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Enhanced methane producing microbial electrolysis cells for wastewater treatment using poly(neutral red) and chitosan modified electrodes†

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Microbial electrolysis cells (MECs) consisting of a bioanode and biocathode offer a promising solution for wastewater treatment. These systems can degrade organic substances at the bioanode while converting carbon dioxide (CO_2), a major greenhouse gas, to a value-added fuel, methane (CH_4) at the biocathode. The bioelectrodes were inoculated with a mixed culture under anaerobic conditions. By applying a constant potential of 0.40 V *vs.* Ag/AgCl (3 M NaCl), the long-term performance of MECs has been studied by monitoring the removal of chemical oxygen demand (COD) in the anolyte which contained synthetic wastewater and CH_4 generation in the cathode chamber. To investigate the effect of electrode modification, poly(neutral red) and chitosan modified carbon felt electrodes were prepared, and applied in MECs. The results revealed that MECs with modified electrodes showed remarkably enhanced overall performance. The average COD removal efficiency, faradaic efficiency towards CO_2 reduction to CH_4 and CH_4 production yield of modified MECs were up to 67%, 55% and 0.14 L_{CH_4}/g_{COD}, respectively.

Introduction

The rise in anthropogenic carbon dioxide (CO_2) content, mainly due to fossil fuel burning, leads to an increase in global temperature and further to climate change. This has been first recognized and manually calculated by Svante Arrhenius in his paper back in 1896.[1] Not only the environmental effects, but also the depletion of such fuels is critical. Therefore, cleaner and more sustainable sources of energy are urgently needed.[2–4] During the last few decades, bio-electrochemical systems (BESs) have gained a lot of interest.[3] The BES is, by definition, an electrochemical system where at least one of the redox reactions is microbially assisted and the electrode at which such a reaction occurs is called a bioelectrode (bioanode and biocathode).[5]

BESs can be implemented in broad applications including electricity generation,[5] wastewater treatment,[6–8] bioremediation[9,10] and production of energy storage chemicals *e.g.* methane (CH_4),[2] formate,[11] and ethanol.[12] Such BESs are operated normally under mild working conditions, show high selectivity towards the desired product, and offer great availability and self-regeneration properties.[5] The first evidence of electrical interaction between microorganisms, which were later named electroactive microorganisms, and an electrode was reported by Potter *et al.* in 1911.[13] This extracellular electron transfer[3,4,13] includes two mechanisms: direct and indirect/mediated electron transfer.[14,15] Direct electron transfer is the inherent interaction between a bacterium, for example *Geobacter sulfurreducens*, and an electrode through redox proteins such as cytochromes or conductive pili, mentioned as nanowires.[14–19] On the other hand, indirect electron transfer describes an interaction between a microorganism and an electrode with the aid of electron shuttles, either endogenous moecules[20] (generated by microorganisms themselves as their secondary metabolites, like flavin,[20] phenanzine[21] and quinones[18]) or exogenous molecules[22,23] (externally added like methylene blue[23] and methyl viologen[24]).

Bioanodes have been investigated for their capabilities of oxidizing organic matter, like glucose,[25] acetate[26] and more complex substrates like landfill leachate[27] and wastewater from various sources.[6] Such oxidation generates electrons, which are

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transferred to the anode. For example, 24 electrons could be harvested from the oxidation of glucose, as shown in eqn (1) ($E^{0'}$ = −0.41 V *vs.* standard hydrogen electrode (SHE)).[28] A system, which contains a bioanode and an abiotic cathode, is called a microbial fuel cell (MFC).[4] This technology is considered as a promising wastewater treatment system due to its capability of organic oxidation concomitant with energy harvesting directly in electricity form.[6]

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \tag{1}$$
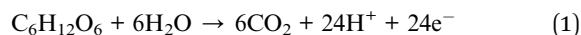
In conventional MFCs, protons are generated from the oxidation of organic matters and coupled with oxygen, an electron acceptor, generating water at the cathode. To improve the efficiency of the system, introduction of prompted catalysts (like platinum) for useful chemical generation in the cathode chamber has been investigated.[29] Instead of using expensive materials, microbial biocathodes are an alternative potential option.[17,30–33] The direct electron uptake from the cathode through microbes has been adopted for several applications, for example, treatment of heavy-metals (bioremediation),[34] reduction of nitrate (denitrification)[35] and reduction of CO_2 to value-added fuels such as formate[11] and CH_4 (eqn (2), $E^{0'}$ = −0.24 V *vs.* SHE).[2] Such BESs, where energy is invested to enhance reaction kinetics or overcome thermodynamic energy barriers, are called microbial electrolysis cells (MECs).[5] The electrochemical CO_2 reduction to CH_4 usually suffers from large overpotential (for example, an overpotential of 1.22 V from using Cu electrodes[36]). Our group has shown the utilization of such microbial cathodes in a MEC for CO_2 reduction to CH_4 with a relatively low over-potential of 0.25 V [37] and it has been reported that CH_4 production was observed in a broad range of production rates.[38] The combination of a bioanode and a biocathode now shall accomplish two tasks simultaneously and utilize the advantages of introducing microorganisms to both electrodes.[39–42] The coupling of CO_2 reduction with the aforementioned microbial oxidation of organic substances is, in principle, feasible by using electrons, protons and CO_2 which are generated during oxidation on the bioanode.[42] The required voltage of such systems could theoretically be eliminated due to a positive

electrochemical cell voltage for CH_4 production with glucose oxidation (E_{cell} = 0.17 V).

