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Bioelectrochemical reduction of CO_2 to CH_4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture

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ABSTRACT

This study describes the performance of a microbial biocathode, based on a hydrogenophilic methanogenic culture, capable of reducing carbon dioxide to methane, at high rates (up to 0.055 ± 0.002 mmol d^{-1} $mgVSS^{-1}$) and electron capture efficiencies (over 80%). Methane was produced, at potentials more negative than −650 mV vs. SHE, both via abiotically produced hydrogen gas (i.e., via hydrogenophilic methanogenesis) and via direct extracellular electron transfer. The relative contribution of these two mechanisms was highly dependent on the set cathode potential. Both cyclic voltammetry tests and batch potentiostatic experiments indicated that the capacity for extracellular electron transfer was a constitutive trait of the hydrogenophilic methanogenic culture.

In principle, both electrons and carbon dioxide required for methane production could be obtained from a bioanode carrying out the oxidation of waste organic substrates.

1. Introduction

Bioelectrochemical systems (BES) have recently been proposed as a new and sustainable technology for energy generation from wastes: in a BES, bacteria interact with solid-state electrodes by exchanging electrons with them, either directly or via redox mediators (Rabaey et al., 2005; Rabaey et al., 2007; Thrash and Coates, 2008). The most extensively studied BES is the microbial fuel cell (MFC), in which microorganisms oxidize the organic matter contained in a wastewater by using a solid-state anode as terminal electron acceptor. Electrons flow from the anode to the cathode through an external conductive wire generating a current, while protons (also produced from organic matter oxidation) diffuse to the cathode through a proton-exchange membrane. In the cathode compartment electrons and protons react with oxygen, typically provided from air, to form water. The anode potential of a MFC strongly depends on the free energy of the substrate and the bacterial metabolism involved in organic substrate oxidation. As an example, with acetic acid as the substrate, and under typical MFC operating conditions, the anode potential is about −0.3 V (vs. a standard hydrogen electrode, SHE) (Logan et al., 2006; Rabaey and Verstraete, 2005).

By eliminating oxygen from the cathode compartment, the electrons released by bacteria can combine with protons to generate useful hydrogen gas instead of electricity. This modified MFC is named microbial electrolysis cell (MEC) and needs a small external voltage ($\geqslant$0.114 V), in addition to that generated by bacteria, to make the reaction thermodynamically feasible (Cheng and Logan, 2007; Logan et al., 2008; Rozendal et al., 2006, 2007).

Currently, one of the main disadvantages of BES, and particularly MEC, is the requirement of expensive materials, such as platinum, as cathodic catalysts. The choice for platinum in MEC is due to its excellent electrocatalytic activity towards hydrogen evolution, even though its performance is negatively affected by several different components often present in waste streams. The need for cheaper and more sustainable cathodes, to be employed for bioenergy generation in BES, has prompted research into the development of alternative cathode catalysts, such as microbial biocathodes (Clauwaert et al., 2007b; Rozendal et al., 2008). Recently, microbial biocathodes are being explored also for other applications, such as the biological reduction of oxidized pollutants in bioremediation systems (Aulenta et al., 2008, 2009a,b), or the biological reduction of nitrate to nitrogen gas (Clauwaert et al., 2007a).

Another possible application is the electrochemical reduction of carbon dioxide to methane according to the following reaction:

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \quad (1)$$

Indeed, both the electrons and the carbon dioxide released at the anode during the microbial oxidation of the organic matter contained in a waste stream can be in principle exploited for the cathodic generation of methane, according to the schematic drawing reported in Fig. 1. At standard conditions, this reaction requires

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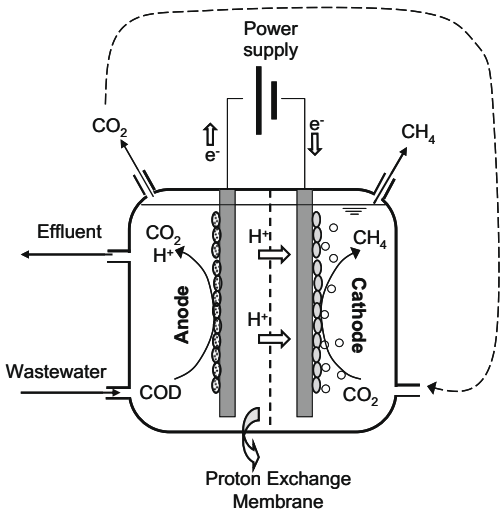

Fig. 1. Schematic drawing of a bioelectrochemical system for wastewater treatment and simultaneous CH_4 production based on bioelectrochemical CO_2 reduction.

a theoretical voltage of −0.244 V (vs. SHE) at pH 7, but it is usually affected by quite large overpotentials that, however, could be possibly reduced by using a microbial biocathode.

