Electrochemical reduction of CO_2 catalysed by *Geobacter sulfurreducens* grown on polarized stainless steel cathodes

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A B S T R A C T

Polarized stainless steel cathodes in pure cultures of *Geobacter sulfurreducens* generated reduction currents of up to 30 A/m^2 even when the sole electron acceptor contained in solution was completely reduced. It was shown here that these currents were driven by the carbon dioxide that was provided to the solution. It was postulated that CO_2 reduction consumed succinate and produced glycerol, which remained stored inside the cells and was released under the effect of stress.

Keywords:
Biocathode
Geobacter sulfurreducens
CO_2 reduction
Microbial electrocatalysis

1. Introduction

The electrochemical reduction of carbon dioxide (CO_2) coupled with a renewable source of electrical energy may be a promising way to produce fuels or other chemical compounds without tapping into fossil resources. Recently, microbial catalysts have opened up exciting new avenues [1–3]. Some microorganisms have been shown to be able to extract electrons from a cathode, use them in their metabolic reactions and finally transfer them to CO_2, which is reduced to various compounds. Such microbial electrosynthesis has very interesting features: only cheap electrode materials are required (carbon, graphite, stainless steel), microorganisms grow spontaneously on the cathode surface, and they self-maintain provided conditions stay appropriate. Nevin et al. have reported the reduction of CO_2 to acetate with cultures of various acetogenic bacteria colonizing a graphite electrode [4,5]. Villano et al. [6,7] have reduced CO_2 to methane on a carbon electrode with a culture of *Methanobacterium palustre*. The current densities related to CO_2 reduction are still quite low, in the 0.01 to 1.3 A/m^2 range. Similarly, it has been shown that *Geobacter sulfurreducens* cells growing on the surface of a graphite cathode can catalyse the reduction of fumarate to succinate [8]:

$$C_4H_4O_4 + 2H^+ + 2e - \rightarrow C_4H_6O_4. \quad (1)$$

The current density of fumarate reduction, of the order of 0.5 A/m^2 on graphite cathodes, increases to 20.5 A/m^2 when graphite is replaced by stainless steel [9,10]. To the best of our knowledge, *G. sulfurreducens* is not known to be able to reduce CO_2. This work was a first step in this direction. The results suggest that this reaction may be possible when *G. sulfurreducens* cells are grown on a cathode used as the sole electron source and can give current densities one order of magnitude higher than reported so far for the reduction of CO_2 with other microorganisms.

2. Materials and methods

2.1. Media and culture conditions

G. sulfurreducens strain PCA (ATCC51573) was purchased from DSMZ. The growth medium was as previously described [9] and contained 10 mM acetate as electron donor and 50 mM fumarate as electron acceptor. *G. sulfurreducens* (10% v/v from an inoculum stored at −20 °C) was incubated at 30 °C for 4 days in the growth medium to obtain a final suspension with absorbance of 0.3 at 620 nm. Secondly, 10% v/v of the suspension was inoculated into fresh medium and incubated for 2 days so that the final absorbance at 620 nm was 0.4. For each new inoculation, the DSMZ culture was inoculated at 2% v/v into a fresh growth medium and the incubation lasted 4 days so that the final absorbance at 620 nm was around 0.4. The reactor medium used for the electrochemical experiments was the same as the growth medium, except that the sodium fumarate concentration was 25 mM and there was no acetate, so the cells were forced to use the cathode as their electron source.

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2.2. Electrochemical reactors

Reactors containing 0.45 L solution were equipped with a single stainless steel electrode (0.1-cm-thick 254SMO stainless steel plate from Outokumpu, composition 0.01% C, 19.9% Cr, 6% Mo, 17.8% Ni, 0.5% Mn and 0.02% N), while the 1.85 L reactors had 3 electrodes, which were individually addressed thanks to an N-STAT device (Bio-Logic, SA). The stainless steel electrodes were cleaned first with an ethanol/acetone (50–50%) mixture to dissolve adsorbed organic species and then with a fluoridic/nitric acid (2–20%) solution to dissolve the oxide layer. Auxiliary electrodes were Pt-Ir grids (Platecxis) cleaned in a flame. A silver wire coated with silver chloride (stable potential 0.31 V/SHE in the medium) was used as the reference and all potentials are expressed with respect to it.