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \tag{2}$$

A schematic of an MEC system where a bioanode and a biocathode are combined, is demonstrated in Fig. 1a. Such systems have been investigated for various applications *e.g.* oxygen reduction to hydrogen peroxide,[30] denitrification[35] and CO_2 reduction to CH_4.[42,43] The MEC approach for CH_4 production offers several advantages, for example the tunability of the degradation/generation rate.[41] Therefore, the MEC could be an effective addition to the existing anaerobic digestion systems by improving biogas quality.[5,44,45] The performance of BESs largely depends on the quality of electroactive biofilms on electrodes and also on the electron transfer process.[46,47] Therefore, several attempts have been made to investigate the effects of different electrode materials and modifications.[48] Various electrodes including carbon-based materials (*e.g.* graphite, carbon felt, carbon cloth and carbon mesh) and other inert materials (*e.g.* stainless steel) are commonly used in BESs.[14,29,46] Further, the surfaces of these materials were modified, aiming to improve microbial growth and electron transfer processes. One example is the modification of carbon-based electrodes with positively charged materials like ammonia,[49] chitosan,[50] or conductive polymers, for example, polyaniline[51] and polypyrrole.[52] Chitosan is a bio-polymer and a derivative of chitin, a component of arthropod exoskeletons. It has been applied in many applications due to its low cost, biocompatibility, non-toxicity and high chemical and thermal stability.[53] These modifications resulted in improved bacterial colonization and enhanced electron transfer, which further improved the overall performance of BESs.[54,55] Another interesting material is poly(neutral red). It is a conductive polymer which could be prepared electrochemically in a slightly acidic to neutral pH solution[56] and its monomer, neutral red, is a staining dye which was used in various bio-electrochemical systems as a redox mediator to facilitate electron transfer between the microbe and electrode.[57–59] Recently, we reported on the contribution of poly(neutral red) modified carbon felt cathodes, for the enhancement of microbial electrochemical reduction of CO_2 to formate by
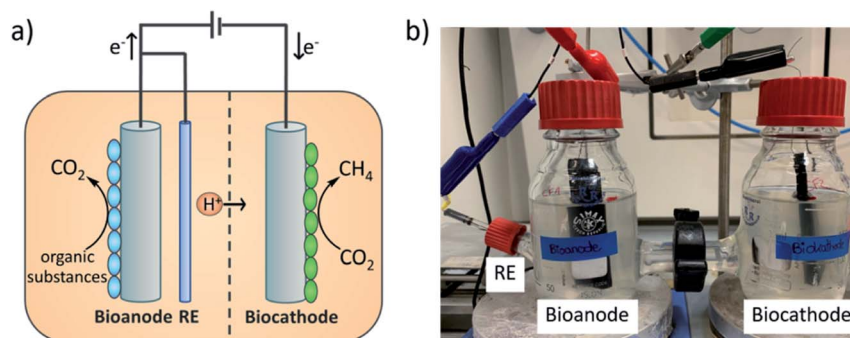

Fig. 1 (a) Schematic two-compartment MEC consisting of a bioanode, a reference electrode (RE) and a biocathode, where the oxidation of organic substances occurs at the bioanode and the reduction of CO_2 to CH_4 takes place at the biocathode and (b) a picture of cell geometry used in this study.

Methylobacterium extorquens. The study showed an improvement in electron transfer processes leading to higher formate production rates, as compared to non-treated electrodes.[60] However, to the best of our knowledge, each of the chitosan and poly(neutral red) electrode modifications has not been investigated for the anode and cathode at the same time in a MEC. Therefore, this idea has attracted our interest in order to investigate the effect of modified electrodes not only on one redox reaction but also on both redox processes simultaneously.

In this study, we report a full assembly of an MEC (Fig. 1b), equipped with bioelectrodes, for two applications: wastewater treatment at the bioanode and CO_2 reduction to CH_4 at the biocathode. The long-term performance of the methane-producing MEC for synthetic wastewater treatment was monitored. The system was evolved by using bioelectrodes (bioanode and biocathode) inoculated with the same mixed culture microorganisms. The experiments were performed at a controlled anode potential of 0.40 V *vs.* Ag/AgCl (3 M NaCl) and the oxidation of synthetic wastewater was observed by monitoring the change of the anolyte chemical oxygen demand (COD) value, which reflected directly the efficiency of wastewater treatment. Moreover, electromethanogenesis at the biocathode was tracked by CH_4 generation in the headspace. Furthermore, carbon felt electrodes were modified with chitosan and poly(neutral red), serving as supports for microorganisms for both the cathode and anode. These two materials were chosen due to the previously reported results, low material costs, good biocompatibility and facile synthesis.[52,55] The performance of all three MECs namely MEC 1 (equipped with non-modified carbon felt electrodes), MEC 2 (equipped with poly(neutral red) modified carbon felt electrodes) and MEC 3 (equipped with chitosan modified carbon felt electrodes) were compared, reflecting the effect of electrode modifications.

Results and discussion

Two different carbon felt electrode modifications were investigated by using poly(neutral red) and chitosan. The poly(neutral red) modified carbon felts were prepared by electropolymerization of neutral red, as reported in our previous work.[60] The chitosan modified electrodes were prepared chemically through carbodiimide chemistry (Scheme 1). Firstly, the carbon felt surface was oxidized under strong acid conditions of concentrated HNO_3. The resulting carboxylic acid groups were later activated through coupling reactions of *N*-hydroxy-3-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) prior to reacting with primary amine groups of chitosan.

To investigate the long-term performance of MECs, three MECs namely MEC 1, 2 and 3 were built. In MEC 1, non-modified carbon felts were used as the anode and cathode, while MEC 2 and 3 were equipped with poly(neutral red) and chitosan modified electrodes, respectively.

