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Lactate Oxidation Coupled to Iron or Electrode Reduction by *Geobacter sulfurreducens* PCA▽

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Geobacter sulfurreducens **PCA completely oxidized lactate and reduced iron or an electrode, producing pyruvate and acetate intermediates. Compared to the current produced by** ***Shewanella oneidensis*** **MR-1,** ***G. sulfurreducens*** **PCA produced 10-times-higher current levels in lactate-fed microbial electrolysis cells. The kinetic and comparative analyses reported here suggest a prominent role of** ***G. sulfurreducens*** **strains in metal- and electrode-reducing communities supplied with lactate.**

There is great interest in the study of bacteria capable of transferring electrons to solid electron acceptors, such as metal oxides or electrodes, in different types of bioelectrochemical systems (BESs). The two most-studied exoelectrogenic bacteria are *Geobacter sulfurreducens* PCA and *Shewanella oneidensis* MR-1 (1, 2, 9, 23), but these microorganisms have never been studied under exactly the same conditions in BESs. *S. oneidensis* MR-1 can grow anaerobically using lactate, but it produces acetate, which it cannot further oxidize under these conditions. 16S rRNAs sequences similar to those found with *G. sulfurreducens* are commonly found in mixed-culture microbial fuel cells (MFCs) and sediments supplied with lactate, while sequences similar to those found with the *Shewanella* genus are relatively rare in such studies (13–15, 28). It has been suggested that the presence of sequences matching those found with *G. sulfurreducens* was primarily due to acetate consumption sustained by other bacteria that incompletely oxidized lactate to acetate (13–15, 28). This assumption was probably based on earlier work indicating that *G. sulfurreducens* PCA could not oxidize lactate, as the authors reported similar levels of Fe(II) in both lactate-supplied cultures and negative controls after 5 days (3). Although *G. sulfurreducens* PCA was thought not to respire with lactate (3), its genome includes genes for putative lactate permeases (GSU1622 and GSU0226) and lactate dehydrogenases (GSU1620 and GSU1621), which are highly conserved among lactate-oxidizing bacteria, including those of the *Shewanella* genus (25).

It is now known that several *Geobacter* species can oxidize lactate (11, 24, 27), and it has recently been shown that *G. sulfurreducens* PCA can oxidize lactate with fumarate reduction (21, 30). Gene regulation in lactate- versus acetate-fed cultures of *G. sulfurreducens* PCA has been examined (30), but no data have yet been presented on lactate oxidation kinetics or intermediate product formation by strain PCA. There are indications of direct lactate oxidation in previous MFC studies with communities containing sequences matching those found with *G. sulfurreducens* (13, 15). For example, there was immediate current production by established mixed-culture MFC biofilms at the onset of lactate fed-batch cycles (13, 14, 16, 20, 29). Immediate current generation is unlikely to be a result of lactate conversion to acetate, as indirect conversion results in a lag of current generation of several hours. Direct current production from lactate oxidation is more plausible in these MFCs, yet bacteria capable of this type of metabolism, such as *Shewanella* species, produce lower current densities than mixed cultures under identical conditions (12, 14, 31). Furthermore, many *Shewanella* species use electron shuttles (22) that would be removed when the medium was replaced prior to fed-batch operation. This suggests that strains of *G. sulfurreducens* capable of high current production directly from lactate oxidation exist within mixed-community biofilms fed with lactate.

In order to better understand lactate utilization in BESs, we examined current generation by *G. sulfurreducens* PCA and *S. oneidensis* MR-1 in microbial electrolysis cells (MECs) under identical conditions (medium, electrode, and temperature). Iron reduction by *G. sulfurreducens* PCA was also examined to relate lactate oxidation kinetics to previously reported rates of acetate oxidation.

Electrode reduction using lactate. Biofilms of *G. sulfurreducens* PCA (strain obtained from laboratory stocks of ATCC 51573 frozen at −80°C) were initially established on graphite plate anodes (anode specific surface area [A_A] = 92 m^2/m^3) in serum bottle (5 ml)-based MECs (4, 18) using stainless steel mesh cathodes (cathode specific surface area [A_C] = 86 m^2/m^3) (5). MECs (triplicate reactors) were supplied with acetate (10 mM) in a freshwater (FW) medium (0.1 g KCl, 0.3 g NH_4Cl, 0.6 g NaH_2PO_4, 2.5 g $NaHCO_3$, 10 ml vitamins, and 10 ml minerals per liter; headspace of 80:20 mixture of N_2/CO_2) (6) and operated in fed-batch mode at an applied voltage (E_{AP}) of 0.7 V at 30°C (Fig. 1A). This applied voltage was chosen because it allowed for relatively fast cycle times (<2 days) compared to those obtained with lower applied voltages, and this voltage was previously used for culturing *Geobacter* sp. in MECs (6).

After current production stabilized, the electron donor was switched to lactate (10 mM). The current density (I_A; normal-

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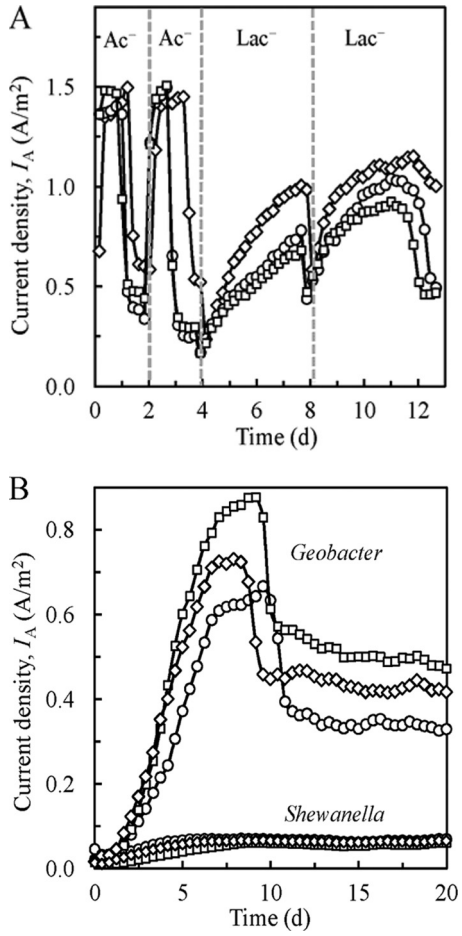

