growth are effective approaches to increase the rates of CO₂ reduction in MES.

Introduction

Carbon dioxide (CO₂) emissions are reported to have near-linear relationship with the global rising of temperature.¹ The need to reduce the level of CO₂ emissions from fossil fuel burning are expediting the development of novel technologies for renewable energy generation besides novel approaches for chemical production. Microbial electrosynthesis (MES) has been realized as a technology to reduce CO₂, a greenhouse gas, into value-added products, e.g. methane and acetate, through microbial catalysis with little electrical energy input.^{2,3} In MES, chemolithoautotrophic microbes growing on the surface of the MES cathode utilize the electrons (directly or indirectly as hydrogen) generated from the cathode to reduce CO₂ to multi-carbon organics.^{4,5} Various strategies have been adopted to enhance cathode specific surface area and

cathode-microbe interaction in order to facilitate the bacterial biofilm formation and electron transfer efficiency.⁶⁻⁹ These efforts mainly focused on surface modification of cathode and optimization of cathode architecture to enhance biocompatibility, porosity, catalytic properties and effective bacterial colonization area.⁶⁻⁹ In previous MES studies using submerged cathode materials, CO₂ was usually bubbled or sparged into the MES catholyte to serve as carbon source for chemolithoautotrophs on the cathode.^{8, 10-12} In these configurations, gas-liquid mass transfer could be one of the limiting factors for the CO₂ reduction process, mainly because of the low solubility of gaseous CO₂ in water.^{13,14} Gas diffusion electrodes (GDEs) in MES are able to enhance the direct CO₂ transfer to chemolithoautotrophs and thus improve the production of volatile fatty acids (VFAs), emphasizing the importance of direct mass transfer from the cathode to microbes in CO₂ conversion.¹⁵ However, the low specific surface area of flat GDEs makes them unsuitable for scale up of MES technology where high surface-to-volume ratio of the cathodes is desirable. Therefore, the design of cathodes possessing high specific surface area and optimum gas-liquid mass transfer are required for CO₂ reduction in MES systems. Metal-based Porous hollow fiber (PHF) cathodes using nickel (Ni) have been recently developed to recover clean water and biogas in electrochemical membrane bioreactors (EMBRs).¹⁶⁻¹⁸

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Applying porous Ni-PHF cathodes with small radial dimensions in MES can allow for direct delivery of CO₂ to chemolithoautotrophs through the pores of PHFs, thus reducing mass-transfer limitation.¹⁸ In addition to solving the issue of gas-liquid mass transfer limitations, the application of this type of cathode architecture in MES has several other advantages summarized as follows: (1) the high specific surface area of the cathode maximizes the diffusion of CO₂ gas; (2) the small radial dimensions of the hollow fiber cathodes maximize the surface-area-to-volume ratio, thus solving the issue of cathode packing density for large-scale applications; and (3) most importantly, this cathode architecture makes the MES process highly attractive for on-site carbon capture and utilization of CO₂ gas generated from various industrial sources.

In addition to mass-transfer limitation, low bacterial density on the cathode due to poor biocompatibility and insufficient electron transfer rates from the electrode to microbes can limit CO₂ conversion rates.¹⁹ Carbon nanotubes (CNTs) are known to be highly conductive and can promote electron transfer^{20–22}, which makes CNTs excellent electrocatalyst for hydrogen evolution reaction.^{23, 24} Also, they have high specific surface area, excellent mechanical strength and chemical stability.^{22, 25} Jourdin et al.^{7, 26} demonstrated the biocompatibility of multi-walled carbon nanotubes (MWCNTs) for mixed microbial cultures in CO₂ reducing MES showing uniform biofilm formation and high electro-catalytic currents. In this study, we report for the first time the development of a novel cathode material by first fabricating Ni-PHF and then coating them with MWCNTs by electrophoretic deposition, creating an advanced functional material with potentially high biocompatibility. The electrodes were explored for their CO₂ reduction performance in MES systems inoculated with *Sporomusa ovata*, which is commonly used species in MES studies for acetate production.^{8, 27} The combined advantages of Ni-PHF and CNTs (Ni-PHF/CNTs) were investigated with regards to specific surface area, electron transfer capacity, CO₂ adsorption and delivery methods which can lead to the improved CO₂ conversion rates to value-added products.