All bioelectrodes in each MEC were inoculated with a mixed culture taken from sewage sludge in a two-compartment electrochemical cell with a controlled potential of 0.40 V *vs.* Ag/AgCl (3 M NaCl). During the adaptation phase, the bioanode was fed once a week with synthetic wastewater consisting of organic substances like glucose and acetate, while the biocathode was fed with glucose and CO_2 to ensure enough biomass formation. After a 4 week adaptation, the faradaic currents were observed at around 2 mA, indicating successfully developed bioelectrodes.[61]

After the adaptation period, the cathodic solution was replaced with a glucose-free medium. All three developed MECs showed capabilities of organic oxidation in the anodic compartment and simultaneous CH_4 generation in the cathodic compartment. To investigate the systems' performance, long-term electrolyses of MEC 1, 2 and 3 were carried out, by applying a constant potential of 0.40 V *vs.* Ag/AgCl (3 M NaCl) at the anode in a batch operation mode. During the operation of around 90 days, half of the anodic solution was replaced twice a week with a freshly prepared medium, consisting of synthetic wastewater with an average COD concentration of 600 mg L^{-1}, while cathodic chambers were purged with CO_2 two times a week. The systems were monitored for 3 running cycles, through analysis of organic degradation by COD determination of anodic solutions and headspace analysis of CH_4 production in cathodic chambers.

The accumulated COD removal in each running cycle (red circle data point) was plotted together with total electrical charge (Q) (blue triangle data point) over the entire running time, as shown in Fig. 2a–c for MEC 1, 2 and 3, respectively. The data were fitted linearly as presented in dashed lines, showing reaction rates in each cycle. The total COD removal, COD removal rate, average COD removal efficiency, total Q and Q rate are summarized in Table 1.

The results revealed that accumulated COD removal in MEC 1 increased from 1.0 g L^{-1} in the first cycle to 1.8 and 2.2 g L^{-1} in the second and third cycles, respectively, showing an enhancement of the oxidation process over the running cycles. Significantly higher COD removal values were observed at 1.6 and 1.9 g L^{-1} in the first cycle of MEC 2 and 3. In the second cycle, the accumulated COD values found in MEC 2 and 3 were enhanced by 1.0 and 0.5 g L^{-1} from the first cycle, respectively, while a slight drop was detected in the third cycle. The removal rates obtained from the slope of fitting lines, were found to be in the range of 40–85 mg per L per day. The results revealed that the removal rate increased largely from 40 mg per L per day in the first cycle to 70 mg per L per day in the third cycle of MEC 1,
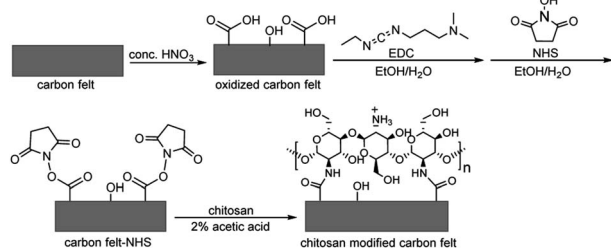
Scheme 1 Schematic preparation of chitosan modified carbon felt. EDC: *N*-hydroxy-3-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide; NHS: *N*-hydroxysuccinimide.
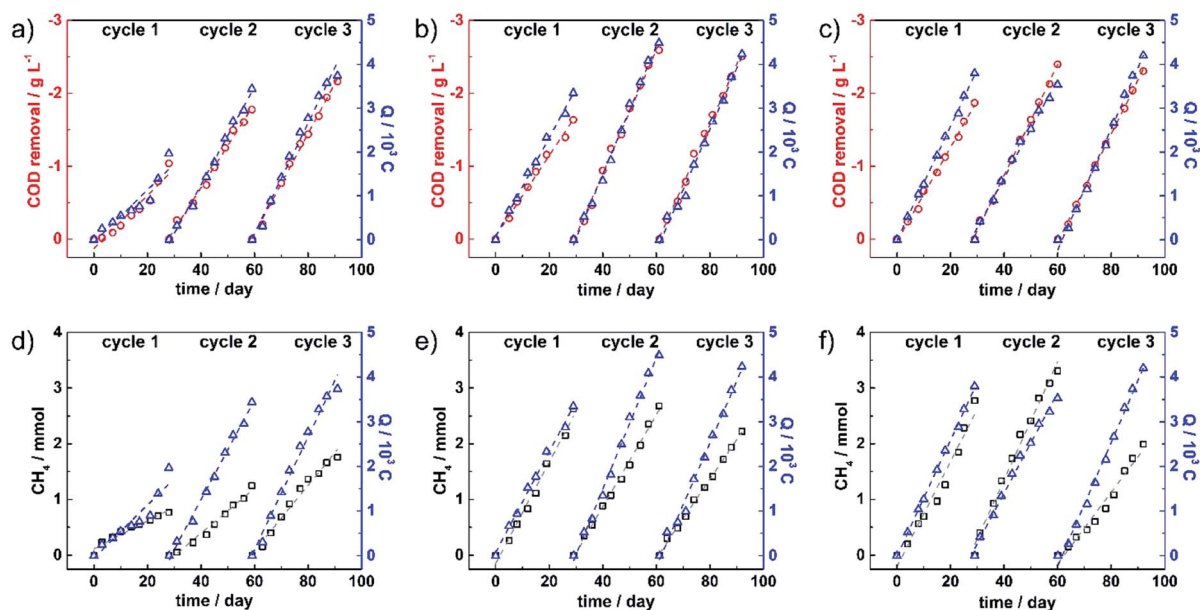
Fig. 2 Plots of accumulated COD removal (red circle) of (a) MEC 1, (b) MEC 2 and (c) MEC 3, and CH_4 production (black square) of (d) MEC 1, (e) MEC 2 and (f) MEC 3, together with accumulated charges (blue triangle) over the entire running time. The dashed lines represent linear-fitting curves of each data set.

