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Selective microbial electrosynthesis of methane by a pure culture of a marine lithoautotrophic archaeon

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ABSTRACT

Reduction of carbon dioxide to methane by microorganisms attached to electrodes is a promising process in terms of renewable energy storage strategies. However the efficient and specific electrosynthesis of methane by methanogenic archaea on cathodes needs fundamental investigations of the electron transfer mechanisms at the microbe–electrode interface without the addition of artificial electron mediators. Using well-defined electrochemical techniques directly coupled to gas chromatography and surface analysis by scanning electron microscopy, it is shown that a pure culture of the marine lithoautotrophic *Methanobacterium*-like archaeon strain IM1 is capable to utilize electrons from graphite cathodes for a highly selective production of methane, without hydrogen serving as a cathode-generated electron carrier. Microbial electrosynthesis of methane with cultures of strain IM1 is achieved at a set potential of −0.4 V vs. SHE and is characterized by a coulomb efficiency of 80%, with rates reaching 350 nmol d^{-1} cm^{-2} after 23 days of incubation. Moreover, potential step measurements reveal a biologically catalyzed hydrogen production at potentials more positive than abiotic hydrogen evolution on graphite, indicating that an excessive supply of electrons to strain IM1 results in proton reduction rather than in a further increase of methane production.

1. Introduction

The biological synthesis of methane is an essential part of the global carbon cycle, driven by strictly anaerobic methanogenic archaea commonly found in anoxic environments, including but not limited to freshwater and marine sediments, marshes and swamps, bogs, tundra, rice paddy fields, water clogged soils, geothermal habitats as well as gastrointestinal tracts of ruminants and insects [1–3]. Methanogenesis is the terminal decomposition of organic matter in those environments which generally lack inorganic electron acceptors except for protons and carbon dioxide [1–3]. Currently, methanogenic archaea are the only microorganisms known to produce methane as the major end product of their metabolism from a limited source of substrates like $H_2 + CO_2$, formate, methanol and acetate [2,3]. It is commonly recognized, that under environmental conditions, methanogenic archaea operate close to the thermodynamic limit, demanding elaborate mechanisms of energy conservation [2]. For instance, certain methanogenic archaea have been isolated that are able to utilize elemental iron (Fe^0) as the sole electron donor for the production of methane, which has a redox potential close to the thermodynamic equilibrium for CO_2 reduction [4–6]. Especially the *Methanobacterium*-like archaeon strain IM1 [5] and the *Methanococcus maripaludis* strain KA1 [6] were shown to corrode iron by direct electron uptake coupled to metabolic activity; i.e. a mechanism recently termed electrical microbially influenced corrosion [7–9]. Particularly, electron exchange mechanisms between microorganisms and electrodes are of special interest since those interactions are poorly understood, but are of potential practical applications in the environmental and bioenergy fields [10,11]. With regard to renewable energy storage strategies, especially microbial electrosynthesis at cathodes is considered as a valuable approach for the conversion of renewable energy (solar and wind energy) into valuable chemicals [11]. On these grounds, methanogenesis controlled by microbial electrosynthesis (Eq. (1)) could be a promising application to produce methane as an intermediate energy storage molecule by the reduction of carbon dioxide [2,3,11].

$$CO_2 + 8e^- + 8H^+ \rightarrow CH_4 + 2H_2O \qquad E^{0\prime} = -0.24 \text{ V vs. SHE.} \qquad (1)$$

Compared to biomass-based methanogenesis, microbial electrosynthesis of methane is more effective, avoiding for example the high demands and costs of biomass feedstock [1,11]. Moreover, self-regenerating biocatalysts like the methanogenic archaea that are able to accept electrons from cathodes for their metabolism are advantageous over chemical catalysts due to their high efficiency and selectivity. Recently, there have been several studies on microbial electrosynthesis of methane from carbon dioxide reduction with environmental methanogenic mixed populations [12–16]. The suspected direct electron transfer from electrodes to

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the microorganisms however was not shown evidentiary and has been doubted by others, as the results in such an approach might be a complex overlap of electron mediation by hydrogen and the contributions of various other strains in the mixed populations [10,11]. Therefore, investigations in well-defined electrochemical systems with pure cultures of lithoautotrophic methanogenic archaea are of crucial fundamental interest to confirm and elucidate the relevant electron transfer mechanisms, as well as to resolve the individual contribution to the microbial electrosynthesis of methane.