The reactors were filled with the medium, flushed with N_2–CO_2 (80%:20%) and the potential was applied via a multi-channel potentiostat (Bio-Logic SA). Microbial cells (7.5% v/v, $DO_{620nm} = 0.4$) were injected after 12 h polarization in abiotic conditions. The N_2–CO_2 flow was maintained at a lower flow rate after inoculation. Current was recorded every 900 s. Polarization was suspended periodically to record cyclic voltammetry curves (CVs) at 1 mV s^{-1}. The temperature was maintained at 30 °C and pH was measured throughout the experiments. Under N_2–CO_2 (80:20%) or pure CO_2 bubbling, the pH was stable at 6.9 ± 0.2; under pure N_2, alkaline values (8.8 ± 0.1) were recorded. The charge supplied to the system by the cathode was calculated by integrating the current–time curves. When three polarized electrodes were present in the same reactor, the charges supplied by the individual electrodes were summed.

The charge required to achieve complete transformation of the fumarate contained in the solution to succinate was:

$$Q_{Fumarate} = 2\,F C_{Fumarate} V \quad (2)$$

where F is the Faraday constant (96,485 C/mol. electrons), $C_{Fumarate}$ is the initial concentration of fumarate (mol/L), and V is the volume of solution (L).

2.3. Sampling and analysis methods

The head-space gas phase of the reactors was analysed by GC (Varian, CP3800) coupled to flame ionization and thermal conductivity detectors. Sampling was achieved with a sealed gas syringe that was purged several times beforehand with the head-space gas phase. Samples were analysed immediately after collection.

The reactor media were sampled in a sterile way with a 5-mL pipette. Samples were filtered at 0.2 μm and stored at −80 °C. HPLC (Thermo Scientific, France) used a Rezex ROA-Organic acid H^+ (8%), 250 × 4.60 mm phase-reverse column (Phenomenex, France) thermostated at 30 °C and associated with a refractive index detector (Finnigan Surveyor, France) in series with a UV detector. The elution was performed at 170 μL min^{-1} with an aqueous solution of sulphuric acid 9 mM (pH 2.2 ± 0.2). The column was calibrated with a mixture of acetate, fumarate, succinate and glycerol, in the analysis concentration range. Before analysis, samples were thawed, diluted twice in the eluent and filtered at 0.2 μm. Each measurement was repeated 5 times, giving an experimental error of 3%.

The identification and quantification of glycerol were confirmed with a specific enzymatic kit (Megazyme glycerol kit K-GCROL, LIBIOS, France).

3. Results and discussion

3.1. CO_2-dependent reduction current

Two electrochemical reactors were filled with 0.45 L of medium that did not contain acetate (electron donor). Each was equipped with a 2.5 cm^2 stainless steel electrode polarized at −0.6 V vs. Ag/AgCl. No current was recorded before inoculation (current density less than 0.1 A/m^2). The reduction currents started to increase a few days after inoculation and then gave stable values around 16 A/m^2. Currents were still stable when the experiments were stopped after 19 days and charges of 4887 and 3908 C had been passed, while only 2171 C was sufficient to completely reduce the initial amount of fumarate contained in the reactors (Reaction 1). An abiotic control run for 16 days without inoculation gave current density close to zero (less than 0.1 A/m^2) and the total charge passed was only 31 C.

It was concluded that the reduction currents were due to a reaction that was microbially catalysed but not directly linked to the reduction of fumarate to succinate (Reaction 1). Another electron acceptor was considered. At pH 7.0 (stable value of the pH under N_2–CO_2 bubbling), the formal potential of the H^+/H_2 system was −0.413 V/SHE, i.e. −0.71 V vs. Ag/AgCl. The polarization potential of −0.60 V was 110 mV above the formal H^+/H_2 potential. Consequently, hydrogen evolution cannot be evoked to explain the stable current up to 30 A/m^2.

No other electron acceptor was present in the medium, except CO_2, which was continuously provided by N_2–CO_2 bubbling. To explore hypothesis, a more detailed experiment was performed with a 1.85 L reactor equipped with 3 stainless steel electrodes (2.5 cm^2 each). Fig. 1 depicts the evolution of the current density vs. time for the 3 electrodes. Seven phases, denoted 1 to 7 (Table 1), corresponded to different operating conditions in terms of polarization potential (−0.6 or −0.4 V vs. Ag/AgCl) or gas flow (N_2–CO_2, pure N_2, or pure CO_2). The three working electrodes always showed identical behaviour (Fig. 1).

During the first phase, the polarization potential was −0.6 V vs Ag/AgCl and the reactor was flushed with N_2–CO_2. After 2 days of latency corresponding to biofilm formation, the current rose and reached a plateau of 23 A/m^2 on average for around 7 days, with maximum values up to 30 A/m^2. The reduction of the total amount of fumarate contained in the reactor consumed 8926 C, which corresponded to the charge provided to the system during the first 10 days. HPLC analyses at day 12 confirmed the complete consumption of fumarate. Nevertheless, the current showed absolutely no decrease that could correspond to fumarate depletion on any of the three electrodes. It was confirmed that the reduction currents were controlled by an electron acceptor other than fumarate.