Table 1 Comparison of the following parameters: accumulated CH_4 production, accumulated charge (Q), accumulated COD removal, as well as their production and removal rates, faradaic efficiency (FE) towards CO_2 reduction to CH_4, COD removal efficiency and CH_4 production yield for each running cycle (cycle 1, 2 and 3) and average values of MEC 1, 2 and 3

	MEC 1				MEC 2				MEC 3			
	1	2	3	Average ± SD	1	2	3	Average ± SD	1	2	3	Average ± SD
CH_4 production/mmol	0.8	1.3	1.8	1.3 ± 0.5	2.6	2.7	2.2	2.5 ± 0.3	2.8	3.3	2.0	2.7 ± 0.7
$Q/10^3$ C	2.0	3.4	3.7	3.0 ± 0.9	3.3	4.5	4.2	4.0 ± 0.6	3.8	3.5	4.2	3.8 ± 0.4
COD removal/g L^{-1}	1.0	1.8	2.2	1.7 ± 0.6	1.6	2.6	2.5	2.2 ± 0.6	1.9	2.4	2.3	2.2 ± 0.3
CH_4 production rate/μmol per day	25	40	60	42 ± 18	90	80	70	80 ± 10	90	110	60	87 ± 25
Q rate/C per day	60	110	130	100 ± 36	110	150	140	133 ± 21	130	110	140	127 ± 15
COD removal rate/mg per L per day	40	60	70	57 ± 15	55	85	80	73 ± 16	65	80	70	72 ± 8
Average% FE_{CH_4}	39	29	38	35 ± 6	66	47	44	52 ± 12	57	72	36	55 ± 18
Average% COD removal efficiency	25	52	55	44 ± 17	52	67	72	64 ± 10	56	73	71	67 ± 9
CH_4 yield/L_{CH_4}/g_{COD}	0.09	0.08	0.09	0.09 ± 0.01	0.18	0.12	0.10	0.13 ± 0.04	0.16	0.15	0.10	0.14 ± 0.04

while the highest removal rates were observed in MEC 2 and 3 with 85 and 80 mg per L per day in cycle 2, respectively. The large increase in COD removal from the first to the second cycle in all MECs might be related to biofilm growth in the first cycle. Over the whole experiment, average COD removal rates were observed at 57, 73 and 72 mg per L per day in MEC 1, 2 and 3, respectively. Additionally, average COD removal efficiencies were calculated giving the overall efficiencies of 44%, 64% and 67% in MEC 1, 2 and 3, respectively. These results reflected that enhanced organic degradation strongly related to both modifications.

Furthermore, the electrical charge plots revealed that during all three cycles, electrons were consumed continuously in all MECs. In MEC 1, accumulated charges increased from 2.0×10^3 C in the first cycle to 3.4×10^3 and 3.7×10^3 C in the second

and third cycles, respectively. The accumulated charges collected in the first cycle of MEC 2 and 3 were found to be almost two times higher than that of MEC 1, suggesting an enhanced electron transfer process at the first cycle in the modified electrode containing systems. This observation might relate to better coverage of biofilms on modified carbon felts. The accumulated charges were further improved in the second cycle and dropped slightly in the third cycle of MEC 2. While those of MEC 3 were of 3.5×10^3 and 4.2×10^3 C in cycle 2 and 3, respectively. Average rates for electron flux were observed for MEC 1, 2 and 3 at 100, 133 and 127 C per day, respectively. The highest electron flux was observed in MEC 2 with 150 C per day in cycle 2, followed by MEC 3 with 140 C per day in cycle 3. Although MEC 1 showed a much lower Q rate in the first cycle relative to MEC 2 and 3, it reached the rate of around 100 C per

Table 2 Comparison of methane-producing MECs

Anode material	Cathode material	Cell Operation	geometry	E_{app} or V_{app}	Substrate	%COD removal	%FE$_{CH_4}$	CH$_4$ yield/L$_{CH_4}$/g$_{COD}$	Ref.
Graphite granules	Graphite granules	Continuous	Two chamber	+0.2 V vs. SHE	Synthetic organic mixture	75 ± 10%	61 ± 5%	0.25[a]	65
Graphite granules	Graphite granules	Continuous	Two chamber	+0.2 V vs. SHE	Synthetic organic mixture	70 ± 2%	47 ± 2%	0.17[a]	43
Graphite granules	Nickel mesh	Continuous	Upflow	0.8 V	Beer wastewater	90% (max)	NG	0.14	66
Graphite granules	Graphite granules	Continuous	Two chamber	+0.2 V vs. SHE	Acetate	94%	79 ± 2%	~0.25[a]	67
Carbon felt	Carbon felt	Continuous	Two chamber	0.8 V	Synthetic wastewater	~80%	NG	0.04[a]	68
Carbon felt	Carbon felt	Batch	Two chamber	+0.4 V vs. Ag/AgCl	Synthetic wastewater	44 ± 17%	35 ± 5.5%	0.09	This work
Poly(neutral red) modified carbon felt	Poly(neutral red) modified carbon felt	Batch	Two chamber	+0.4 V vs. Ag/AgCl	Synthetic wastewater	64 ± 10%	52 ± 11.9%	0.13	This work
Chitosan modified carbon felt	Chitosan modified carbon felt	Batch	Two chamber	+0.4 V vs. Ag/AgCl	Synthetic wastewater	67 ± 9%	55 ± 18.1%	0.14	This work

[a] Calculated from the given data; E_{app}: applied potential; V_{app}: applied voltage; NG: not given; max: maximum value.

day which is in the same range as MEC 3 in the second cycle. This observation suggested that biofilm formation on bare carbon felts took longer, as compared to treated carbon felts.

In the cathodic compartment, the CH$_4$ production was quantified twice a week. Fig. 2d–f show the plots of accumulative CH$_4$ concentration (black square data point) and accumulative Q (blue triangle data point) during each running cycle of MEC 1, 2 and 3. The CH$_4$ production rate and the average faradaic efficiencies are summarized in Table 1. The produced CH$_4$ observed in MEC 1 rose continuously from 0.8 mmol in the first cycle to 1.3 mmol in the second cycle to finally 1.8 mmol in the third cycle. In MEC 2, the amount of produced CH$_4$ in cycles 1 and 2 was relatively stable at 2.6 and 2.7 mmol, respectively. However, the production declined to 2.2 mmol in the third cycle, while produced CH$_4$ in MEC 3 increased from 2.8 mmol in cycle 1 to 3.3 mmol in cycle 2 and dropped significantly to 2.0 mmol in cycle 3. The decline observed in MEC 2 and 3 in the third cycle, might result from the instability of the modified electrodes and/or detachment of biofilms. The CH$_4$ production rates in the three MECs were found to be in the range of 25–110 μmol per day and the average production rates over the entire experiments in MEC 1, 2 and 3 were 42, 80 and 87 μmol per day, respectively. Together with the obtained charges, faradaic efficiencies towards the CO$_2$ reduction to CH$_4$ were calculated according to the equation given in the experimental part. The corresponding faradaic efficiencies were averaged within each running cycle. MEC 1 showed efficiencies of 39% in cycle 1, 29% in cycle 2 and 38% in cycle 3, and an overall average efficiency of 35% while the highest faradaic efficiency in MEC 3 was reached at 72% in cycle 2 and then decreased to 36% in cycle 3. The overall average efficiencies of MEC 2 and 3 were reported to be 52 and 55%, respectively.