Experimental

Fabrication of Ni-PHF/CNT cathode

Fabrication of Ni-PHF cathode by a combined phase-inversion/sintering method has been previously reported.^{16, 28, 29} Briefly, Ni powders with an average particle size of ~1 µm were used as raw material and mixed with N-methyl-2-pyrrolidone (NMP, HPLC grade, 99.5%, Alfa Aesar) and Polyether Sulfone (PES, Ultrason E6020P, BASF) with stirring for 18 h. Polyvinylpyrrolidone (PVP, Alfa Aesar, M_w = 630, 000) was added to adjust the viscosity of the polymer solution. After degassing for overnight at room temperature and pressurizing with N₂ at 20 psi, the suspension was extruded through a tube-in-orifice spinneret with tap water as inner and outer coagulant. The PHF green body was dried at room temperature and then heated to 560 °C to remove the polymer compounds, followed by PHF reduction in H₂ atmosphere at 810 °C.

MWCNTs (MWNT-10, Shenzhen Nanotech) were deposited onto Ni-PHF surface through electrophoretic deposition (EPD), using a similar approach described in Jourdin et al.²⁶ After plasma-treatment in air and H₂SO₄/HNO₃ (1:3 in volume) acid etching, the negatively charged MWCNTs were repeatedly harvested by centrifugation (Sorvall ST 16R, Thermo Fisher) and washed with deionized (DI) water before air drying. The MWCNTs were then suspended in DI water at a concentration of 200 mg/L using a sonicator (Qsonica, USA) with a ¼ inch microtip and 40% amplitude for 1 h. A voltage of 35 V was applied (3645A, Circuit Specialists) to carry out EPD of MWCNT with Ni-PHF serving as the anode and stainless-steel mesh bent in a cylinder shape as the cathode (Scheme S1). The distance between Ni-PHF and stainless-steel mesh was ~2 cm. Since the voltage applied was high enough for oxygen evolution reaction on the active surface of Ni-PHF, a large amount of small oxygen bubbles began to accumulate and detach from the anode surface along with some CNTs after 10 mins of EPD, resulting in inefficient CNT coating on Ni-PHF. Thus, the CNT-coated Ni-PHF were oven-dried every 10 min of EPD to avoid violent oxygen evolution reaction and detachment of CNTs from the Ni-PHF surface while MWCNT suspension was sonicated during the Ni-PHF drying period. The EPD process was repeated six times to obtain a thick layer of MWCNT coating on PHF surface.

Fourier-transform infrared spectroscopy (FT-IR)

The functional groups introduced onto the MWCNT surface after plasma and acid treatment were detected with FT-IR (NICOLET iS10, Thermo Fisher). Prior to FT-IR analysis, treated MWCNT samples were vacuum-dried at room temperature for 4 h and grinded into fine powders. The analysis was repeated 3 times for each sample.

CO₂ adsorption and specific surface area

CO₂ adsorption capability and specific surface area of Ni-PHF before and after MWCNT deposition were measured by a pressure sorption analyzer (ASAP 2050, Micromeritics) and a mercury porosimeter (AutoPore IV 9500, Micromeritics), respectively. Samples were first vacuum-dried at 150 °C for 3 h to remove the adhered moisture and gases on the sample surface before the final adsorption test. All the calculations were based on the weight vacuum-dried cathode materials.

Linear sweep voltammetry and electrochemical impedance spectroscopy

Before the start of the MES experiments, linear sweep voltammetry (LSV) was used to evaluate the cathode properties of the Ni-PHF/CNT compared to Ni-PHF at a scanning rate of 1 mV/s from -0.1 V to -1 V (vs. Ag/AgCl). Electrochemical impedance spectroscopy (EIS) was carried out to analyze the electron transfer property of the cathode by applying a sine signal of 5 mV amplitude between cathode and anode, ranging the frequency from 100 kHz to 0.01 Hz. The same media used during the MES experiments (see below) was purged with pure N₂ for 20 min before LSV and EIS analysis. LSV and EIS were carried out using a potentiostat (VMP3, Biologic Science instruments).