When the potential was changed from −0.6 V to −0.4 V vs. Ag/AgCl at day 12 (phase 2), the currents of the three electrodes dropped to 6–8 A/m^2, consistently with common electrochemical kinetics.

When N_2–CO_2 bubbling was replaced by pure CO_2 (phase 2′), current increases were observed (7.5 to 10 A/m^2) in a reproducible way, which expressed the dependence of the current density on the CO_2 partial pressure. The same shift from N_2–CO_2 to pure CO_2 made at day 18 (phase 6) gave similar current increases.

During the third phase (days 13–14), the operating conditions were put back to the initial conditions (−0.6 V vs. Ag/AgCl, N_2–CO_2 bubbling) and currents recovered their initial values. When the N_2–CO_2 bubbling was replaced by pure N_2, the currents dropped to zero very quickly (phase 4, days 14–15) and they recovered their initial values when the N_2–CO_2 bubbling was restored (phase 5, days 15–18). The fast annihilation of the current of the three electrodes in the absence of CO_2 and the fast recovery when the reactor was fed with CO_2 again confirmed that the currents were directly dependent on CO_2.

The last phase (phase 7) showed a slow decrease in the currents, which was attributed to biofilm ageing and/or depletion of some oligoelements in the reactor medium.

CV curves did not detect any redox reaction just after the cell injection. At the maximum current under N_2–CO_2 bubbling (day 5), CVs exhibited a zero-current potential around −0.25 V vs. Ag/AgCl. Consistently with the results obtained under polarization, the current fell to zero in the whole potential range when the reactor was no longer fed with CO_2 and recovered the maximum value when

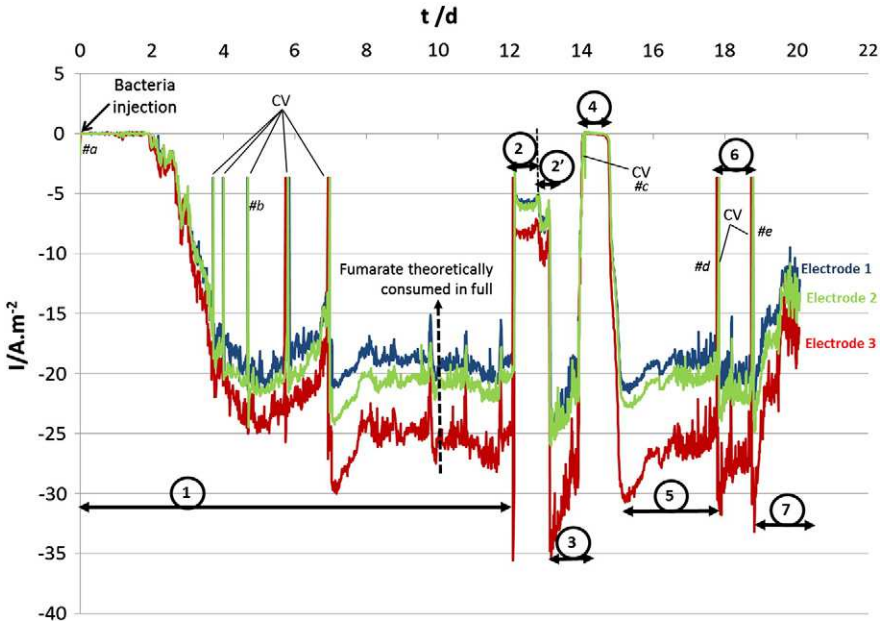

Fig. 1. Evolution of the current density with time on three polarized stainless steel electrodes exposed to *Geobacter sulfurreducens* in a 1.85 L reactor. Seven phases were distinguished, corresponding to the different operating conditions described in Table 1. Injection of 7.5% (v/v) of bacteria; medium contained 25 mM fumarate as electron acceptor and no acetate. (#) indicates polarization interruptions for CV recording: after bacteria injection (# a), when the biofilm was formed under N_2–CO_2 bubbling (# b), under N_2 flush (# c), again under N_2–CO_2 bubbling (# d) and under CO_2 bubbling (# e). Perturbations of the current corresponded to CV recordings.

bubbled again with N_2–CO_2. The slight increase of currents under pure CO_2 also confirmed the observations under polarization.