Considering CH$_4$ production relative to the removed COD (CH$_4$ yield), MEC 1 revealed a relatively stable CH$_4$ yield at 0.09 L$_{CH_4}$/g$_{COD}$ while the yield observed from MEC 2 and 3 decreased from the first cycle to the third cycle and the average yields were of 0.13 and 0.14 L$_{CH_4}$/g$_{COD}$, respectively.

Concerning the performance in both cathodic and anodic chambers, MEC 2 and MEC 3 showed significantly higher organic degradation efficiencies, faradaic efficiencies toward the conversion of CO$_2$ to CH$_4$ and CH$_4$ yield, as compared to those of MEC 1. Moreover, higher numbers of electrons were delivered in the modified electrode containing systems. This might be due to biofilm coverage and/or facilitated electron transfer by the coating. As suggested in the previous report, a positively charged electrode modification with chitosan coating, could enhance the interaction between the electrode and Gram-negative microorganisms like *Sporomusa ovata*, resulting in improved microbial electrolysis rates.[54] However, the instability of the modified systems for CO$_2$ reduction to CH$_4$ was observed during the long-term running.

Compared to the reported studies on methane-producing MECs using non-treated carbon felt electrodes (Table 2), our results (MEC 1) showed lower COD removal efficiency but significantly higher CH$_4$ yield. While, with the electrode modifications (MEC 2 and 3), the higher efficiencies were now
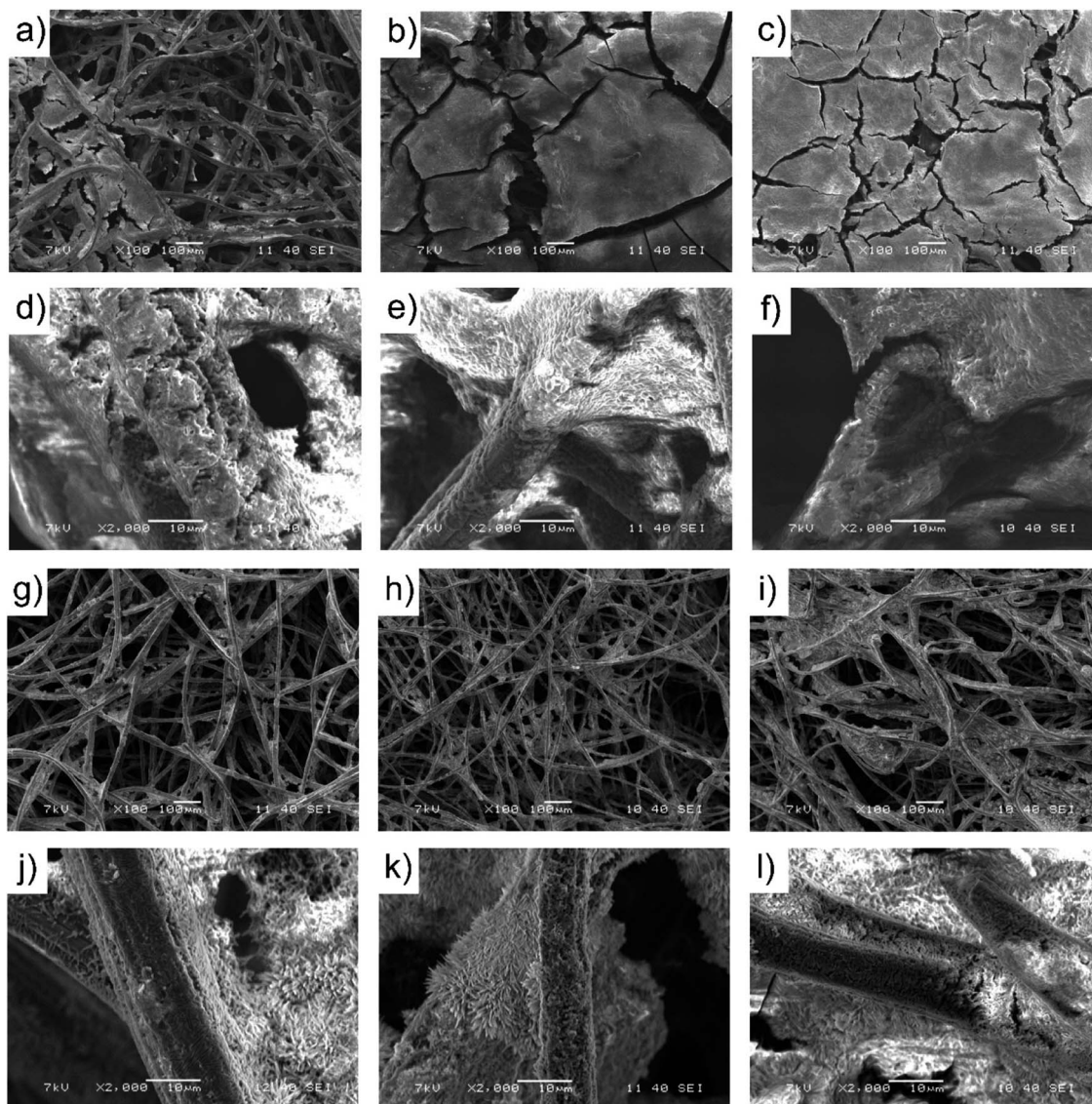
Fig. 3   SEM images of bioanodes of MEC 1 (a and d), MEC 2 (b and e) and MEC 3 (c and f) and biocathodes of MEC 1 (g and j), MEC 2 (h and k) and MEC 3 (i and l).

