Bioresource Technology 102 (2011) 3193–3199

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Bioelectrochemical hydrogen production with hydrogenophilic dechlorinating bacteria as electrocatalytic agents

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ARTICLE INFO

Article history:
Received 11 June 2010
Received in revised form 6 October 2010
Accepted 7 October 2010
Available online 4 November 2010

Keywords:
Bioelectrochemical systems
Biocathode
Dechlorinating bacteria
Hydrogen production
Redox mediator

ABSTRACT

Hydrogenophilic dechlorinating bacteria were shown to catalyze H_2 production by proton reduction, with electrodes serving as electron donors, either in the presence or in the absence of a redox mediator. In the presence of methyl viologen, *Desulfitobacterium*- and *Dehalococcoides*-enriched cultures produced H_2 at rates as high as 12.4 μeq/mgVSS (volatile suspended solids)/d, with the cathode set at −450 mV vs. the standard hydrogen electrode (SHE), hence very close to the reversible H^+/H_2 potential value of −414 mV at pH 7. Notably, the *Desulfitobacterium*-enriched culture was capable of catalyzing H_2 production without mediators at cathode potentials lower than −700 mV. At −750 mV, the H_2 production rate with *Desulfitobacterium* spp. was 13.5 μeq/mgVSS/d (or 16 μeq/cm^2/d), nearly four times higher than that of the abiotic controls. Overall, this study suggests the possibility of employing dechlorinating bacteria as hydrogen catalysts in new energy technologies such as microbial electrolysis cells.

1. Introduction

The production of hydrogen as energy source should ideally be based on renewable resources and sustainable processes (Turner, 2004). Microbial electrolysis cells (MEC) have the potential to replace conventional electrolytic systems operating with platinum or other precious metals. In a MEC "exoelectrogenic" microorganisms (Logan, 2009; Rabaey and Verstraete, 2005) oxidize (waste) organic matter by releasing electrons to a solid-state electrode (anode) and carbon dioxide and protons into solution. By applying a small external voltage to the system, electrons can be forced to travel from the anode to the cathode and combine with protons to produce molecular H_2 (Logan et al., 2008). Typically, the equilibrium anode potential generated from the bacterial oxidation of organic substrates (e.g., acetate) is around −300 mV (vs. the standard hydrogen electrode, SHE). Under standard conditions and pH 7, the reversible potential of H^+/H_2 is −414 mV, and therefore, the minimum theoretical voltage that has to be applied in order to produce H_2 at the cathode is 114 mV. This voltage is very low compared to the voltage necessary for conventional water electrolysis (i.e., 1.6–2.0 V) (Zeng and Zhang, 2010).

Currently, a major limitation of the process is that noble metal (e.g., Pt-based) catalysts are typically used on the cathode to enhance the rate and efficiency of hydrogen production. These noble metal catalysts are expensive and susceptible to poisoning. Various non-noble materials have been also studied (Call et al., 2009), but they typically exhibit insufficient chemical stability and/or reactivity at neutral pH for efficient MEC operation (Daftsis et al., 2003; Highfield et al., 1999; Rabaey and Keller, 2008; Selembo et al., 2009).

Microbial biocathodes, in which microorganisms are the electrocatalytic agents of the desired cathodic reaction, are potential alternatives to chemical cathodes since they are inexpensive, self-regenerating and can operate under neutral pH. Lojou et al. (2002) showed that a pure culture of *Desulfovibrio vulgaris* immobilized onto a carbon electrode could catalyze H_2 production with methyl viologen (MV) ($E^{o\prime}$ = −446 mV) as a redox mediator and Rozendal et al. (2008) tested a MEC with a mixed culture biocathode of unknown microbial composition which catalyzed H_2 production likely via direct extracellular electron transfer. The occurrence of methanogens in the mixed culture was the main limiting factor which required the removal of bicarbonate from the medium to maintain acceptable H_2 yields.

Based on our earlier findings that a dechlorinating culture released some H_2 in the presence of excess MV, in addition to dechlorinating trichloroethene (TCE), as a strategy to dispose of an excess of reducing equivalents (Aulenta et al., 2008), we established TCE dechlorinating (*Desulfitobacterium*-enriched) and *cis*-DCE dechlorinating (*Dehalococcoides*-enriched) cultures to produce H_2 in short-term potentiostatic tests (i.e., 8 h) either at −450 mV (vs. SHE) in the presence of MV concentrations ranging from 0 to 2.5 mM or without exogenous redox mediators, at cathode potentials ranging from −650 to −900 mV (vs. SHE).