Growth and enrichment of *S. ovata*

S. ovata DSM 2662 was acquired from Deutsche Sammlung Mikroorganismen und Zellkulturen (DSMZ). *S. ovata* was initially grown in DSMZ 311 media. Followed by growth, cells were activated for autotrophic growth in a separate serum vial under a H₂:CO₂ (80:20) atmosphere. Casitone, betaine and yeast extract were omitted from the growth medium during the enrichment with CO₂ as the only carbon source and H₂ as the electron donor. Betaine, casitone, yeast extract, cysteine, Na₂S and resazurin were omitted from the media for MES tests at all time.

Construction and operation of MES cells

The MES cells were constructed using a dual-chamber reactor separated by a Nafion 117 ion-exchange membrane (VWR, UK). All reactors were run in duplicates. The anode was made of IrO₂-coated carbon cloth (0.5 mg/cm² IrO₂/C, 24 cm²). Ni-based PHF cathodes (four Ni-PHF or Ni-PHF/CNT with projected surface area of 13.5 cm² or 14.5 cm², respectively) were bundled together using a nickel wire and conductive epoxy. The bottom end of each fiber was sealed with epoxy, while the other end was inserted into a silicone tubing connected to a 250-ml gasbag filled with 20% CO₂. The junction of silicone tubing and the hollow fiber bundle was covered with epoxy glue to make the PHF module gas-tight before submersion into 125 mL of DSMZ 311 media. The cathode potential was set at -600 mV vs. Ag/AgCl reference electrode using VMP3, BioLogic potentiostat. H₂/CO₂-enriched *S. ovata* was transferred to the cathodic chamber under a H₂:CO₂ (80:20) atmosphere with mild stirring (150 rpm). The mixed gas was switched to N₂:CO₂ (80:20) stored in the gasbag connected to the silicone tubing after first batch and delivered through the pores of the Ni-based PHF cathodes. The MES cells were operated for over 100 days in a fed-batch mode before we took a small piece from the Ni-PHF/CNT cathode for SEM imaging and before changing the CO₂ delivery method. In the last two batches, the gas delivery method was changed from direct delivery of N₂:CO₂ (80:20) through the pores of Ni-PHF/CNT cathodes to sparging of 50 ml of N₂:CO₂ (80:20) into the media every 1.5 days. Each batch lasted for about 7-8 days and new DSMZ 311 media (lacking betaine, casitone, yeast extract, cysteine, Na₂S and resazurin) was used for each batch test.

High performance liquid chromatography (HPLC) and Gas chromatography (GC)

The samples (1~2 ml) were collected every 1 or 2 days from the cathode chambers for measuring acetate concentration. The acetate concentration in the MES was measured with HPLC (Accela, Thermo Fisher) as previously reported.³⁰ A solution of 5 mM H₂SO₄ (0.5 ml/min) was used as a flow carrier. Where mentioned, acetate production rates were normalized to the outer projected surface area of Ni-based PHF cathodes.

For off-gas analysis, a GC (310C, SRI Instruments) was used¹⁷ to quantify the volume of hydrogen gas produced in the reactor headspace and the gasbag connected to the top of the reactor at the end of each batch cycle.

Cathode surface and biofilm characterization

The surface of the cathodes before and after MES operation with direct CO₂ delivery were analyzed with scanning electron microscopy (SEM) equipped with an Everhart-Thornley detector (Quanta 600, FEI). Energy-dispersive X-ray spectroscopy (EDS) was used to determine the elemental composition on the surface of Ni-PHF and Ni-PHF/CNT cathodes. Biofilms on Ni-PHF and Ni-PHF/CNT cathodes were first fixed with a phosphate buffer solution (PBS) containing 3% glutaraldehyde overnight at 4 °C and then dehydrated in a series of graded ethanol solutions. After 4 nm iridium coating, samples were characterized with SEM at an accelerating voltage of 5 V under high vacuum condition.

The LIVE/DEAD® BacLight™ Bacterial Viability Kit (Thermo Fisher) was used to determine the viability of *S. ovata* cells on Ni-PHF and Ni-PHF/CNT cathodes, as previously reported³¹ and CLSM (LSM 710 upright, Zeiss) images were taken.