Here we report for the first time on selective microbial electrosynthesis of methane by a pure culture of the marine lithoautotrophic *Methanobacterium*-like archaeon strain IM1, which was previously shown to cause serious corrosion of iron by direct electron uptake [5]. In order to quantify methane production rates, cultures of the *Methanobacterium*-like archaeon strain IM1 were studied in bio-electrochemical cells that were directly coupled to gas chromatography for online analysis of the gas effluent. Besides microbial electrosynthesis analysis, observations with scanning electron microscopy have been performed to further demonstrate surface interactions of strain IM1 at the microbe–electrode interface on cathodes.

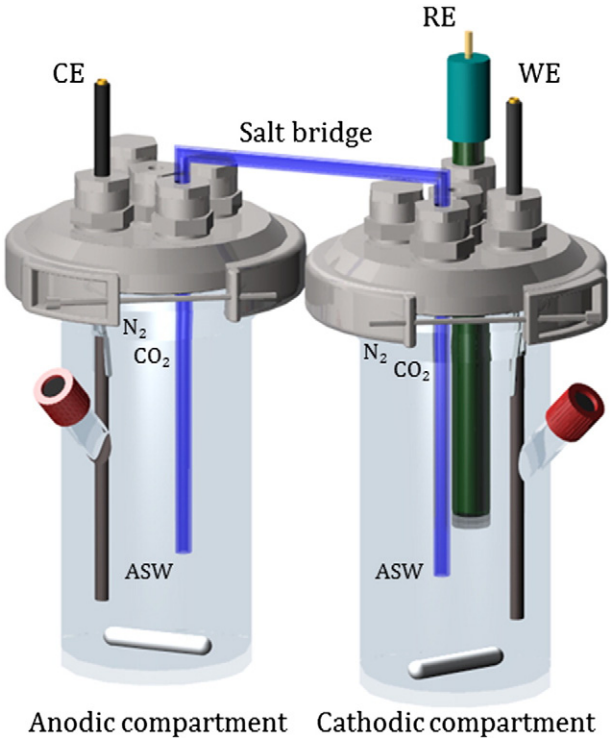
2. Materials and methods

2.1. Organisms and cultivation

Cultures of the marine lithoautotrophic *Methanobacterium*-like archaeon strain IM1 [5] were routinely grown in anoxic, buffered (CO_2/HCO_3^-) artificial seawater medium [17] without sulfate, under an anoxic N_2–CO_2 (9/1, by volume) headspace at room temperature (21 °C). The inoculum of strain IM1 was grown on an iron specimen (30 × 10 × 1 mm, ARMCO® Pure Iron, 99.877% Fe; <0.06% Mn; <0.03% Cu; <0.01% C; <0.005% P; <0.005% N; <0.005% Co; <0.005% Sn; <0.003% S; AK Steel GmbH, Cologne, Germany) and prior to transfer into experimental cultures (1% inoculum) the inoculum in the serum bottle was flushed with N_2-CO_2 to prevent introduction of methane. In addition to a sterile control, an authenticated culture of *M. maripaludis* (DSMZ 2771) from the German Collection of Microorganisms and Cell Culture (Braunschweig, Germany) was deployed as a hydrogenotrophic control, to further exclude possible influences of hydrogen evolution on the polarized electrodes. The inoculum of *M. maripaludis* was grown under the same conditions mentioned above for strain IM1, but with the addition of 1 bar of hydrogen serving as the electron donor instead of an iron specimen. Prior to transfer into experimental cultures (1% inoculum) the inoculum of *M. maripaludis* was sufficiently flushed with N_2–CO_2 to prevent introduction of methane.