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doi:10.1016/j.biortech.2010.10.146

2. Methods

2.1. Microbial dechlorinating cultures

The TCE dechlorinating and *cis*-DCE dechlorinating cultures were established by enrichment from contaminated sediments of the Venice Lagoon (Aulenta et al., 2002) and maintained in anaerobic bioreactors with a liquid volume of 1.4 and 0.6 L for the TCE dechlorinating and the *cis*-DCE dechlorinating culture, respectively. The reactors consisted of continuously stirred borosilicate glass bottles, sealed with a Teflon-faced butyl rubber stoppers and aluminum crimp seals. The bioreactors were operated at 25 ± 1 °C in fill and draw mode. Every 7 days, they received a spike of neat trichloroethene (TCE) or *cis*-dichloroethene (*cis*-DCE) to a nominal concentration (i.e., neglecting the partitioning of compounds into the gas phase) of 0.5 mM. Thereafter, hydrogen gas (99.5+%) was added to the headspace of the reactors to a nominal concentration of 2.1 and 1.4 mM, for the TCE dechlorinating and the *cis*-DCE dechlorinating culture, respectively. Before each feeding, the headspace of each bioreactor was flushed with a N_2/CO_2 (70:30) gas mixture to remove all the volatile compounds. Weekly, a fixed volume of suspended culture was removed from each culture and replaced by anaerobic basal medium, which contained (g/L): NH_4Cl, 0.5; $MgCl_2 \cdot 6H_2O$, 0.1; K_2HPO_4, 0.4; $CaCl_2 \cdot 2H_2O$, 0.05; 10 mL/L of a trace metal solution (Balch et al., 1979), 10 mL/L of vitamin solution (Zeikus, 1977), and 15 mL/L of $NaHCO_3$ (10% w/v). The pH of the medium was 7.5. For both cultures the average cell retention time was maintained at around 60 days. The pseudo steady-state biomass concentration was about 63 mgVSS/L for the TCE dechlorinating culture and about 53 mgVSS/L for the *cis*-DCE dechlorinating culture. During fill and draw cycles, TCE was predominantly dechlorinated to *cis*-DCE and, in lower amounts, also to vinyl chloride (VC) and ethene; in the other bioreactor, *cis*-DCE was predominantly dechlorinated to VC and lower amounts of ethene. The two bioreactors were operated for a period of over 3 years. Prior to the bioelectrochemical experiments, the microbial composition of the two cultures was analyzed by fluorescence in situ hybridization (FISH) and catalyzed reporter deposition (CARD)-FISH in order to quantify the relative abundance of the different dechlorinating bacteria as described previously (Fazi et al., 2008; Rossetti et al., 2008). Hybridizations with the specific probes DSF440 and DSF475 for *Desulfitobacterium* spp.; DHE1259t and DHE1259c for *Dehalococcoides* spp. were carried out simultaneously with DAPI and/or probes EUB338, EUB338-II, and EUB338-III combined in a mixture (EUB), specific for most *Bacteria*. Details on oligonucleotide probes are available at probeBase (Loy et al., 2007). The probes were synthesized with 5′-FITC and -Cy3 labels and purchased from MWG AG Biotech (Germany). The ratios of the cells binding the group-specific probes and of cells staining with DAPI or binding the EUB probes were established for at least 20 different, randomly selected fields. Images were captured with Olympus F-view CCD camera and handled with AnalySIS software (SIS, Munster, Germany).

2.2. Bioelectrochemical cell setup

The bioelectrochemical cell setup 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 boiled successively in H_2O_2 (3% v/v), DI water, then in 0.5 M H_2SO_4, and DI water each for 2 h, and stored in DI water. The cathodes used were either a piece (50 × 10 mm) of carbon paper (E-TEK; nominal surface area ~8 cm^2) or a glassy carbon rod (HTW GambH, Germany; 5 mm diameter, 50 mm length, nominal surface area ~8 cm^2); the anode was a glassy carbon rod (HTW GambH, Germany; 5 mm diameter, 50 mm length, nominal surface area ~8 cm^2). The distance between the anode and cathode was around 10 cm. 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., Milan, Italy). 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

The cathode and anode compartments of the bioelectrochemical cell were anaerobically filled with 150 mL of the dechlorinating culture and 150 mL of mineral medium, respectively, and flushed with a N_2/CO_2 (70:30 v/v) gas mixture.