Biomass on the surface of Ni-based biocathodes was measured based on Lowry method³² and previous reports by Chen et al.^{33, 34} Briefly, protein in cathodic biofilm was harvested and determined by DC-protein assay kit (BIO-RAD Laboratories, Inc.) after being re-suspended in DI water, with a series of graded Bovine Serum Albumin (BSA, Sigma) solution as standards.

Results and discussion

Fabrication and characterization of Ni PHF-based cathode

MWCNTs were plasma oxidized and acid treated to create negatively-charged surface by introducing hydrophilic sites and some functional groups, such as -COOH, -OH and >C=O, which were essential for the preparation of homogeneous deposition solution.^{35, 36} The FT-IR spectrum for the acid-treated MWCNTs, clearly showed two significantly enhanced adsorption peaks at 1635.42 and 3444.24 cm⁻¹ (Fig. 1), which typically resulted from the >C=O carbonyl vibrations of carboxylic groups and the -OH stretching vibration, respectively. This means -COOH group was introduced to the surface of MWCNTs and thus the hydrophilic sites were successfully created.

After EPD of MWCNTs, the Ni-PHF and Ni-PHF/CNT cathodes were characterized with SEM. A uniform distribution of macro pores (average pore size: 1-2 μm) was exhibited on the Ni-PHF surface before CNT coating (Fig. 2A and 2B). EDS analysis indicated that the Ni-PHF surface was composed of 97.6 wt% Ni, 1.6 wt% O and 0.8 wt% C (Fig. S1A), demonstrating excellent reduction of the Ni element with H₂ at high temperature during its fabrication. After EPD, the Ni-PHF surface was fully covered by a uniform layer of MWCNTs (Fig. 2C and 2D) with 93.22 wt% C, 4.65 wt% Ni and 2.13 wt% O as shown in EDS analysis (Fig. S1B). This further enhanced the surface roughness and pore density of PHFs (average pore size: 500 nm – 3 μm). The cross-section of Ni-PHF/CNT cathodes exhibited a ~ 50 μm thick CNT layer closely attached to the Ni-PHF surface (Fig. 2F) after 60 mins of EPD. Sixty minutes were sufficient to obtain a full coverage of MWCNT on the Ni-PHF. The specific surface area of Ni-PHF/CNT cathodes (20.6 m²/g) measured with a mercury porosimeter was increased by 5.02 m²/g compared to Ni-PHF cathodes (15.6 m²/g). Since the

specific surface area and roughness play an important role in enhancing bacterial adhesion and colonization^{37–39}, thick MWCNT layers are expected to attract more biomass on the cathode surface and thus improve the CO₂ conversion efficiency.

The CO₂ adsorption of Ni-PHF and Ni-PHF/CNT cathodes was analyzed by a pressure sorption analyzer, to test the capability of the cathode to capture CO₂. The CO₂ adsorption capability was significantly improved after CNT deposition on Ni-PHFs, and at around atmospheric pressure the CO₂ adsorption of Ni-PHF/CNT cathode was 11.1 ± 0.3 times higher than Ni-PHF (Fig. S2), suggesting more CO₂ could be available on the cathode surface for chemolithoautotrophic activities. Since most of CO₂ adsorption is a combination of physisorption and chemisorption, strong van der Waals forces between adsorbents and CO₂ molecules are required for high CO₂ adsorption if no chemical interaction occurs at room temperatures.⁴⁰ Since CO₂ has been reported to easily desorb from nickel surface due to weak van der Waals forces and chemical interactions⁴¹, the 11.1 times higher CO₂ adsorption in the Ni-PHF/CNT cathode is possibly due to the deposited CNT layers, which possess strong physical forces with CO₂ molecules.^{40, 42} Also, improved CO₂ adsorption on the Ni-PHF/CNT could be due to the large surface area and geometry of CNTs, which exhibit different regions and sites for adsorption.⁴³