Compared to conventional anaerobic digestion, this process could offer some specific advantages, such as the physical separation of the waste organic matter oxidation stage from the methane production one. This would allow to protect the methanogenic consortia against inhibitory compounds possibly present in the waste streams and to produce methane with lower content in carbon dioxide and other impurities. Moreover, since the wastewater only flows through the anodic chamber (which can even be operated at ambient temperature), less energy is required to maintain the cathode at the desired temperature (e.g., 35 °C). As suggested in the literature (Clauwaert et al., 2008; Clauwaert and Verstraete, 2009), anaerobic digestion and methane-producing MEC could also operate in series, because of the possibility to remove the residual organics contained in the effluent of a conventional anaerobic digester with such a bioelectrochemical system, which is typically effective even at low substrate concentrations.

The ability of microorganisms to produce methane from CO_2 reduction by using an electrode as direct electron donor, has been reported for the first time only very recently (Cheng et al., 2009). The authors of this study observed that methane was produced at cathode potentials more negative than −0.7 V (vs. Ag/AgCl) (corresponding to −0.5 vs. SHE). At −1.0 V (vs. Ag/AgCl) (corresponding to −0.8 vs. SHE) current capture into methane was 96%. Electrochemical measurements made by voltammetry showed that the biocathode enhanced current densities compared to an abiotic cathode, which produced only small amounts of hydrogen. According to the authors, the increased current densities and limited amounts of abiotic hydrogen production suggested that methane production derived directly from current and not from hydrogen gas. The biocathode, originally inoculated with a mixed culture from the anode chamber of an existing two-chamber MEC, was enriched in *Methanobacterium palustre*.

The present work aimed to gain a deeper understanding of the performance of microbial biocathodes for CH_4 production. To accomplish this objective, bioelectrochemical experiments at controlled cathode potentials were performed on a hydrogenophilic methanogenic culture not previously acclimated to the electrochemical system. Main attention was paid at analyzing the contribution of both biotic and abiotic processes on methane generation, as a function of the set cathode potential.

2. Methods

2.1. Source microbial culture

The methanogenic culture, used in the hereafter described experiments, was enriched in a 0.570-L bioreactor consisting of a continuously stirred borosilicate glass bottle (liquid volume 0.350 L). Initially, the reactor was inoculated with anaerobic sludge from a packed bed biofilm reactor fed with a synthetic mixture of fatty acids and alcohols, simulating the composition of an industrial wastewater. The reactor was operated in fill and draw mode: every 7 days, it was flushed with a N_2/CO_2 (70:30 v/v) gas mixture and fed with H_2. During the initial 40 days of operation, the applied substrate (i.e., H_2) load rate was approximately 2.5 mgH_2 L^{-1} d^{-1}, then it was increased to 14 mgH_2 L^{-1} d^{-1}, and maintained at this value for the remainder of the study. On average, 75 mL of suspended culture were weekly removed from the reactor and replaced by anaerobic basal medium, which contained (g L^{-1}): $(NH_4)Cl$, 0.5; $MgCl_2{\cdot}6H_2O$, 0.1; K_2HPO_4, 0.4; $CaCl_2{\cdot}2H_2O$, 0.05; 10 mL L^{-1} of a trace metal solution (Balch et al., 1979), and 10 mL L^{-1} of vitamin solution (Zeikus, 1977). The resulting average cell retention time was ∼33 days. The pH of the medium was maintained between 7.3 and 7.6 with bicarbonate buffer and the temperature was controlled at 35 ± 1 °C. During pseudo-steady-state operation, the concentration of microorganisms in the reactor (as volatile suspended solids, VSS) was of about 78 ± 4 mg L^{-1}.