The bioelectrochemical cell was connected to the potentiostat and the cathode potential was set at the desired value. A set of batch experiments was carried out either in the absence or in the presence of methyl viologen (MV). In the latter case, appropriate volumes of a 100 mM stock solution of MV were added to achieve concentrations of 0–2.5 mM. Each batch experiment lasted 8 h and at regular intervals (e.g., every 2 h), gaseous samples were taken from the headspace of the compartments using gastight, sample-lock Hamilton (Reno, NV) syringes, and 500 and 40 μL samples were analyzed by gas-chromatography for hydrogen and methane, respectively. In parallel, control tests were performed under the same operating conditions in the absence of the microbial culture or the redox mediator. The bioelectrochemical reactor was maintained at 25 °C in a water bath, under vigorous magnetic stirring to ensure that current generation was not substantially affected by mass transfer phenomena.

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_{H_2}) that were used for the formation of H_2 were calculated from the measured amounts of H_2, considering the corresponding molar conversion factor of 2 μeq/μmol. Coulombic efficiency (CE) for H_2 was accordingly calculated as $CE\ (\%) = (\mu eq_{H_2}/\mu eq_i) \times 100$. The energy recovery was calculated as $\eta_E\ (\%) = W_{H_2}/W_{IN}$, where $W_{H_2} = n_{H_2} \times \Delta G_{H_2}$ is the energy content (kJ) of the produced H_2, calculated from the total amount of H_2 produced (n_{H_2}, mol) and the molar Gibbs free energy of H_2 oxidation by oxygen to water ($\Delta G_{H_2} = -237.1$ kJ/mol); W_{IN} is the electricity input determined as $C_P \times E_{APP}$, where C_P is the total Coulombs calculated by integrating the current over time and E_{APP} is the hypothetical applied voltage. This latter value was calculated by assuming a hypothetical anode potential of −0.150 V, as typical of acetate-fed bioanodes.

2.4. Analytical methods

The concentration of microorganisms in the source culture reactor was determined as volatile suspended solids (VSS), according to standard methods (APHA, 1995). H_2 was analyzed in a 500 μL gaseous sample by a Trace Analytical TA3000R reduction gas detector (RGD) (H_2 detection limit is 0.02 ppmv) (Menlo Park, CA). The H_2 level above the range of the RGD (i.e., ~100 ppmv) was quantified using a Varian 3400 (Lake Forest, CA, USA) gas-chromatograph (stainless-steel column packed with molecular sieve, Supelco, He carrier gas 18 mL/min; oven temperature 180 °C; thermal-conductivity detector (TCD) temperature 200 °C) (Aulenta et al., 2005). 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; He carrier gas at 18 mL/min; oven temperature at 50 °C; flame ionization

detector (FID) temperature 260 °C). Headspace concentrations were converted to aqueous-phase concentrations using tabulated Henry's law constants (Gossett, 1987).

2.5. Chemicals

Hydrogen (99.5+%) and all the other chemicals were purchased from Sigma–Aldrich (Milan, IT) (except where differently indicated). The chemicals used to prepare the mineral medium were of analytical grade and were used as received.

3. Results and discussion

3.1. Molecular characterization of the dechlorinating cultures used in the bioelectrochemical tests

Over 91.5% and 99.5% of the microorganisms stained by DAPI in the TCE dechlorinating and *cis*-DCE dechlorinating cultures, respectively, were also stained with the eubacterial probe EUB (Fig. 1). In the TCE dechlorinating culture, 83.0% and 5.6% of EUB-positive cells were stained with the FISH probes targeting *Desulfitobacterium* and *Dehalococcoides* species, respectively. In contrast, in the *cis*-DCE dechlorinating culture, *Dehalococcoides* spp. accounted for over 96% of bacterial cells (Fig. 1). FISH images of *Desulfitobacterium* spp. and *Dehalococcoides* spp. cells after hybridization with specific probes can be found in Supplementary material. These results clearly demonstrate that the long-term operation of the bioreactors with TCE and *cis*-DCE as the sole electron acceptors, and H_2 as the sole electron donor, was highly effective in selecting for *Desulfitobacterium* spp. and *Dehalococcoides* spp., respectively. These findings are also consistent with *Desulfitobacterium* spp. being capable to dechlorinating TCE to *cis*-DCE, using H_2 or other substrates as electron donors (Nonaka et al., 2006) and with *Dehalococcoides* spp. being the only isolated microorganism capable to dechlorinating *cis*-DCE and VC with H_2 as the sole electron donor (Maymo-Gatell et al., 1997).