Since VFA production from CO₂ could be achieved through direct electron transfer and/or hydrogen-mediated electron transfer in MES^{4, 5, 44}, LSV and EIS were performed in the double-chamber reactors before MES operation, to characterize hydrogen evolution reaction and electron transfer resistance of Ni-PHF and Ni-PHF/CNT cathodes, respectively. LSV curves showed that the Ni-PHF/CNT cathode had similar onset potentials compared to Ni-PHF (sharp increase of current at ~ -0.55 V vs. Ag/AgCl, Fig. 3A). However, Ni-PHF/CNT had much better catalytic activity for hydrogen evolution reaction (HER) with regards to the current density at -1.0 V, which was 1.66-fold higher than that of the Ni-PHF (2 vs. 1.2 mA/cm², Fig. 3A). The influence of MWCNT layers on the HER activity of Ni-PHF cathode was further proven with Tafel plots (Fig. 3B), since Tafel slopes derived from LSV data are commonly believed to be an indication of HER catalytic property of electrode materials.⁴⁵ The Ni-PHF/CNT cathode exhibited a 15 % lower Tafel slope (342 mV dec⁻¹) compared to Ni-PHFs (393 mV dec⁻¹), demonstrating Ni-PHF/CNT as a better HER catalyst. The lower Tafel slope of Ni-PHF/CNT could be attributed to low electron transfer resistance after MWCNT coating and more electro-catalytical active sites formed on the surface of Ni-PHF/CNT networks for hydrogen evolution reaction.^{23, 46}

The electron transfer resistance of the cathodes was estimated by best fitting the Nyquist plots with EC-Lab software (Fig. 3C). It is stated that a slope of the Nyquist plot at the low-frequency region close to 90° exhibits excellent capacitive behavior, which means better electron charge-discharge capability of the electrode.⁴⁷ The fitting curve for Ni-PHF/CNT cathodes displayed a steeper slope compared to Ni-PHF

cathodes, indicating better capacitive behavior for Ni-PHF/CNT (5.83×10^{-4} F for Ni-PHF/CNT vs. 4.98×10^{-4} F for Ni-PHF obtained from the equivalent circuit). The real-component axis intercept at the high-frequency region represents the sum (R_s , which is R_1 in the equivalent circuit) of the solution resistance and the contact resistance between electrode and current collector, and the diameter of the semicircle impedance loop (R_{ct} , which is R_2 in the equivalent circuit) could be interpreted as the faradaic charge-transfer resistance in the electrode materials. It was clear that the Ni-PHF cathodes exhibited lower R_s (~ 2.80 ohm) than Ni-PHF/CNT (~ 5.06 ohm), owing to the lower contact resistance (Fig. 3C). However, the Ni-PHF/CNT cathode showed a much smaller semicircle than Ni-PHF in Nyquist plot, which indicates lower charge-transfer resistance (0.66 ohm for Ni-PHF/CNT vs. 2.79 ohm for Ni-PHFs) between electrolyte and CNT layer and within CNTs. The low charge-transfer resistance is expected to enhance the electron transfer between cathode and microbes in MES.^{47, 48}