2.2. Bioelectrochemical cell setup

The bioelectrochemical cell used in this study consisted of two gastight borosilicate glass bottles (with a total volume of about 270 mL per bottle) separated by a 3 cm^2 cross-sectional area, Nafion® 117 proton-exchange membrane (PEM). The PEM was pretreated by boiling in H_2O_2 (3% v/v), then in 0.5 M H_2SO_4, and finally in DI water, each for 2 h, and then stored in DI water prior to being used. The cathode was a piece (50 mm × 10 mm) of carbon paper (E-TEK; working surface area ∼8 cm^2) and the anode was a glassy carbon rod (HTW GambH, Germany; 5 mm diameter, 50 mm length, working surface area ∼7 cm^2). The reference electrode (placed in the cathode chamber) was a KCl saturated Ag/AgCl electrode (+199 mV vs. standard hydrogen electrode, SHE) (Amel S.r.l., Milano, Italy). Throughout the manuscript, all voltages are reported with respect to SHE. Electrochemical potentiostatic measurements and monitoring were performed using a Galvanostat/Potentiostat Amel 551 (Milan, Italy).

2.3. Bioelectrochemical experiments

For the bioelectrochemical batch experiments, the cathode compartment of the bioelectrochemical cell was anaerobically filled with 75 mL of the source culture and with 75 mL of mineral medium. In parallel, the anode compartment was filled with 150 mL of the same mineral medium and the two compartments were flushed with a N_2/CO_2 (70:30 v/v) gas mixture.

Thereafter the bioelectrochemical cell was connected to the potentiostat and the cathode potential was set in the range from −650 to −900 mV, to evaluate the ability of microorganisms to use the negatively polarized cathode as direct electron donor for the production of methane. Each test lasted 8 h and at regular intervals gaseous samples were removed from the headspace of the compartments, using gastight, sample-lock Hamilton (Reno, NV) syringes, and analyzed by gas-chromatography for methane (40 μL) and hydrogen (500 μL). In parallel, control tests were also performed under the same operating conditions, but for the absence of the microbial culture. In all tests, the bioelectrochemical

reactor was maintained at 35 °C in a water bath, under vigorous magnetic stirring to ensure that current generation was not substantially affected by mass transfer.

The cumulative electric charge (μeq_i) that was transferred at the electrodes was calculated by integrating the current (A) over the period of electrode polarization. Cumulative reducing equivalents (μeq_p) that were used for the formation of reduced products were calculated from the measured amounts of CH_4 and H_2, considering the corresponding molar conversion factor of 8 μeq μmol^{-1} and 2 μeq μmol^{-1}, respectively. Coulombic efficiency for reduced products was accordingly calculated as η_P (%) = ($\mu eq_p/\mu eq_i$) × 100.

2.4. Cyclic voltammetry

Cyclic voltammetry (CV) tests were carried out in a 50 mL glass cell, with a PAR model 273 potentiostat/galvanostat (Princeton Applied Research, EG&G, USA) and a conventional three electrode setup. A glassy carbon electrode (0.196 cm^2 surface area) was used as the working electrode; a platinum electrode served as the counter electrode and a saturated calomel electrode was used as a reference electrode (E = +0.241 V, vs. SHE). CV tests were carried out either in the presence of the mixed culture or in the presence of its filtered (0.22 μm) growth medium (i.e., filtered culture). To assess the kinetic performance of the methanogenic culture, CV data were analyzed according to Tafel equation which relates the reaction overpotential (η) to the current density (i):

$$\eta = a + b \log i \quad (2)$$

where for large negative overpotentials: $\eta = (E - E°)$, with $E°$ corresponding to the reversible potential of the reaction; $a = (2.3RT/\alpha_C F)\log i_o$; where i_o is the exchange current density and α_C is the cathodic charge transfer coefficient; and $b = (-2.3RT/\alpha_C F)$.

CV scans were converted to Tafel plots by plotting η as a function of log i. Tafel slope and intercept, and accordingly i_o and α_C, were obtained from Tafel plots, via linear regression of experimental data.

2.5. Analytical methods

The concentration of microorganisms in the source culture reactor was determined as volatile suspended solids (VSS), according to standard methods (APHA, 1995). Methane was analyzed by injecting 40 μL of sample headspace (with a gas-tight Hamilton syringe) into a Varian (Lake Forest, CA, USA) 3400 gas chromatograph (GC; 2 m × 2 mm glass column packed with 60/80 mesh Carbopack B/1% SP-1000, Supelco; N_2 carrier gas at 18 mL min^{-1}; oven temperature at 50 °C; flame ionization detector temperature 260 °C) (Aulenta et al., 2005, 2006).