3.2. Bioelectrochemical H_2 production at −450 mV (vs. SHE) in the presence of methyl viologen (MV)

Both dechlorinating cultures showed the ability to catalyze H_2 production, with specific production rates showing a saturation dependency on the liquid phase MV concentration (Fig. 2). Under all the experimental conditions, methane was not detected in the headspace of the cell. Importantly, over the entire range of MV concentrations investigated, H_2 production was negligible in abiotic (control) tests (data not shown).

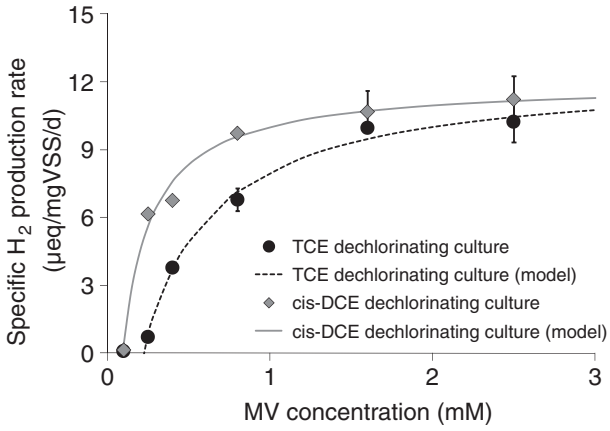

Fig. 2. Influence of methyl viologen (MV) concentration on H_2 production rate by the microbial dechlorinating cultures in batch experiments at −450 mV (vs. SHE). For each culture, two MV concentration levels were assayed in duplicate (namely, 0.8 and 2.5 mM for the TCE dechlorinating culture; 1.6 and 2.5 mM for the *cis*-DCE dechlorinating culture); for these experiments the average value (±1 standard deviation) of duplicates is reported. Lines are simulations based on Michaelis–Menten model, modified to account for a MV threshold concentration.

Experimental data were fitted to a Michaelis–Menten-type model, modified to account for a MV threshold concentration (i.e., the lowest MV concentration which sustained a measurable H_2 production) (Fennell and Gossett, 1998). Estimated maximum specific H_2 production rates (k_{MAX}) were similar for the two cultures (12.4 ± 0.6 μeq/mgVSS/d and 11.9 ± 0.6 μeq/mgVSS/d, for the TCE and the *cis*-DCE dechlorinating culture, respectively). Conversely the half-velocity coefficients (K_m) and the MV threshold concentrations were substantially different, with the *cis*-DCE dechlorinating culture exhibiting a greater affinity for the mediator, namely a lower K_m (0.177 ± 0.038 mM) and MV threshold concentration (0.096 ± 0.009 mM) than the TCE dechlorinating culture (estimated K_m and MV threshold concentration were 0.429 ± 0.076 and 0.222 ± 0.016 mM, respectively).

The values of current densities, recorded during each test, almost linearly increased with the MV concentration, up to around 0.1 mA/cm^2 (at a mediator concentration of 2.5 mM), regardless the culture used.

For both cultures, the coulombic efficiency (CE), calculated at the end of the 8-h tests, reached a maximum value when MV

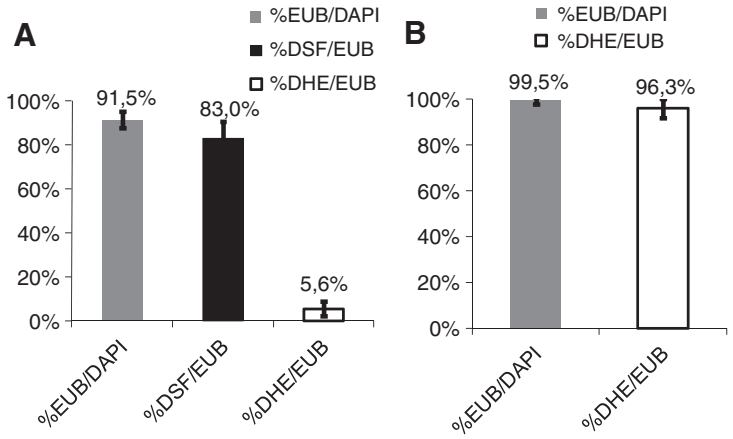

Fig. 1. Molecular characterization of the TCE (A) and *cis*-DCE (B) dechlorinating cultures by fluorescence in situ hybridization using probes for *Bacteria* (EUB probes: EUB338, EUB338-II, and EUB338-III), *Desulfitobacterium* spp. (DSF probes: DSF440 and DSF475 helper) and *Dehalococcoides* spp. (DHE probes: DHE1259t and DHE1259c); error bars are 1 standard deviation of replicated measurements.