3.2. Products resulting from CO_2 reduction

GC analyses performed on the head-space gas of the reduction reactors at the end of the experiments did not detect any gas species other than N_2 and CO_2. There was notably no trace of methane, which might be expected among the products of CO_2 reduction. HPLC analyses of the medium indicated that fumarate was no longer present in the reactor from day 12, as predicted by the charge calculations. Succinate was found at significant concentrations, which confirmed that fumarate was reduced into succinate in a first step, according to Reaction (1) [8]. The succinate concentration decreased linearly from 11.5 ± 0.5 mM at day 12 to 7.5 ± 0.2 mM at the end of the experiment. No acetate was detected. HPLC analyses evidenced the formation of only one product: glycerol, the identification of which was confirmed by an enzymatic kit specific to glycerol. No glycerol was found at day 12 but it appeared at day 13 with a concentration of 8.7 ± 0.3 mM. The glycerol concentration remained in the 6.0 to 9.0 mM range throughout the rest of the experiment.

Table 1
Operating conditions for the 1.85 L reactor equipped with 3 polarized stainless steel electrodes, for which the current density variation with time is depicted in Fig. 1, and related mean current densities recorded. The mean current densities were calculated by integrating the current of the three polarized electrodes over the period considered, summing the results of the integrals and dividing the sum by 3 and by the duration of the period.

Phase	Period (j)	Vp vs. Ag/AgCl (V)	Bubbling (%)	Mean current density ($A.m^{-2}$)
1	Days 0–12.1	−0.6	N_2:CO_2 (80:20%)	23
2	Days 12.1–12.8	−0.4	N_2:CO_2 (80:20%)	6–8
2′	Days 12.8–13.1	−0.4	CO_2 (100%)	8–10
3	Days 13.1–14	−0.6	N_2:CO_2 (80:20%)	23–30
4	Days 14–15	−0.6	N_2 (100%)	0
5	Days 15–17.8	−0.6	N_2:CO_2 (80:20%)	18–26
6	Days 17.8–18.8	−0.6	CO_2 (100%)	20–28
7	Days 18.8–20.1	−0.6	N_2:CO_2 (80:20%)	17

A similar experiment was carried out at −0.4 V vs. Ag/AgCl with three stainless steel electrodes having surface areas of 16, 16 and 25 cm^2 in 1.85 L solution that contained 25 mM fumarate. Stable current densities in the 2.5 to 4.5 A/m^2 range were maintained for more than 12 days. The final analysis of the solution confirmed the complete consumption of fumarate and the presence of glycerol at 6.5 mM. Once again, the final succinate concentration of 13.7 mM was smaller than the initial concentration of fumarate.

3.3. Discussion of a possible reaction pathway

The linear decrease of the succinate concentration that was shown by the HPLC analyses suggested that succinate was involved in CO_2 reduction. The reduction of CO_2 may consequently have produced glycerol ($C_3H_8O_3$) by consuming succinate ($C_4H_6O_4$) according to:

$$1/2C_4H_6O_4 + HCO_3^- + 8H^+ + 7e- \rightarrow C_3H_8O_3 + 2H_2O \quad (2)$$

where CO_2 was assumed to react through its bicarbonate form resulting from its reaction with water.

No glycerol was detected in solution before day 13. It may be postulated that glycerol was stored inside the cells and then massively released at day 13, in response to a stress for instance. Here the change of polarization potential from −0.6 to −0.4 V vs. Ag/AgCl, which drastically decreased the ability of the cathode to provide cells with electrons, may have been the triggering event.

After day 15, the total charge provided to the system by the cathode continued to increase linearly, while the concentration of succinate continued to decrease linearly. Meanwhile, the concentration of glycerol was almost stable, which is consistent with the postulate that glycerol was produced and stored inside the cells. Finally, one molecule of glycerol requires 7 electrons coming from the cathode and 1 electron from succinate. Current monitoring indicated that the cathode provided 500 C/h, while the rate of succinate decrease provided 70 C/h. This ratio of around 7 (500/70) fitted the electron ratio of Reaction (2) perfectly. The standard potential (E_0) of Reaction (2) was 0.538 V/SHE, i.e. 0.23 V vs. Ag/AgCl, which gave a formal potential $E_0' = -0.25$ V vs. Ag/AgCl at pH 7.0. This value was

consistent with the experimental zero-current potential around −0.25 V vs. Ag/AgCl observed on CVs. Reaction (2) fitted the experimental data obtained here but obviously requires further investigation before it can be established. Finally, a biochemical pathway allowing *G. sulfurreducens* cells to reduce CO_2 remains to be identified, because planktonic *G. sulfurreducens* cells are not known to be able to do this. A reverse Krebs cycle pathway could be envisaged [11,12], which may have been induced by the specific culture conditions using a cathode as sole electron donor. This possibility may be investigated in further studies.

4. Conclusion

A current driven by carbon dioxide was recorded, with stable current densities of 23 A/m^2 and maximum values up to 30 A/m^2, which led to the formation of glycerol. These preliminary results open up a new route for the exploitation of carbon dioxide via microbial electrosynthesis. The mechanisms now need to be elucidated by further in-depth investigations.

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