H_2 was analyzed in a 500 μL gaseous sample by a Trace Analytical TA3000R reduction gas detector (RGD) (Menlo Park, CA). When the H_2 level was above the range of the RGD (i.e., >0.5 μM) it was quantified using a Varian 3400 gas chromatograph (stainless-steel column packed with molecular sieve, Supelco; N_2 carrier gas 18 mL min^{-1}; oven temperature 180 °C; thermal-conductivity detector (TCD) temperature 200 °C) (Aulenta et al., 2006). Headspace concentrations were converted to aqueous-phase concentrations using tabulated Henry's law constants (Gossett, 1987).

2.6. Chemicals

Methane (99.0%) and hydrogen (99.5+%) were purchased from Sigma–Aldrich (Milano, IT). All the other chemicals used to prepare the mineral medium were of analytical grade and were used as received.

3. Results and discussion

3.1. Performance of the enriched methanogenic culture

Fig. 2 shows the performance of the hydrogenophilic methanogenic culture used in the bioelectrochemical experiments: during the first month of fill and draw operation with H_2 as the sole electron donor, the maximum rate of methane production was approx. 2000 μeq d^{-1} (corresponding to 0.25 mmol d^{-1}) and then gradually increased over time up to ~12,000 μeq d^{-1} (i.e., 1.5 mmol d^{-1}, corresponding to a specific production rate of ~0.055 mmol d^{-1} mgVSS^{-1}) after about 100 days of operation. Thereafter, it remained roughly constant around this value over the following 200 days. This initial increase mirrored the applied substrate (i.e., H_2) load rate which was increased from approximately 2.5 mgH_2 L^{-1} d^{-1} to 14 mgH_2 L^{-1} d^{-1}, during this initial period. A similar trend was also observed for the yield of CH_4 formation from added H_2, that gradually increased from ~30% to ~100%.

From the pseudo steady-state operation of the reactor, an average observed growth yield of 0.024 ± 0.002 mgVSS mgCOD^{-1} was estimated. From the average cell retention time (i.e., 33 d) and assuming a published (Pavlostathis and Giraldo-Gomez, 1991) decay coefficient (i.e., 0.088 d^{-1}), a maximum specific growth rate value of 0.38 ± 0.06 d^{-1} was also estimated. All these values fall within the range of those typically reported for hydrogenophilic methanogenic cultures (Pavlostathis and Giraldo-Gomez, 1991).

All the bioelectrochemical experiments hereafter described were carried out after the culture had reached a stable performance (i.e., starting from day 120).

3.2. Preliminary investigations of the electrochemical activity of the hydrogenophilic methanogenic culture: CV tests and Tafel plots

To verify the ability of the methanogenic culture to directly accept electrons from a polarized carbon electrode, CV tests have been carried out, at a scan rate of 10 mV s^{-1}. For each test, the current recorded in the presence of the methanogenic culture was compared to that recorded in abiotic controls carried out in the presence of filtered growth medium from the same culture. As shown in Fig. 3, when the electrode potential was in the range from −600 to −800 mV, the current recorded in the methanogenic culture and in a solution constituted by the filtered growth medium, was nearly the same. On the other hand, at more negative potentials (<−800 mV), the current measured in the presence of the methanogenic culture was significantly higher than that in its absence (filtered growth medium). This suggested that microorgan-

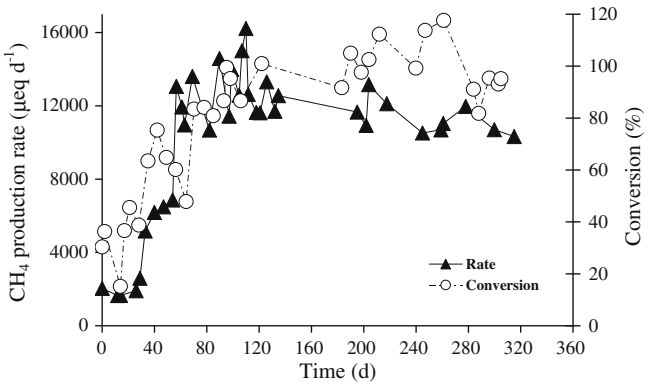

Fig. 2. Performance of the hydrogenophilic methanogenic culture: methane production rate and yield of hydrogen conversion into methane.

