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Influence of setup and carbon source on the bacterial community of biocathodes in microbial electrolysis cells

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ABSTRACT

The microbial electrolysis cell (MEC) biocathode has shown great potential as alternative for expensive metals as catalyst for H_2 synthesis. Here, the bacterial communities at the biocathode of five hydrogen producing MECs using molecular techniques were characterized. The setups differed in design (large versus small) including electrode material and flow path and in carbon source provided at the cathode (bicarbonate or acetate). A hydrogenase gene-based DNA microarray (Hydrogenase Chip) was used to analyze hydrogenase genes present in the three large setups. The small setups showed dominant groups of *Firmicutes* and two of the large setups showed dominant groups of *Proteobacteria* and *Bacteroidetes*. The third large setup received acetate but no sulfate (no sulfur source). In this setup an almost pure culture of a *Promicromonospora* sp. developed. Most of the hydrogenase genes detected were coding for bidirectional Hox-type hydrogenases, which have shown to be involved in cytoplasmatic H_2 production.

1. Introduction

Hydrogen gas (H_2) is a valuable product as a renewable energy carrier and as a reductant in the chemical industry [1]. H_2 can be formed by steam-reforming of natural gas, gasification of fossil or renewable materials and by water electrolysis. Water electrolysis, however, is energetically costly. An interesting alternative is microbial electrolysis. In a microbial electrolysis cell (MEC) microorganisms degrade organic compounds (e.g. acetate) at the anode to CO_2, protons and electrons. At the cathode, protons and electrons derived from the anode and energized by a power supply are combined to H_2. The anode and the cathode are generally separated by an ion selective membrane [2]. By using this technology, the fossil energy requirements for H_2 production can be diminished more than five times compared to H_2 production from direct water electrolysis [3,4].

Platinum is generally used to catalyze H_2 production at the cathode, as it significantly reduces the cathode overpotential. However, platinum is expensive, non-renewable, and very ineffective in catalyzing CO_2 reduction, and it is susceptible to poisoning by sulfur and carbon [5] monoxide. A low cost alternative for a platinum cathode is the biocathode.

A biocathode can be defined as an electrode made of cheap material (e.g. carbon or graphite) at which microorganisms catalyze the cathodic reaction (in this case: H_2 production). In a microbial cell, H_2 formation is thermodynamically confined and dependent on the available energy [6]. Microorganisms can produce H_2 through dark fermentation, with a low conversion efficiency of substrate to H_2, or by addition of energy in the form of light or heat [7,8]. The required energy can alternatively be provided by an electrode. In MEC cathodes inoculated with biomass originated from a wastewater treatment plant, the possibility of electron transfer from the electrode to microorganisms for the production of H_2 was

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demonstrated [9,10]. Little information is available on the types of microorganisms that develop at a biocathode in a microbial electrolysis cell and on the mechanism of electron transfer from the cathode to the microorganism to produce H_2.

Biocathode microorganisms have been studied mainly in cathodes for oxygen reduction [11], but also fumarate [12] and nitrate reduction, dechlorination and product formation (e.g. methane or acetate) with an electrode as electron donor have been shown [13,14]. Only a few studies describe the microbiology in H_2 producing biocathodes [15–19]. Microbial H_2 production involves hydrogenases, the enzymes that catalyze the reversible reaction $2H^+ + 2e^- \leftrightarrow H_2$. Hydrogenases are categorized according to their (redox active) metal site. The three groups of hydrogenases are (1) nickel-iron (NiFe)-hydrogenases of which a sub-group contains also selenium, (2) iron–iron (FeFe)-hydrogenases and (3) iron (Fe)-hydrogenases. The last group was previously characterized as iron-free hydrogenases because they do not contain a redox active iron. The catalysis of H_2 production has been mostly associated with the FeFe-hydrogenases and, H_2 oxidation (consumption) mostly with NiFe-hydrogenases. Nevertheless, some NiFe-hydrogenases, mainly the cytoplasmic ones, can also catalyze H_2 production in vivo [20,21]. The Fe-hydrogenases, which are found in several methanogens, are involved in methane formation from CO_2 and H_2 rather than in H_2 production [22]. No knowledge is currently available on the hydrogenases involved in H_2 production in the MEC biocathode.