Microbial electrosynthesis of acetate with Ni-PHF and Ni-PHF/CNT cathodes

Both types of cathodes were tested abiotically (free of bacterial cells) in MES reactors for H₂ production from HER and acetate production from CO₂ with the cathode potential set at -0.6 V vs. Ag/AgCl. No acetate was detected in the abiotic experiments. After 7.5 days, a small amount of H₂ was produced by the Ni-PHF/CNT (0.23 ± 0.10 ml) and Ni-PHF (0.14 ± 0.03 ml) cathode, which was much lower than the minimum threshold for acetate production from hydrogen by acetogens⁵. The very small hydrogen production was due to the relatively high cathode potential (-0.6 V vs. Ag/AgCl) tested in this study, which was close to the onset HER potential of both electrodes (-0.55 V vs. Ag/AgCl from LSV curves, Fig. 3a). Since *S. ovata* is presumably able to bio-electrochemically produce acetate with no H₂ involved, the reactors were then inoculated with pure culture of *S. ovata*. The acetate production in MES became obvious after 2-month enrichment of *S. ovata* on Ni-based PHF cathodes. The volumetric acetate and biogas production rate was calculated based on the cathode chamber volume (125 mL) and the outer projected surface area (Ni-PHF: 13.5 cm², Ni-PHF/CNT: 14.5 cm²) of the cylindrical cathode morphology. Acetate production rate for Ni-PHF/CNT cathode in MES reactor was 247 ± 17 mM/m²/day compared to 145 ± 4 mM/m²/day for Ni-PHF cathode (Table 1 and Fig. 4), which means a 1.7-fold faster acetate production rate. Higher current density was also observed for Ni-PHF/CNT cathodes (332 ± 24 mA/m² vs. 214 ± 14 mA/m² for Ni-PHF). Since Ni-PHF/CNT has lower electron transfer resistance in EIS analysis and little or no hydrogen was observed in the abiotic and biotic experiments at -0.6 V vs. Ag/AgCl, the increase in acetate production rates and current densities of the Ni-PHF/CNT cathodes could be largely attributed to a combination of factors including lower electron transfer resistance, higher specific surface area and better CO₂ adsorption capability, even though we could not rule out completely the possibility of H₂-mediated acetate production. It should be noted that a deeper mechanistic understanding of CO₂ reduction (i.e. direct electron transfer or H₂-mediated) via

S. ovata was not within the scope of this study. Jourdin et al.^{7, 26, 49} compared reticulated vitreous carbon/CNT (RVC/CNT) cathodes with bare RVC cathodes for CO₂ reduction to acetate in MES. The authors suggested that better biocompatibility and higher specific surface area induced by CNTs were responsible for the higher acetate production from CO₂ compared with bare RVC cathode. However, it has been reported that better CO₂ adsorption is one of the key factors determining the CO₂ conversion efficiency and probably lead to enhanced electrochemical or photocatalytic CO₂ reduction.^{50–52} This principle could be applied in aqueous MES reactors as well. For example, Zhang et al.⁵¹ reported high CO₂ reduction to formate and high coulombic efficiency (CE: 87%) in aqueous media using nitrogen-doped carbon nanotube/Polyethylenimine (PEI) composite materials. Several reasons for the CO₂ reduction enhancement, such as the excellent CO₂ adsorption capability based on van der Waals forces and greatly reduced overpotential of the N-doped CNT/PEI, were deduced from their analysis. Also, higher volatile fatty acid (VFA) production in MES⁵³ and power output in MFC⁵⁴ were reported due to the reduced overpotential and improved charge transfer of the biocathodes. All of these reports reveal the importance of enhanced CO₂ adsorption, reduced charge transfer resistance and overpotential in MES operations.

The average acetate production rates reported in the current study using *S. ovata* and delivering CO₂ directly through the pores of Ni-based PHF cathodes, are much higher than carbon cloth (88 ± 8 mM/m²/day), carbon felt (136 ± 43 mM/m²/day) or graphite stick cathodes (137.2 ± 28 mM/m²/day) using also *S. ovata* as inoculum.^{6, 8, 27} Higher acetate production rates indicate the advantage of the porous PHF cathode architecture for direct CO₂ delivery over flat electrodes. Considering the somewhat less negative set-potential for the cathodes (−0.6 V vs. Ag/AgCl), the acetate production with Ni-based PHF cathode in *S. ovata* MES reactors is at least 2 times higher compared with carbon cloth (30 ± 7 mM/m²/day) or graphite stick cathode (76 mM/m²/day) under similar growth conditions (i.e., set potential).^{55, 56}

In the last two batches of the experiment, the method of CO₂ delivery was changed from direct delivery through the pores of the Ni-PHF/CNT cathode to CO₂ bubbling into the media every 1.5 days. The pH of the MES media changed from 7.35 to 8.5 before the next CO₂ sparging. To maintain the pH and enhance the CO₂ dissolubility, the CO₂ sparging was conducted at a slower rate (< 1 ml/min). The acetate production rate obtained with CO₂ diffusion through the media to *S. ovata* cells on the Ni-PHF/CNT surface was 158 ± 3 mM acetate /m²/day with a CE of 69 ± 1% (Fig. 4C and Table 1). The acetate production rate (student's t-test, *P* < 0.05) was significantly lower compared to those obtained with CO₂ delivered directly through the pores of Ni-PHF/CNT cathode, clearly illustrating the importance of direct CO₂ delivery through PHF pores for enhancing conversion rates in MES systems.