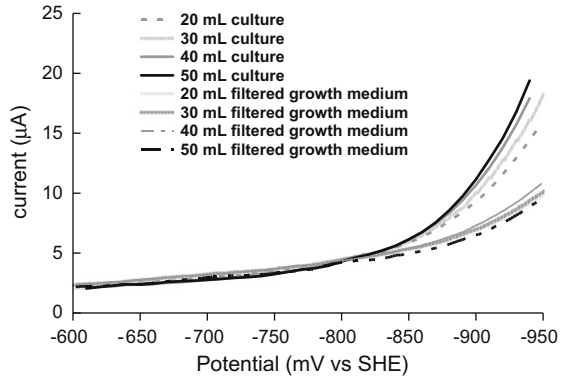

Fig. 3. Linear sweep voltammograms in the presence of increasing volumes of methanogenic culture or filtered growth medium (scan rate of 10 mV s^{-1}).

isms catalyzed the formation of reduced end-products (e.g., hydrogen or methane) by directly accepting electrons from the surface of the electrode and/or by converting into methane the abiotically produced hydrogen gas.

Biotic and abiotic CV tests were repeated in the presence of increasing volumes of methanogenic culture and filtered growth medium, respectively. In abiotic tests, the current did not increase when increasing volumes of filtered growth medium were added to the cell. In contrast, the current steadily increased with the volume of methanogenic culture added to the cell. One possible explanation of these results is that by increasing the volume of the methanogenic culture, hydrogen (abiotically produced) was more quickly converted into methane, thereby resulting in a higher current. Otherwise, these results could also reflect the ability of the methanogenic culture to engage in extracellular electron transfer processes with the cathode serving as direct electron donor, where a higher number of microorganisms yields higher electron transfer rates.

To verify if the observed behavior was specific to the enriched methanogenic culture, CV tests were also carried out under identical conditions on an aerobic mixed culture enriched on volatile fatty acids as carbon and energy source. In this case, the current observed in the presence of microorganisms was almost indistinguishable from that measured in abiotic control tests (data not shown).

The catalytic activity of the methanogenic culture was also evaluated on the basis of the calculated slopes and intercepts of Tafel equation, where smaller values of these parameters indicate better catalytic activity (i.e., for any given η, the smaller the absolute value of Tafel parameters, the higher the cathodic current). The absolute value of calculated Tafel slope and intercept decreased from 0.27 to 0.17 V and from 2.26 to 1.72 V, respectively, with the volume of added methanogenic culture, consistently with the microorganisms reducing the activation overpotentials. However, the rate of decrease of Tafel parameters progressively diminished with the volume of added culture, likely suggesting that the "free" space on the electrode surface, available for microbial interaction, could become progressively limiting the rate of electron transfer. This latter finding also suggests that the mechanisms by which microorganisms enhance the current are related to their direct interaction with the electrode surface.

3.3. Batch experiments with the enriched methanogenic culture

In order to quantify the products of the electrochemical reductions catalyzed through direct electron transfer by the methanogenic culture, batch experiments were conducted under potentiostatic conditions setting the working electrode potentials