was in the range 0.25–0.75 mM, then it gradually decreased as the MV concentration increased. Notably, the highest CE values for the *cis*-DCE dechlorinating culture (i.e., over 40% at 0.25 mM) were typically higher than those observed with the TCE dechlorinating culture (i.e., around 25% at 0.75 mM). A possible explanation for the relatively low CE values is the irreversible reduction of the radical $MV^{+\cdot}$ (i.e., the species thought to be involved in the electron transfer with the microorganisms) to MV^0, as well as the formation of dimerization products from coupling reactions between $MV^{+\cdot}$ radicals.

Regarding the mechanisms of H_2 production in the presence of dissolved MV, it is worth noting that many dechlorinating bacteria, including *Desulfitobacterium* spp. and *Dehalococcoides* spp. possess multiple periplasmic hydrogenases which probably directly reacted with electrically reduced MV. A similar mechanism has been previously suggested for the sulfate-reducing bacterium *Desulfovibrio vulgaris* (Lojou et al., 2002).

3.3. Bioelectrochemical H_2 production in the absence of exogenous redox mediators

Short-term (i.e., 8 h) H_2 production tests were also carried out in the absence of exogenous redox mediators, in a range of cathode potentials from −650 to −900 mV. The aim of these tests was to explore the ability of the dechlorinating cultures to directly catalyze H_2 production (via direct electron transfer) upon adsorption onto the surface of the polarized electrode. For these tests, carbon paper cathodes having a greater surface area than the glassy carbon cathodes (used in the tests with MV) were employed, in order to maximize bacterial adsorption onto the electrode surface.

Fig. 3 compares the time course of H_2 production, with the cathode potential set at −750 mV, for the two dechlorinating cultures and the abiotic control (from separate batch tests under identical experimental conditions). Differently from the tests carried out in the presence of soluble MV, the *Desulfitobacterium*-enriched culture showed a greater affinity for the cathode than the *Dehalococcoides*-enriched culture. The H_2 production rate in the presence of the TCE dechlorinating (*Desulfitobacterium*-enriched) culture was nearly 3.7 times higher than in the abiotic control with a current density of 0.025 mA/cm^2; conversely the rate of H_2 production in the presence of the *cis*-DCE dechlorinating (*Dehalococcoides*-enriched) culture was not significantly (95% confidence) higher (<40%), than that of the abiotic control despite the fact that the two cultures had a very similar cell concentration.

As expected, the specific rate (i.e., normalized with respect to the nominal surface area of the electrode) of H_2 production increased with decreasing cathode potential (i.e., by setting the cathode at more reducing potentials) in the biotic and abiotic tests (Fig. 4A). Notably, in the range of cathode potentials from −700 to −900 mV, the specific rate of H_2 production in the presence of the TCE dechlorinating culture was always higher than that measured in abiotic tests and hence purely due to electrochemical H^+ reduction. At −900 mV it reached a value of 329 ± 36 μeq/cm^2/d (or 278 ± 31 μeq/mgVSS/d) with a current density of 0.44 mA/cm^2. In contrast, H_2 production in the presence of the *cis*-DCE dechlorinating culture was almost indistinguishable from that measured in the abiotic control tests over the entire range of cathode potentials. These findings demonstrate the superior ability of *Desulfitobacterium* spp., compared to *Dehalococcoides* spp., to catalyze H_2 production with solid electrodes serving as electron donors. Also in these tests, no methane was ever detected in the headspace of the cells.

The TCE dechlorinating culture did not only result in consistently higher H_2 production rates than those of the abiotic controls, but also in higher CE and therefore reaction selectivity (Fig. 4B). Remarkably, the beneficial effect of the biocatalyst was greater at cathode potentials from −700 to −800 mV where the abiotic reaction was sluggish. As shown in Fig. 4, the CE approached 100% for cathode potentials lower than −800 mV in the presence of the TCE dechlorinating culture as well as in abiotic controls.