The current consumption of the Ni-PHF/CNT cathode was also enhanced by 1.55 times with a CE of 83 ± 8%, which was slightly higher than that of the Ni-PHF cathode (75 ± 5%) (Table 1). Higher coulombic efficiency of Ni-PHF/CNT is due to the

combined effect of higher CO₂ adsorption and enhancement of electron transfer between cathode materials and *S. ovata* biofilm at the electrolyte-electrode interface.^{2, 57} Since ohmic losses and biomass growth were proposed to consume electrons,⁵⁷ lower EIS charge transfer resistance means less electron would be wasted before microbial catalytic CO₂ conversion, while higher CO₂ adsorption and mass transfer on hydrophilic surface indicate more CO₂ available for bacterial growth and faster CO₂ conversion possibility.

The biofilm coverage on the surface of the cathodes after MES operation with direct CO₂ delivery through PHF pores was characterized using SEM and CLSM images. By comparing Fig. 5 A and C, we could clearly observe more *S. ovata* cells (rod-shaped) on the Ni-PHF/CNT surface, indicating enhanced biocompatibility with CNT layers^{7, 26}. Bacterial cells in higher-resolution SEM images (Fig. 5B & D) exhibited complete cellular structures without any detectable cell membrane damage. Apart from more clumped biofilm, white solid particles were more visible on the Ni-PHF/CNT surface (Fig. 5). Further EDS analysis indicated that the white dots mainly originated from calcium carbonate (CaCO₃) and calcium phosphate precipitates (40.9 At% C, 35.41 At% O, 8.9 At% P and 7.12 At% Ca). Given the low concentration of soluble CO₂, acetate production by microbial electrochemical reduction of CO₂ resulted in a pH increase (from 7.35 to 8.55 due to proton consumption) and this may have resulted in the precipitation of CaCO₃ and calcium phosphate. Probably due to the excellent salt and CO₂ adsorption capability of MWCNTs induced by high surface area and negatively charged functional groups of MWCNT^{58, 59}, calcium-related precipitates are much easier to precipitate on Ni-PHF/CNT surface than Ni-PHF.

The biocathodes were cut into thin slices after fixation using a microtome (CM3050 S, Leica) to check the biofilm thickness and uniformity by CLSM imaging. CLSM images with live *S. ovata* cells stained in green confirmed that a thicker active biofilm was enriched on the Ni-PHF/CNT cathode (~10 μm) than Ni-PHF cathode (~5 μm) (Fig. 6A and 6B). Dead bacterial cells stained in red were more continuous and uniformly distributed on the Ni-PHF/CNT surface (Fig. 6C and 6D). The concentration of protein on the Ni-PHF/CNT cathode was 157 ± 13 μg protein/cm², compared to 62 ± 38 μg protein/cm² on Ni-PHF surface. These values suggest that growth of *S. ovata* on Ni-PHF/CNT was greatly enhanced compared to Ni-PHF electrode since protein concentration is usually correlated with biomass growth.⁶⁰ Upadhyayula and Gadhamshetty⁶¹ reported that bulk CNT aggregates might produce interstitial space and groove space between CNT bundles, which falls in the range of mesopores or macropores and would be suitable for the adsorption of microbes. This agrees with our SEM observation where interstitial and groove pores formed between CNT bundles with pore sizes ranging from a few hundred nanometers to 1–5 micrometers (Fig. 2D), which are large enough for bacterial colonization (Fig. 5D). Since biomass density on the MES cathode, CO₂ availability and microbe-cathode electron transfer are crucial for VFA production from CO₂, the Ni-PHF/CNT cathode proved to have much better performances than Ni-PHF.

Conclusions

This study developed a simple but effective method to fabricate porous PHF cathodes for direct CO₂ delivery to chemolithoautotrophs in MES system. By functionalizing the Ni-PHF with superconductive MWCNTs, the specific surface area, CO₂ adsorption capability and charge transfer of the cathode were significantly enhanced, and these in turn facilitated bacterial adhesion and acetate production rate, as well as electron utilization.