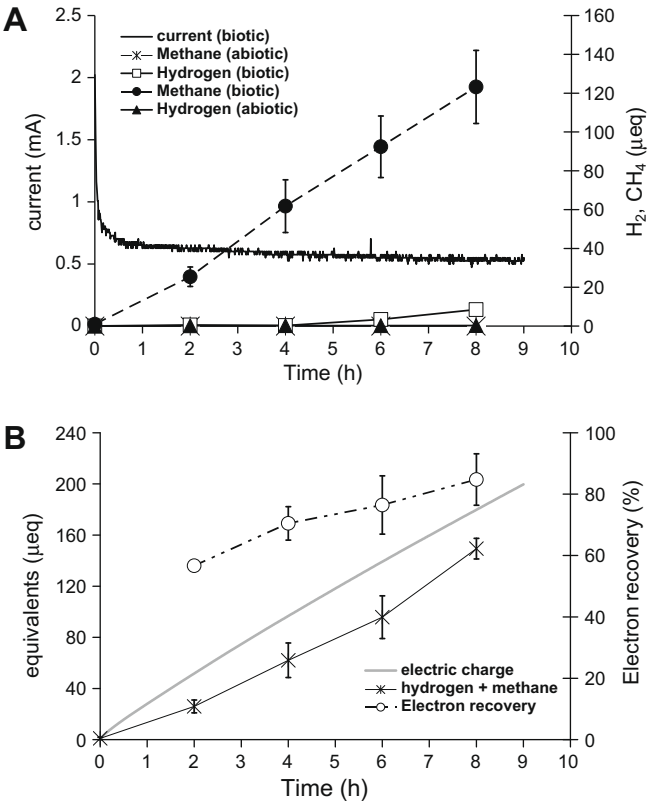

Fig. 4. Bioelectrochemical experiment at $V = -750$ mV with the enriched methanogenic culture. (A) Time course of H_2, CH_4 and current. (B) Cumulative electric charge transferred, equivalents recovered as H_2 and CH_4 and total electron recovery (total coulombic efficiency). Error bars are standard deviations of triplicate batch experiments.

in the range from −650 to −900 mV. As an example, Fig. 4 shows the results of tests carried out at −750 mV with the filtered growth medium (abiotic control) and with the microbial culture (biotic test). In the abiotic control (Fig. 4A), hydrogen production was negligible and no production of methane was observed. The lack of electrochemical hydrogen production was probably due to the high overpotential of this reaction at the carbon electrode. In the presence of microorganisms, methane gas was almost linearly produced at a rate of ∼374 ± 57 μeq d^{-1} (corresponding to 0.008 ± 0.001 mmol d^{-1} $mgVSS^{-1}$). Also in this case, the hydrogen production was very low. Fig. 4A also shows the time course of the measured cathodic current during the biotic test. After an initial sharp drop, it remained nearly constant at values between −0.6 and −0.5 mA, throughout the remainder of the test.

The cumulative electric charge transferred during the test was very close to the cumulative equivalents recovered as CH_4 plus H_2 (Fig. 4B) and, as a consequence, the overall coulombic efficiency (total electron recovery) of the process was high (∼85 ± 2%), mostly due to methane formation (∼76 ± 7%).

The catalytic effect of the methanogenic culture, was observed at cathode potentials more negative than −700 mV, as shown in Fig. 5. Indeed, in the range from −650 to −700 mV the total rate of reduced end-products formation (e.g., the sum of the rate of formation of CH_4 and H_2) in the presence of the microorganisms was very close to that observed in control tests; differently, starting from cathode potentials more negative than −700 mV, it was always higher than in abiotic tests (Fig. 5A).

In abiotic experiments hydrogen was the only reduced end-product, whereas in the biotic ones methane was the major product formed along with lower amounts of hydrogen. In particular, in abiotic tests the rate of hydrogen production (Fig. 5B) was very low