For growth, biocathodic bacteria require a carbon source. Carbon dioxide is a low cost carbon source but in lab scale experiments acetate is often used as carbon source because it is an end product of dark fermentation. Acetate seems to be a preferred energy and carbon source for high efficiencies in microbial fuel cell (MFC) anodes [23] and recently it was also shown that in MEC biocathode systems acetate is a preferred carbon source over bicarbonate, for rapid startup of a biocathode [24]. The effect of different carbon sources on the growth and further development of the active microbial population of an MEC biocathode has not been studied before.

In the present study five MEC biocathode samples from an experiment that was described before by Jeremiasse et al. [24] were analyzed. The microbial communities were determined by 16S rRNA gene analysis and the hydrogenases of three of the samples were analyzed by using a Hydrogenase Chip developed previously [25]. Two small setups were operated of which one was supplemented with acetate (AcS) and the other with bicarbonate (BicS) and three large setups of which one received acetate (AcL), one bicarbonate (BicL) and the third one contained catholyte with acetate but without any sulfate (AcnSL), this to prevent loss of electrons by reduction of (the low amounts of) sulfate present in the growth medium. We hypothesize that the carbon source will have a major impact on the development and composition of the microbial population.

2. Material and methods

2.1. Operational conditions of the microbial electrolysis cell cathode

All setups were operated as described by Jeremiasse et al. [24]. In short, two different setups were used. The small setup, described by Ter Heijne et al. [26], consisted of a 22 cm^2 graphite paper cathode, and the large setup, described by Jeremiasse et al. [27], consisted of 100 cm^2 projected surface area of a 0.25 cm thick graphite felt cathode. The cathode was fed with anaerobic mineral salts medium containing (g L^{-1}) KH_2PO_4, 0.68; K_2HPO_4, 0.87; KCl, 0.74; NaCl, 0.58; NH_4Cl, 0.28; $CaCl_2{\cdot}2H_2O$, 0.1; $MgSO_4{\cdot}7H_2O$, 0.01 and 0.1 mL L^{-1} of a trace element mixture [28], supplemented with either 0.01 mol L^{-1} sodium bicarbonate or 0.001 mol L^{-1} sodium acetate. The cathode and anode were separated by a cation exchange membrane (Ralex CMH-PES, Mega A.S., Prague, Czech Republic). In the anode compartment 0.1 mol L^{-1} potassium hexacyanoferrate(II) was used, circulated from a 5 L tank which was refreshed weekly. No crossover of cyanoferrate was observed between compartments in all experiments. First the optimal potential for operation was determined in two series of small setups with a total cathodic circulation volume of 192 mL which was constantly refreshed with medium at a rate of 36 mL h^{-1}. The small setups were inoculated with 10 mL of biomass from the effluent and the biofilm of previously operated MEC anodes and cathodes. The two series of the small setups consisted of four MECs that were operated at potentials of −0.5, −0.6, −0.7 and −0.8 V (vs SHE) for more than 60 days. Those values were chosen because at those cathode potentials no significant chemical H_2 is produced at carbon electrodes in those systems [24]. A cathode potential of −0.7 V (vs. SHE) resulted in the highest catalytic activity. After operation, samples were collected from the electrode material of the −0.7 V setups (AcS and BicS). The biomass including electrode material was resuspended in catholyte solution and used as inoculum for the large setups (10 mL per setup). The large setups contained a 100 cm^2 (projected surface area) flow-through graphite felt electrode, a total volume of 100 mL and nutrient solution dosed at a rate of 156 mL h^{-1}. The large setups were operated at −0.7 V and supplemented with acetate (AcL, inoculated from AcS) or bicarbonate (BicL, inoculated from BicS). A third large setup (AcnSL) was inoculated from the AcL setup (10 mL of electrode biomass resuspended in catholyte) and run without any added sulfate to exclude that sulfate was used as an electron acceptor instead of protons. For this setup the $MgSO_4$ in the nutrient solution described above was replaced by $MgCl_2$. H_2 production was determined in a 48 h yield test for the BicL and AcnSL setup and in a 6 h yield test for the AcL setup as described previously [24]. After operation 1 cm^2 of a representative part of the electrode material (visual inspection) was cut from all five cathodes (AcS, BicS, AcL, BicL and AcnSL). The samples were stored at −20 °C for DNA analysis or processed further for SEM imaging.