2.2. Bio-electrochemical cell setup

Two modified bioreactors (SR0200SS, DASGIP, Jülich, Germany) were connected via a salt bridge (3 mol L^{-1} KCl, 2% Agar) to be employed in a dual compartment configuration as a bio-electrochemical cell with a three electrode setup (Scheme 1). The separation of the anodic and cathodic compartments excluded any influences of oxidation reactions in the anodic compartment to the electrochemical measurements in the cathodic compartment. The bio-electrochemical cell was operated as a batch culture, in which the working volume of each compartment (350 mL) was filled with anoxic artificial seawater (ASW) under an anoxic headspace of N_2–CO_2 (50 mL, 9/1 by volume) as described elsewhere [18]. The three electrode setup consisted of a graphite rod working electrode (graphite rods 100 mm, ø: 5 mm, 13 cm^2 immersed surface area, Müller & Rössner GmbH & Co. KG, Berlin, Germany) and a directly immersed standard Ag/AgCl/3M KCl reference electrode (Metrohm, Herisau, Switzerland) in the cathodic compartment, as well as a graphite rod counter electrode (same dimensions as working electrode) in the anodic compartment of the bio-electrochemical cell. Before usage in electrochemical experiments, the graphite rods were treated with HCl (1 mol L^{-1}) to especially remove contaminations with iron particles and were autoclaved afterwards.



Scheme 1. Bio-electrochemical cell setup. Bio-electrochemical cell in a dual compartment configuration connected via a salt bridge (3 mol L^{-1} KCl, 2% Agar). Working- (WE) and counter electrode (CE) consisting of graphite rods (ø 5 mm, length 100 mm), Ag/AgCl/3 M KCl reference electrode (RE), working volume of glass vessel (350 mL) filled with anoxic artificial seawater (ASW) under anoxic headspace (50 mL, N_2–CO_2, 9/1). Note that the gas purging lines of the cathode compartment and the connection to the GC are not shown in the sketch for clarity reasons.

In potentiostatic measurements, the potential of the graphite cathode was constantly set to −0.4 V vs. SHE, serving as the only electron source for microbial growth. The bio-electrochemical cell was incubated at room temperature (21 °C) and a magnetic stir bar realized an adequate stirring (200 rpm) of the growth medium. For the online product analysis of microbial electrosynthesis the cathodic compartment of the bio-electrochemical cell was coupled to gas chromatography by a constant gas flow of N_2–CO_2 (9/1, by volume) at a controlled flow rate of 10 mL min^{-1} (mass flow controller F201 CV, Bronkhorst Mättig GmbH, Kamen, Germany), purging the artificial seawater medium (electrolyte). At the gas in – and outlet single use filter units (Minisart, pore size 0.2 µm, Sartorius AG, Göttingen, Germany) ensured sterile conditions. The gas effluent was continuously sampled from the headspace of the bio-electrochemical cell and analyzed by a gas chromatograph (Light Gas Analyser ARNL3878 modified model 4016, PerkinElmer, Waltham, Massachusetts, USA) containing a column set consisting of HayeSep N 60/80 and Molecular 13× 45/60 column (PerkinElmer, 45 °C–100 °C, Ar as a carrier gas) and a thermal conductivity detector (TCD) as well as a flame ionization detector (FID), optimized for the quantitative analysis of hydrogen, ethylene, carbon dioxide, oxygen, nitrogen, methane and carbon monoxide.

2.3. Coupled electrochemical measurements

All electrochemical measurements have been operated in separate bio-electrochemical cells and were independently performed and reproduced. However, correct replication of the experiments was hampered by an undefined cell count in the inoculation volume of strain IM1. Since strain IM1 was exclusively isolated with elemental iron serving as the electron donor for metabolic activity and strain IM1 hardly