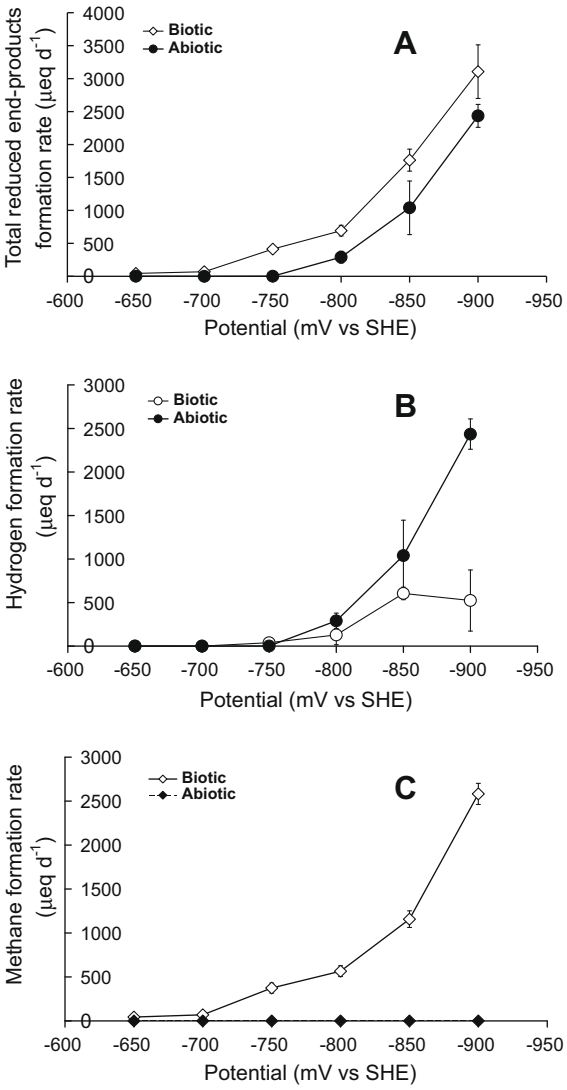

Fig. 5. Bioelectrochemical experiments at different cathode potentials with the methanogenic culture.

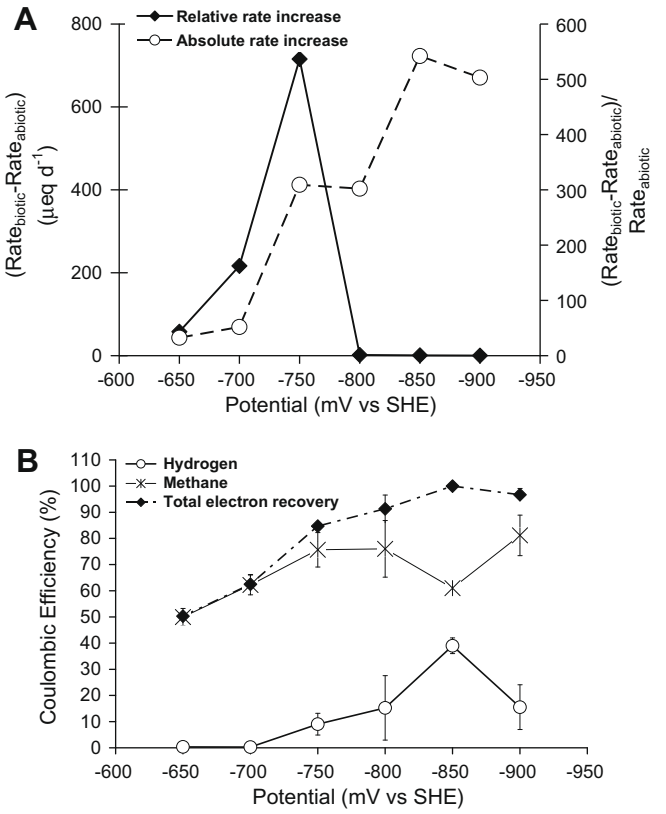

Fig. 6. (A) Absolute and relative rate increase and (B) contribution of methane and hydrogen production reactions to the total electron recovery at different cathode potentials with the methanogenic culture.

when the cathode potential was set between −650 and −750 mV, then it increased almost exponentially at more negative potentials.

As for the biotic tests, the H_2 production rate was similar to that measured in the abiotic tests polarizing the electrode from −650 to −800 mV; instead at more negative potentials the observed rate of hydrogen production was significantly lower than in the abiotic tests. This fact is likely due to the rapid interconversion, catalyzed by the methanogenic culture, of the produced H_2 into CH_4, as also indicated by the trend of the CH_4 production rate (Fig. 5C). There was no methane production in abiotic tests, whereas in biotic tests, the rate of CH_4 production increased as the electrode potential became more negative, up to $\sim 2582 \pm 120$ μeq d^{-1} (i.e., 0.32 ± 0.01 mmol d^{-1}) at $V = -900$ mV. This rate corresponded to 0.055 ± 0.002 mmol d^{-1} $mgVSS^{-1}$, a value that is very similar to that reached in the enrichment bioreactor, in the presence of excess hydrogen, showing that the electrochemical system did not exert negative effects on the activity of the microbial culture.

The difference between the total electron transfer rate in biotic and abiotic experiments is actually a measure of the rate of microbial extracellular electron transfer with the cathode. This rate, defined as "absolute rate increase" (Fig. 6A), showed a 20-fold increase when the cathode potential was decreased from −650 mV to −900 mV.

In Fig. 6A, the "relative rate increase" was defined as the ratio between the absolute rate increase and the abiotic reduced product formation rate. The observed trend of this parameter showed that the maximum microbial contribution to electron transfer was obtained at an applied potential of −750 mV; when there was very low hydrogen production in the abiotic tests and the overall electron transfer was mostly due to direct transfer.