For any given cathode potential, the contribution of bacterial catalysis to the rate of H_2 production was calculated by subtracting the abiotic H_2 production rate from the total biotic H_2 production rate measured in the presence of the microbial culture. For the TCE dechlorinating culture, the H_2 production due to bacterial catalysis accounted for at least 70% of the total H_2 production at −750 mV (Fig. 5), and then gradually decreased at more negative cathode potentials where most of the H_2 was derived from abiotic processes. At −900 mV, less than 15% of the H_2 was produced via bacterial catalysis (Fig. 5). It is also worth mentioning that, for

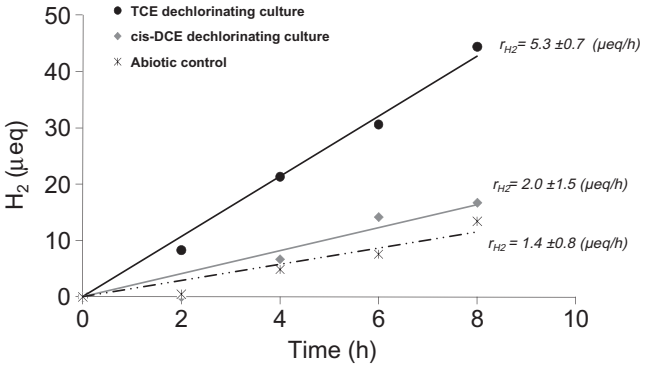

Fig. 3. H_2 production without exogenous mediators by the dechlorinating cultures and the abiotic control: time course of produced H_2 equivalents in batch experiments at −750 mV (vs. SHE). Calculated H_2 production rates are average values (±1 standard deviation) of replicated experiments.

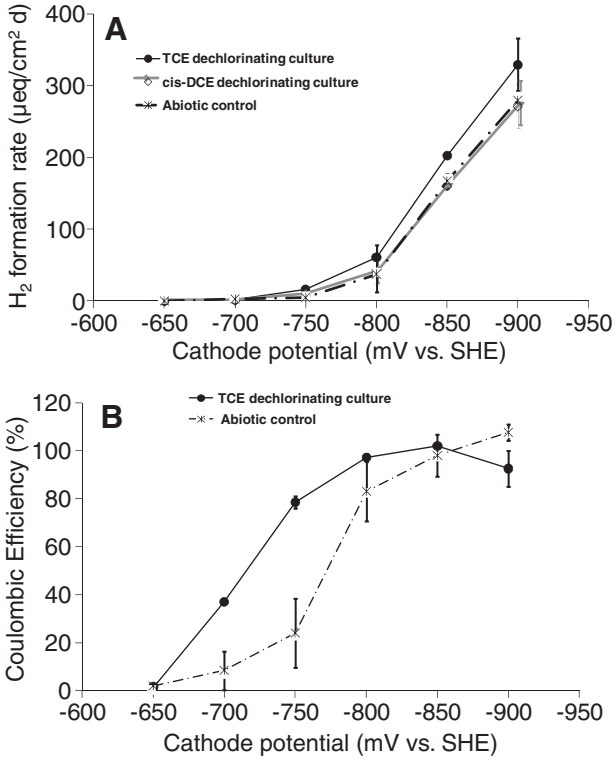

Fig. 4. Effect of cathode potentials on: (A) H_2 production rate by the dechlorinating cultures and the abiotic control and (B) coulombic efficiency of H_2 production by the TCE dechlorinating culture and the abiotic control. Error bars are 1 standard deviation of replicated tests.

the TCE dechlorinating culture, the net biocatalytic H_2 formation rate almost linearly increased with decreasing cathode potential and accordingly by increasing the driving force for the electron transfer (Fig. 5). The latter observation suggests that the rate of electron transfer from the electrode to the microorganisms, rather than the intrinsic biocatalyst activity, was limiting the rate of hydrogen production.