2.2. Scanning electron microscopy (SEM)

Electrode samples were fixed in 2.5% glutaraldehyde (w/v) for 2 h at room temperature and washed twice with 0.01 mol L^{-1} PBS buffer (pH 7.4). Subsequently, the samples were dehydrated in a graded series of ethanol (10%, 25%, 50%, 75%, 90% and twice in 100% during 20 min for each step) and dried in a desiccator. The samples were coated with gold and examined in a JEOL JSM-6480LV Scanning Electron Microscope (acceleration voltage 6 kV, HV-mode, SEI detector).

2.3. DNA extraction and amplification of 16S rRNA genes

Genomic DNA was extracted from the electrode samples using the Fast DNA spin kit for soil (Bio101, Vista, CA, USA) using the manufacturer's instructions. Bacterial 16S rRNA genes were amplified using the primers Bact27F and Univ1492R [29]. PCR reaction mixtures consisted of 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 3 mM $MgCl_2$, 200 mM each of the four deoxynucleoside triphosphates (dNTP), 2.5U of Taq polymerase (Invitrogen), 200 nM of each primer and 2 μL of appropriately diluted template DNA in a final volume of 50 μL.

PCR settings were initial denaturation for 2 min at 95 °C, followed by 25 cycles of 30 s denaturation at 95 °C, 40 s annealing at 52 °C and 1.5 min elongation at 72 °C. Post-elongation was 5 min at 72 °C. The PCR products were tested on a 1% agarose gel for amount, integrity and size of the amplicon. For DGGE analysis partial bacterial 16S rRNA genes were amplified using primers Bact968F (including GC clamp) and 1401R [30] and partial archaeal 16S rRNA genes were amplified using primers Arch109(T)F and GC515R (including GC clamp) [29,31]. PCR conditions were as above, except that 35 cycles were applied and an annealing temperature of 56 °C was used.

2.4. Clone library construction and analysis

For all five cathode samples PCR-amplicons of almost complete bacterial 16S rRNA genes were purified using Nucleo Spin Extract II kit (Macherey-Nagel, Düren, Germany) and ligated into pGEM-T easy vector system I (Promega, Madison, WI, USA). After ligation the vectors were cloned in XL-1 blue competent *Escherichia coli* cells (Stratagene, Santa Clara, CA, USA) and grown on LB-agar containing 100 mg L^{-1} ampicillin, 0.001 mol L^{-1} isopropyl-1-thio-β-D-galactopyranoside (IPTG) and 40 mg L^{-1} 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). After blue-white screening, 96 white colonies were transferred to 1 mL liquid LB medium with 100 mg L^{-1} ampicillin. After overnight incubation at 37 °C all clones were transferred to a GATC 96 well nutrient agar plate with 100 mg L^{-1} ampicillin and sent to GATC (GATC Biotech AG, Konstanz, Germany) for sequencing.

Electropherogram analysis and sequence assembly were performed with DNAbaser version 2.71.0 (Heracle Software, Lilienthal, Germany) and phylogenetic affiliation of the sequences was examined with an NCBI BLAST identity search. All sequences were aligned using the online Silva alignment tool [32] and merged with the ARB database using ARB software package version 5.1 [33]. A Chimera check was performed using the Ribosomal Database Project website [34]. A phylogenetic tree was constructed using the ARB Neighbour Joining Algorithm with Jukes Cantor correction.

The microbial diversity per setup was calculated using Shannon's diversity index [35] using:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$