Since the tests were carried out under vigorous stirring of the liquid phase, it is unlikely that current generation was substantially affected by any increase of mass transfer rate due to microbial consumption, in the bulk liquid, of the produced hydrogen (if any). Therefore, the results confirmed the ability of the methanogenic culture to enhance the formation of reduced end-products, namely methane, by directly accepting electrons from the polarized electrode.

In Fig. 6B, the contribution of both methane and hydrogen production reactions to the total electron recovery is reported. The coulombic efficiency of carbon dioxide reduction to methane increased from 50% to about 80% as the cathode potential decreased. The equivalents recovered as hydrogen were negligible at −650 and −700 mV, whereas at more negative values, increased up to ~30%. As a consequence, the total electron recovery approached about 100% at −900 mV.

3.4. Electron transfer mechanisms

The present study provided new insights into the recently discovered process of methane generation from bioelectrochemical carbon dioxide reduction (the process is also known as "electromethanogenesis"). Methane production was found to proceed on plain carbon cathodes, polarized at potentials more negative than −650 mV, in the presence of a hydrogenophilic methanogenic cul-

ture. In the whole range of cathode potentials investigated, only a fraction of methane was produced via extracellular electron transfer processes; the remainder was biologically produced via hydrogenophilic methanogenesis, using hydrogen abiotically produced from water reduction. The relative contribution of these routes to overall methane production was highly dependent on the set cathode potential.

Both CV tests and batch potentiostatic experiments seemed to indicate that the capacity for extracellular electron transfer with solid-state electrodes was a constitutive trait of the hydrogenophilic methanogenic culture. This is a substantial new evidence with respect to what reported by Cheng and colleagues where "electromethanogenesis" was observed with a culture previously enriched in a bioelectrochemical system. Also in that case, however, a hydrogenophilic methanogen appeared to be responsible of the observed methane production, and the rates were comparable to those obtained in this study [e.g., ~400 mmol CH_4 d^{-1} m^{-2} at $V = -900$ mV, both in this study and the previous one (Cheng et al., 2009)]. Some researchers have suggested that this capacity was developed by microbes to enable intraspecies and interspecies electron transfer (Cheng et al., 2009; Dinh et al., 2004; Gorby et al., 2006; Rabaey et al., 2004; Reguera et al., 2005).

Interestingly, this study revealed that the rate of extracellular electron transfer was strongly dependent on the cathode potential: a 20-fold increase was observed when the potential was decreased from −650 mV to −900 mV. Nonetheless, in the same interval, the enhancement of abiotic electron transfer processes (i.e., hydrogen gas production) was much greater; overall the relative contribution of extracellular electron transfer displayed a maximum at −750 mV. It is expected that the rate of extracellular electron transfer, as well as its relative contribution to the overall electron transfer, could be further increased through the formation of an electroactive biofilm on high specific surface electrodes.

Even though this study revealed that the hydrogenophilic methanogenic culture was "electroactive" (i.e., catalyzed the formation of reduced end-products by directly accepting electrons from the cathode), it did not allow to elucidate the exact pathway and mechanisms of the extracellular electron transfer-driven methane production reaction. As an example, it is possible that methanogens directly accepted the electrons from the electrode and intracellularly delivered them to the final acceptor (i.e., carbon dioxide) with resulting methane generation. On the other hand, previous studies (Aulenta et al., 2008; Rozendal et al., 2008) have shown that microorganisms possessing hydrogenases are able to undergo direct electron transfer at the electrode and release hydrogen. Hence, it is still possible that the observed methane production was sustained via interspecies hydrogen transfer between electroactive H_2-producing microorganisms and H_2-utilizing methanogens. Clearly, further studies in this direction are required.

4. Conclusions

This study confirmed the feasibility of reducing carbon dioxide to methane, at coulombic efficiencies exceeding 80%, using a carbon electrode as electron donor and a hydrogenophilic methanogenic culture as the catalytic agent. In principle, the voltage required for cathodic methane production could be obtained, at least partially, in a MEC from the anodic biological oxidation of waste organic materials, including diluted wastewaters. Depending on applications, this reaction also offers the opportunity for converting (renewable) electrical energy into a gaseous biofuel.

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