The mechanisms of H_2 production by the TCE dechlorinating culture in the absence of exogenous redox mediators could not be determined. A possible mechanism could have involved the direct interaction of adsorbed bacterial cells with the surface of the polarized electrode and routing of electrons to hydrogenases via redox active components (e.g., cytochromes) located on the outer membrane of the microorganism (Rosenbaum et al., 2011). This hypothesis is in agreement with the fact that the genome of *Desulfitobacterium* sp. encodes several *c*-type cytochromes (Nonaka et al., 2006) whereas the genome of *Dehalococcoides* strains does not (Seshadri et al., 2005). It cannot be excluded that self-produced soluble mediators and/or products of bacterial lysis (including hydrogenases) may have also contributed to the shuttling of electrons from the electrode to the microorganisms or may have catalyzed the H_2 production themselves (Freguia et al., 2010; Marsili et al., 2008). Importantly, it should be noted that the enhancement of H_2 production rate (with respect to the abiotic controls) was observed only with the TCE dechlorinating culture, but not with *cis*-DCE dechlorinating culture, though the two cultures had a very similar cell concentration. This finding indicates that the mechanism of H_2 production was specific to the TCE dechlorinating culture and likely ascribable to the presence of *Desulfitobacterium* species; however, further investigations are needed to shed light on this fundamental issue.

3.4. Energetic analysis of mediated and mediatorless bioelectrochemical H_2 production

The use of MV allowed production of H_2 at −450 mV, a value that is very close to the reversible H^+/H_2 potential, whereas a potential more negative than −700 mV was needed if the redox mediator was lacking. In other words, the mediator reduced the overpotentials for H_2 evolution by at least 300 mV, thereby potentially reducing the energy input to the H_2-producing electrolyzer. A higher CE was typically obtained in the tests carried out in the absence of MV, thereby indicating a more efficient usage of the electric current. To account for these factors, we calculated the energy yield (i.e., the energy recovery as H_2 relative to the electrical energy input) for the mediatorless and mediated H_2 production. For this calculation we considered a hypothetical applied voltage for an electrolyzer or MEC having an anode potential of −150 mV [a value that is typical for acetate-fed MECs or MFCs (Aelterman et al., 2008)] and the different cathode configurations tested in this work (Fig. 6). For the tests carried out in the presence of different MV concentrations, only those yielding the highest energy yields are shown in Fig. 6 (i.e., 0.25 mM for the *cis*-DCE dechlorinating culture and 0.75 mM for the TCE dechlorinating culture). As shown in Fig. 6, in the presence of MV, the *cis*-DCE dechlorinating culture (MV = 0.25 mM) resulted in a higher energy yield (about 170%) than the TCE dechlorinating culture (about 100% with MV concentration of 0.75 mM). This fact reflects the relatively higher CE (over 40%) obtained with the *cis*-DCE dechlorinating culture than the TCE dechlorinating culture at a relatively low hypothetical applied voltage (300 mV) (i.e., with the cathode at −450 mV). Similar energy yields were obtained in the absence of MV, with the highest value (over 180%) observed in the presence of the TCE dechlorinating culture at a hypothetical applied voltage of 650 mV (i.e., at a cathode potential of −800 mV). These values of energy yield are comparable with those reported in the literature with metal catalysts (Call et al., 2009; Selembo et al., 2009; Tartakovsky et al., 2009).

Clearly, identification of the optimal biocathode conditions requires that other factors, besides energetic ones, are taken into consideration; these include the cost of the mediator (which is also function of its chemical and electrochemical stability under selected operating conditions) as well as the rate of H_2 production needed. Along this line, it needs to be considered that the maximum volumetric H_2 production rate in the presence of excess MV (e.g., 0.011 $m^3/m^3/d$ for the TCE dechlorinating culture) was nearly the same than that obtained at −750 mV in the absence of MV (i.e., 0.010 $m^3/m^3/d$, for the TCE dechlorinating culture), but much higher production rates were obtained in the absence of MV at more negative potentials.

It is expected that both for the mediated and the mediatorless biocathodes, H_2 production rates could be greatly enhanced by increasing biomass density. The most appropriate strategies to achieve this will have to be identified in future investigations.