comparable to those of the continuous-operating MECs equipped with graphite granules and nickel mesh. Furthermore, using carbon felt offers several advantages as it is highly flexible, has high active surface area, high conductivity and is less corrosive.[62] However, it is important to be noted that apart from electrode material, other parameters like cell configuration, inoculation, substrate and operation parameters are of importance.[63] Therefore, this comparison was aimed to show the general view of this field and parameters which could be further optimized.

After the long-term operation of around 90 days, samples of bioanodes and biocathodes of all MECs were dried under ambient conditions overnight for SEM measurements. Fig. 3 presents SEM images of bioanodes and biocathodes of MEC 1, 2 and 3. The bioanodes obtained from modified carbon felt electrodes showed better coverage and thicker biofilms (Fig. 3b

and c). High magnification images (Fig. 3d–f), from both modified and non-modified carbon felt electrodes are colonized from different cell shape bacteria, revealing mixed microbial strains. In the SEM images of all biocathodes, rod-shaped cells of bacteria were found to be located on carbon fibers (Fig. 3j–l). This characteristic shape refers to some species from phylum Firmicutes like *S. ovata*[64] and the Euryarchaeota *Methanobacterium palustre*,[2] which are capable of growing autotrophically by using a cathode as the sole electron donor and CO_2 as the carbon source.

The utilization of electrochemical impedance spectroscopy (EIS) in BESs is highly significant since extensive information of the systems can be extracted, such as the charge transfer resistances and the mechanism of the electron transfer, among many others. In this present study, the electrical loss in the system was evaluated.
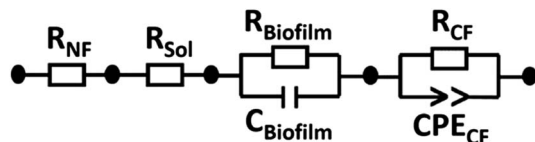
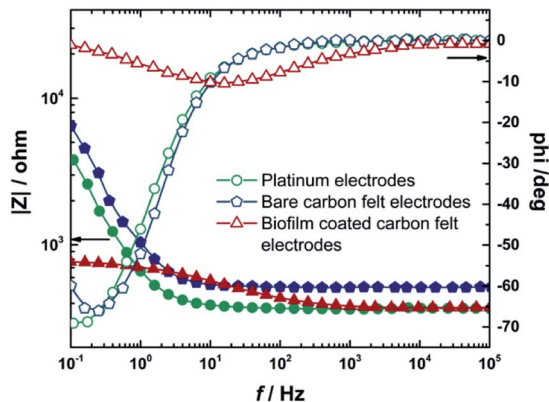**Fig. 4** Electrical circuit used for the fitting of the cell parameters.

Fig. 5 Bode plots of three different two-electrode cell configurations equipped with platinum electrodes (green circle), bare carbon felt electrodes (blue pentagon) and biofilm on carbon felt electrodes (red triangle).

All impedance spectra were recorded in the frequency range of 10^{-1} to 10^5 Hz with a perturbation amplitude of 50 mV. Firstly, the resistance of the medium as an electrolyte solution (R_{sol}) was determined in a one-compartment cell, having platinum electrodes as the working (WE) and counter (CE) electrodes, showing 8.9 Ω. The platinum electrodes were then transferred to a two-compartment electrochemical cell containing a medium to determine the resistance of a Nafion membrane (R_{NF}). The electrical circuit used for data fitting is shown in Fig. 4. In this configuration, R_{NF} was found to be 530 Ω and connected in series with R_{sol}, the resistance of carbon felt (R_{CF}) and the resistance of biofilm ($R_{biofilm}$). R_{CF} is in parallel with the constant phase element of carbon felt (CPE_{CF}) and $R_{biofilm}$ is in parallel with the capacitance of the biofilm ($C_{biofilm}$), representing the bioanode.

Then, the platinum electrodes were replaced with bare carbon felt electrodes and the impedance spectrum was measured in a similar manner. After that, these carbon felt electrodes were used as electrodes for biofilm formation in MEC 1. With these two bioelectrodes, impedance spectra were recorded to investigate changes of the system in the presence of biofilms. Fig. 5 presents Bode plots for the two-electrode setup

of platinum, bare carbon felt and carbon felt with the biofilm, and the data evaluated from the measurements are summarized in Table 3.

The constant phase element was used for the description of the non-ideal capacity of spongy-like carbon felt electrodes. Thus, the resistances of electrodes (R_{CF}) as bare carbon felt and carbon felt with a biofilm were found to be 1.1 and 0.4 kΩ, respectively. The calculated resistance and capacitance of the biofilm ($R_{biofilm}$, $C_{biofilm}$) were 0.4 kΩ and 0.1 F. Further, the results reveal that, with the biofilm on electrodes, a decrease in capacitive current was observed in a low frequency regime due to enhanced electron transport, indicating the establishment of a conductive biofilm and a larger surface area due to biofilm formation.[61] Moreover, EIS results indicated negligible losses of this MEC.