Besides improving acetate production rates, the integration of porous PHF cathodes in MES system allowed for the direct CO₂ delivery to chemolithoautotrophs and this could address the needs in industries for direct utilization of flue gas, since no further CO₂ treatment is required. The key challenges are now to create a suitable reactor architecture that minimizes anode / cathode spacings, to limit ohmic drop, and to further develop these types of electro-catalytic and porous PHFs to achieve higher level of performance at an increasing scale.

Conflicts of interest

There are no conflicts to declare.

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Ni-PHF/CNT Cathode

Scheme 1 Microbial electrosynthesis cell with Ni-PHF/CNT cathode. 1) Anode compartment; 2) Cathode compartment; 3) Reference electrode; 4) Gasbag for collecting oxygen; 5) Gasbag for collecting biogas; 6) Potentiostat; 7) Gasbag for delivering CO₂.

Figure 1 Fourier-transform infrared spectroscopy (FT-IR) spectra of the original and oxidized MWCNTs after plasma and acid treatment.

Figure 2 Scanning electron micrograph (SEM) images of the surface morphology of the (A, B) Ni-PHF cathode and (C, D) Ni-PHF/CNT cathode. SEM images of the cross-sections of the (E) Ni-PHF and (F) Ni-PHF/CNT cathode.

Figure 3 (A) Linear sweep voltammetry (LSV) obtained for the Ni-PHF and Ni-PHF/CNT electrode in sterile DSMZ 311 media after omitting all organic carbon sources, with a potential window of -0.1 to 1 V vs. Ag/AgCl and scan rate of 1 mV/s. (B) Corresponding Tafel plots derived from LSV data of the Ni-PHF and Ni-PHF/CNT cathodes. The curves in the low current density region were considered for Tafel plots. (C) Nyquist plots of Ni-PHF and Ni-PHF/CNT in sterile blank media. The inset is the equivalent circuit for EIS fitting.

Figure 4 Electron transfer, measured acetate production and current consumption of (A) the Ni-PHF cathode and (B) the Ni-PHF/CNT cathode, both with direct CO₂ delivery through the pores of the hollow fibers. (C) MES performance of the Ni PHF/CNT cathode with CO₂ bubbled into the media. The electron transfer curves represent the acetate production if all the transferred electrons are converted into acetate. The acetate concentration curves correspond to the actual acetate production detected by HPLC in the MES reactors.

Figure 5 Scanning electron micrograph (SEM) images of (A, B) the Ni-PHF cathode and (C, D) the Ni-PHF/CNT cathode after 80 days of fed-batch operation showing well-colonized *S. ovata* biofilm on the surface of the cathodes.

Figure 6 Confocal laser scanning microscopy (CLSM) images of live *S. ovata* cells (green) at the edge surface of (A) the Ni-PHF cathode and (C) the Ni-PHF/CNT cathode in MES reactors. (B) and (D) are the cross-section views of dead *S. ovata* cells (red) on the Ni-PHF and Ni-PHF/CNT biocathodes, respectively.

Table 1 Average current density and acetate production rate from CO₂ in MES.

Cathode	Acetate production rate ^a		Current density ^a (mA/m ²)	Coulombic efficiency ^b
	mM/m ² /day	g/m ² /day		
Ni-PHF	145 ± 4	1.09 ± 0.03	214 ± 15	75 ± 5
Ni-PHF/CNT	247 ± 17	1.85 ± 0.13	332 ± 24	83 ± 8
Ni-PHF/CNT with CO ₂ diffusion from media	158 ± 3	1.19 ± 0.02	244 ± 12	69 ± 1

^a Each value is the mean and standard deviation of two duplicate reactors, and is calculated based on cumulative acetate concentration from HPLC after 7.5 days or 8 days and the projected surface area of the corresponding cathode material. ^b Coulombic efficiency is calculated based on the equation: $CE = \frac{Q_{\text{acetate}}}{Q_{\text{total}}}$ (where Q_{acetate} is the coulomb required for the acetate production (measured by HPLC) in one batch, Q_{total} is the total coulomb produced by current in the corresponding batch).