3.5. Perspectives for application of hydrogenase-containing microorganisms in electrochemical systems

In this study we have shown that living hydrogenophilic dechlorinating bacteria (i.e., *Desulfitobacterium* spp. and *Dehalococcoides* spp.), till now known for their ability to utilize hydrogen as an electron donor to *respire* chlorinated solvents (Holliger and Schumacher, 1994) can also carry out the reverse reaction of hydrogen generation (from water reduction), by using carbon-based

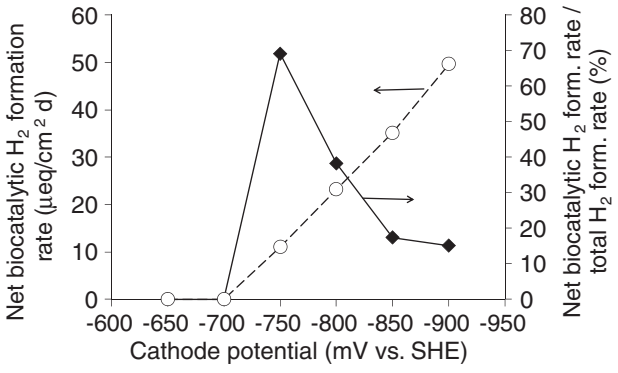

Fig. 5. Effect of cathode potential on the (net) rate of biocatalytic H_2 production and on the relative contribution (%) of the (net) rate of biocatalytic H_2 production on the total H_2 production rate by the microbial TCE dechlorinating culture.

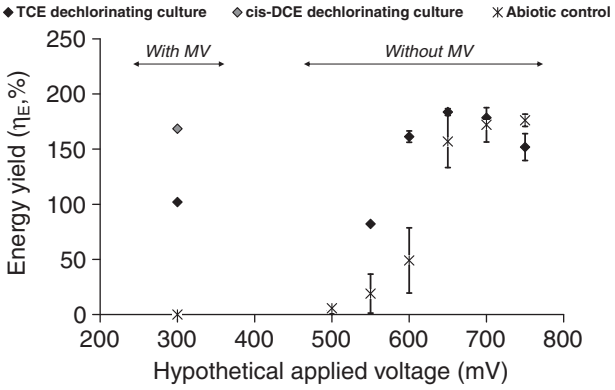

Fig. 6. Effect of hypothetical applied voltage on the energy recovery calculated as the ratio between the energy content of H_2 produced and the electrical energy consumed (by arbitrarily assuming a value of the anode potential of −150 mV vs. SHE). Error bars are 1 standard deviation of replicated experiments.

electrodes as electron donors. These dechlorinating bacteria are highly evolved to utilize H_2 by means of multiple hydrogenase systems (Nonaka et al., 2006; Seshadri et al., 2005), which, most likely, are also involved in the observed bioelectrocatalytic activity toward H_2 generation. Hydrogenases, the enzymes catalyzing the rapid interconversion of hydrogen and water in the microbial hydrogen cycle (Baker et al., 2009; Cracknell et al., 2008; Vincent et al., 2007) are receiving considerable attentions as effective electrocatalysts for fuel cells and electrolyzers. On the other hand, their high costs of isolation, difficulties in attaching these delicate molecules onto electrodic surfaces while protecting their fragile active sites from inactivation, have greatly hampered their practical application (Armstrong et al., 2009; Fourmond et al., 2009). Here we demonstrate that whole cells of dechlorinating bacteria, thus far only considered for bioremediation applications (Lovley, 2001), hold a potential as "novel" hydrogen catalysts for possible applications in new energy technologies such as microbial electrolysis cells. Based on the results of this study, in the future other hydrogenase-possessing microorganisms should also be assayed for similar applications. In this context, autotrophic microorganisms which do not rely on organic carbon for growth hold a significant potential. Nonetheless, further studies will also have to evaluate whether the bioelectrocatalytic H_2 production is linked to energy conservation and microbial growth. This issue will have direct and practical implications on the long-term process durability and sustainability.

4. Conclusions

In a bioelectrochemical system, a *Desulfitobacterium*-enriched culture and a *Dehalococcoides*-enriched culture catalyzed H_2 production with a carbon-based cathode set at −450 mV (a value that is very close to the reversible H^+/H_2 potential of −414 mV), provided a soluble mediator (i.e., MV) was present in the solution.

Notably, the *Desulfitobacterium*-enriched culture was also capable to produce H_2 in the absence of exogenous redox mediators, when the cathode was set at potentials more negative than −700 mV. This suggests that the different dechlorinating microorganisms employ different mechanisms for routing electrons to hydrogenases, which will necessarily have to be identified in order to fully exploit their potential.

Acknowledgements

This study was carried out in the frame of the FITOLISI research project, funded by the Italian Ministry of Agriculture, Food, and Forestry (MIPAAF). The authors thank Prof. Stefania Panero, Dr. Priscilla Reale, and Dr. Judith Serra Moreno (Sapienza University of Rome) for their suggestions and comments with the experimental setup.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2010.10.146.

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