Apart from the observed enhanced performance of the modified electrodes containing MECs, the economic aspects should be addressed as they impact significantly on up-scaling. However, only a few studies have been reported on the cost of electrode modification for this type of MEC. We performed cost studies for such modifications from the price of chemicals which were used in each modification process (see details in the ESI†). The calculation showed that poly(neutral red) modification ($\sim$100 € per m^2) was much cheaper, as compared to chitosan modification ($\sim$5400 € per m^2) due to the costly coupling reagents (NHS and EDC). Compared to the reported metal based electrodes like Ni mesh[66] ($\sim$8600 € per m^2), carbon felt ($\sim$170 € per m^2) modified with poly(neutral red) (in total $\sim$270 € per m^2) are much cheaper and showed similar results with respect to the CH$_4$ yield (Table 2). Moreover, this poly(neutral red) modified carbon felt is more cost effective than the conventional platinum based electrodes (e.g. Pt (60%) carbon cloth is $\sim$4100 € per m^2,[62]) which are normally used and, being the main cost of MFC technology made it less feasible for up-scaling reactors.

Experimental

Chemicals

Low molecular weight chitosan and 3-amino-7-dimethylamino-2-methylphenazine hydrochloride (neutral red) were purchased from Sigma-Aldrich. Carbon felt electrodes were received from Alfa Aesar. Other chemicals were of analytical grade and were used as received. Phosphate buffer solutions were prepared from KH$_2$PO$_4$ and K$_2$HPO$_4$ to achieve the desired pH value. A Nafion perfluorinated membrane (Nafion 117) was received from Chemours. With a slight modification to the reported study,[69] the Nafion membrane was pre-treated prior to use by boiling in H$_2$O$_2$ (30% v/v), deionized water, 0.5 M H$_2$SO$_4$ and

Table 3 Impedance data of MEC 1

WE	CE	R_{sol}/Ω	$R_{CF/Pt}/k\Omega$	R_{NF}/Ω	$R_{biofilm}/k\Omega$	$C_{biofilm}/F$	CPE-T (10^{-4})	CPE-E
Platinum	Platinum	8.9	8.7	530	—	—	6.6	1.1
Carbon felt	Carbon felt	70	1.1	530	—	—	2.5	1.1
Carbon felt with biofilm	Carbon felt with biofilm	8	0.4	530	0.4	0.1	1.6	0.7

deionized water, sequentially. Each step was carried out for 1 h at 80 °C.

Setup

All microbial electrocatalytic experiments were performed in two-compartment electrochemical cells with the same geometrical design (Fig. 1b). Each chamber had a volume of around 250 mL. The cathode and anode chambers were separated by using a pre-treated Nafion 117 membrane allowing proton transport between the two chambers. Carbon felt electrodes (7.0 × 2.5 × 0.6 cm^3) connected with Ti wires were used as anodes and cathodes, while a Ag/AgCl (3 M NaCl) electrode (ProSense) was used as a reference electrode. All electrochemical experiments were performed using an IVIUM CompactStat instrument. The potential values reported in this work referred to Ag/AgCl (3 M NaCl) which were calibrated externally with a $K_3[Fe(III)(CN)_6]$/ $K_4[Fe(III)(CN)_6]$ redox couple of which the potential value of 0.36 V vs. standard hydrogen electrode (SHE) was used. The calibration showed a Ag/AgCl (3 M NaCl) electrode potential of 0.21 V vs. SHE.

Medium

With slight differences from the previous report,[2] a medium was prepared in deionized water containing the following ingredients (per L): KH_2PO_4 (3.0 g), K_2HPO_4 (2.5 g), NaCl (0.13 g), NH_4Cl (0.31 g), $NaHCO_3$ (6.0 g), $MgSO_4 \cdot 7H_2O$ (0.040 g), trace element solution (12.5 mL) and vitamin solution (5 mL). The trace element solution consisted of the following ingredients (per L): HCl (25%, 10 mL), $FeCl_2 \cdot 4H_2O$ (1.5 g), $ZnCl_2$ (0.070 mg), $MnCl_2 \cdot 4H_2O$ (0.10 g), H_3BO_3 (0.006 g), $CoCl_2 \cdot 6H_2O$ (0.19 g), $CuCl_2 \cdot 2H_2O$ (0.002 g), $NiCl_2 \cdot 6H_2O$ (0.024 g), $Na_2MoO_4 \cdot 6H_2O$ (0.036 g), $Na_2WO_4 \cdot 2H_2O$ (0.036 g) and deionized water (990 mL). The vitamin solution contained the following ingredients (per L): biotin (0.002 g), folic acid (0.002 g), pyridoxine hydrochloride (0.010 g), thiamine hydrochloride (0.005 g), riboflavin (0.005 g), nicotinic acid (0.005 g), calcium D-pantothenate (0.005 g), vitamin B12 (0.0001 g), p-aminobenzoic acid (0.005 g), lipoic acid (0.005 g) and deionized water (1 L).

Electrode modification

Preparation of the poly(neutral red) modified carbon felt. As described in the previous study,[60] the modification was done in a one-compartment cell. A carbon felt, a Ag/AgCl (3 M NaCl) and a Pt plate were used as working, reference and counter electrodes, respectively. Before electropolymerization, the carbon felt electrode was pre-treated by sweeping the potentials between 0 and 1.0 V with a scan rate of 50 mV s^{-1} over 20 cycles in a 0.1 M KNO_3 aqueous solution. After rinsing with deionized water, electropolymerization was carried out on the treated carbon felt electrode at potentials between −1.0 and 1.0 V with a scan rate of 50 mV s^{-1} for 20 cycles in 0.1 M phosphate buffer solution (pH 6.0), containing 1 mM neutral red and 0.1 M KNO_3.

Preparation of the chitosan modified carbon felt. With slight modification from the reported study,[54] a carbon felt electrode was pre-treated with concentrated HNO_3 at room temperature for 14 h. After that, the electrode was washed with deionized

water until the rinsed water was neutral and dried under vacuum for 14 h. To covalently modify with chitosan, the oxidized carbon felt was immersed in a coupling reagent of ethanol and water mixture (4 : 1 v/v) containing 50 mM N-hydroxy-3-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and 50 mM of N-hydroxysuccinimide (NHS) at room temperature for 2 h. The electrode was transferred into a 2% acetic acid solution containing chitosan (1% w/v) of which the pH was adjusted to 6.5 with a 1 M NaOH solution. The modification took place at room temperature for 14 h, then the modified carbon felt electrode was carefully washed with ethanol and dried under vacuum.

Adaptation phase

For all MECs, anodes and cathodes were inoculated with the same inoculum prepared by centrifugation (4000 rpm for 10 min) of sewage sludge, collected from a communal wastewater treatment plant (Austria). The bioelectrodes were developed in the aforementioned two-compartment cell in which each chamber contained 20 mL of prepared inoculum. In cathodic chambers, 200 mL of the previously described medium and glucose (0.22 g) were added. While anodic chambers were filled with 200 mL of medium, containing additional carbon sources, to simulate municipal wastewater which included the following composition (per L): peptone (0.138 g), yeast extract (0.075 g), sodium acetate (0.088 g) and glucose (0.336 g).[43] The adaption phase was carried out at room temperature by applying a constant potential of 0.40 V vs. Ag/AgCl (3 M NaCl) at the anode for 4 weeks. In this phase, carbon sources were supplied once a week. For the anode compartment, half of the solution was replaced with a freshly prepared medium containing synthetic wastewater, while glucose (0.22 g) was added into the cathodic compartment without replacing the solution. Both chambers were kept saturated with CO_2 to ensure anaerobic conditions. After 4 weeks, 200 mL of the cathodic solution was replaced with a freshly prepared medium without glucose addition.

Long-term performance studies

After the 4 week adaptation, all three MECs were studied continuously by applying a constant potential of 0.40 V vs. Ag/ AgCl (3 M NaCl) at the anode in a batch operation mode. Headspace samples from cathode chambers and liquid samples from the anolyte were taken twice a week for headspace product analysis and COD measurements, respectively. After sampling, the cathode chambers were purged with CO_2 to keep the chamber saturated, while half of the anodic solution was replaced with fresh medium containing synthetic wastewater with an average COD concentration of 600 mg L^{-1} and the chamber was flushed with N_2 to achieve anaerobic conditions. Every 4 weeks, 200 mL of cathodic solution was replaced with fresh medium to provide microorganisms with sufficient trace elements and vitamins, labelled as running cycles 1–3.

Determination of COD

The COD of liquid samples taken from the anodic solution were analyzed photometrically. Firstly, 2 mL of the liquid sample was

mixed with the reagent in a COD test tube (Nanocolor COD 160 and 1500, Macherey-Nagel). Then, the mixture was heated at 148 °C for 2 h in a thermostat (Nanocolor Vario Mini, Macherey-Nagel). Afterwards, the tubes were allowed to cool down to room temperature. Then COD was determined by using a LED photometer (Compact Photometer PF-3, Machery-Nagel) at wavelength 620 nm. COD removal efficiency can be calculated using the following calculation:

$$\%\text{COD removal efficiency} = \left(\frac{\Delta\text{COD}}{\text{COD}_1}\right) \times 100$$

in which ΔCOD is calculated from the change of COD_1 (COD concentration of anodic solution before reaction) to COD_2 (COD concentration of anodic solution after reaction).

Headspace product analysis

Headspace samples from cathode chambers were taken by using a gas-tight syringe for CH_4 analysis. The analysis was done by using a gas chromatograph (Thermo Scientific GC ultra) equipped with a thermal conductivity detector (TCD). The faradaic efficiency (FE) can be calculated using the following equation:

$$\%\text{FE} = \frac{n_{\text{CH}_4} \times 8 \times F}{\int_0^t I\,\mathrm{d}t} \times 100$$

where n_{CH_4} is the number of moles of CH_4 calculated from the amount of CH_4 produced in the system, 8 is the number of electrons needed for the reduction of CO_2 to CH_4, F is the faradaic constant (96 485.33 C mol^{-1}), I is current recorded during the reaction and t is time of the reaction.

Scanning electron microscopy measurements

The samples were taken by cutting the bottom edge of each bioelectrode (size of 1.5×0.5 cm^2) and allowing them to dry under ambient conditions overnight. Scanning electron microscope (SEM) images of dried bioelectrodes were taken using the JEOL JSM-6360 LV scanning electron microscope at the accelerating voltage of an electron beam of 7.0 kV with a working distance of 15 mm.

Electrochemical impedance

The impedance experiments were carried out in a two-compartment electrochemical cell separated with a pre-treated Nafion 117 membrane (as mentioned above for microbial electrocatalytic experiments) by using an IVIUM CompactStat instrument. The impedance spectra were recorded within the frequency range of 10^5 to 10^{-1} Hz at the perturbation amplitude of 50 mV.

Conclusion

In this study, we investigated the long-term performance of CH_4 producing MECs equipped with bioanodes and biocathodes for wastewater treatment, in terms of COD removal and CH_4 production. By applying a constant potential of 0.40 V *vs.* Ag/

AgCl (3 M NaCl) at the bioanode, organic degradation and CH_4 generation were monitored through COD measurement of the anodic electrolyte solution and cathodic headspace analysis, respectively. In addition, carbon felt electrodes were modified with poly(neutral red) and chitosan, serving as electrodes and supports for microbial colonization in MECs. The results of all three MECs revealed the possibility of oxidation of organic substances at bioanodes and simultaneous CH_4 generation at biocathodes. Compared to non-modified MECs, higher COD removal rates and CH_4 production rates of modified electrode containing systems were observed, especially in the first running cycle. These revealed that modified electrodes with poly(neutral red) and chitosan could enhance biofilm formation and activity. Furthermore, the corresponding efficiencies from those were improved, suggesting efficient electron transfer processes between electrodes and microorganisms. Compared to the previously reported MECs, our MECs showed comparable results even though they were batch operated which limited the substrate input. Further, the cost studies on the electrode material and modification prices suggested that the poly(neutral red) modified electrode is more cost-effective, as compared to other metal based materials, which provides economic feasibility for large-scale operation.

Conflicts of interest

There are no conflicts to declare.

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