FIG. 1. (A) Current densities of *G. sulfurreducens* in MECs fed acetate (Ac^-) or lactate (Lac^-) at an E_{AP} of 0.7 V. Batch cycles were operated in triplicate in the order shown, with dotted lines representing times of replacement of medium. (B) Current densities of *G. sulfurreducens* and *S. oneidensis* in MECs fed lactate during an initial batch cycle at an E_{AP} of 0.7 V. Both cultures were operated in triplicate.

ized to total anode surface area) reached a maximum of 0.8 ± 0.2 A/m^2 (mean ± standard deviation) over a period of 4 days, which was ca. 50% less than the maximum current produced when the cultures were supplied with acetate. When the MECs were emptied, sparged with sterile, anaerobic gas, and refreshed with new medium, 0.3 ± 0.1 A/m^2 was produced immediately after applying the voltage, supporting direct conversion of lactate into current. Improvements in current production for the triplicate samples were noted for the second fed-batch cycle, possibly due to evolution of *G. sulfurreducens* PCA for more efficient lactate utilization. Electrons were recycled via hydrogen gas, as shown by estimated Coulombic efficiencies (C_E) above 100% for both acetate-fed (140% ± 15%) and lactate-fed (140% ± 33%) MECs. To eliminate the possibility that residual hydrogen produced in the MECs was the main contributor to current production, *G. sulfurreducens* PCA was also tested in a two-chamber reactor incorporating a membrane separator (Nafion) between the working and counter chambers (12-cm electrode spacing). The internal resistance of this two-chamber reactor is large, which results in a low current. Therefore, to increase current in this device and to better define the anode conditions, the potential of the anode (graphite rod, 14.5 cm^2) was controlled at +0.4 V (versus a standard hydrogen electrode). This potential was selected to allow comparison with previous reports of acetate-fed reactors at this potential (1, 32). After 20 days, the current reached a maximum of ca. 0.2 A/m^2 (data not shown) with an estimated C_E of 12% ± 1%, confirming that lactate could serve as the sole electron donor for current generation. The higher current densities in the MEC tests (single chamber) than in the two-chamber tests may have been due to different operating conditions (fixed whole-cell potential versus fixed anode potential), a decrease in internal resistance due to closer electrode spacing and no membrane, or the presence of hydrogen, which could serve as an additional electron donor.

The use of lactate by *G. sulfurreducens* PCA allowed for the direct comparison of its electrode-reducing rates to those of *S. oneidensis* MR-1 under identical conditions. *S. oneidensis* MR-1 was grown overnight in Luria-Bertani medium at 30°C to an optical density at 600 nm (OD_{600}) of 1.1 ± 0.0, centrifuged, washed three times in sterile FW medium, and inoculated into sterile MECs (in triplicate) containing the same medium used for testing *G. sulfurreducens* PCA. *G. sulfurreducens* PCA was also inoculated into new, sterile reactors (in triplicate) for comparison. The current production reached a maximum of 0.06 A/m^2 for *S. oneidensis* MR-1, compared to 0.8 A/m^2 for *G. sulfurreducens* PCA (Fig. 1B). The low current densities observed here for *S. oneidensis* MR-1 are consistent with previous reports of low current generation by this bacterium in MFCs and MECs (12, 14, 31).

Iron reduction using lactate. Cells of *G. sulfurreducens* PCA were thawed from frozen stocks, grown at 30°C in anaerobic culture tubes containing acetate (10 mM) and ferric citrate (15 mM) in FW medium (10 ml) until complete Fe(III) reduction occurred, and transferred (10%) three times in FW medium containing lactate (2 mM) and ferric citrate (15 mM) under Fe(III)-limiting conditions. On the fourth transfer, the lactate, acetate, and pyruvate concentrations were recorded using high-performance liquid chromatography (HPLC), the Fe(II) production was measured using a ferrozine assay (19), and cell counts were done using an acridine orange staining procedure (10). After 10 days, *G. sulfurreducens* PCA produced 12.8 ± 0.6 mM Fe(II) and removed 2.6 ± 0.0 mM lactate, indicating a stoichiometry of Fe(II) production to lactate consumption of 4.9 ± 0.2 (Fig. 2). These results are not consistent with incomplete lactate oxidation to acetate via equation 1 (below), as this would have required a stoichiometric ratio of less than four when including substrate conversion to biomass.

$$\text{Lactate}^- + 2H_2O + 4\text{Fe(III)} \rightarrow \text{acetate}^- + HCO_3^- + 5H^+ + 4\text{Fe(II)} \quad (1)$$

$$\text{Lactate}^- + 2\text{Fe(III)} \rightarrow \text{pyruvate}^- + 2\text{Fe(II)} + 2H^+ \quad (2)$$

$$\text{Pyruvate}^- + 2\text{Fe(III)} + 2H_2O \rightarrow \text{acetate}^- + 2\text{Fe(II)} + HCO_3^- + 3H^+ \quad (3)$$

Additional tests under conditions of limited lactate [1 mM lactate, 30 mM Fe(III); conducted in triplicate] confirmed that complete oxidation of lactate occurred. Pyruvate was detected,

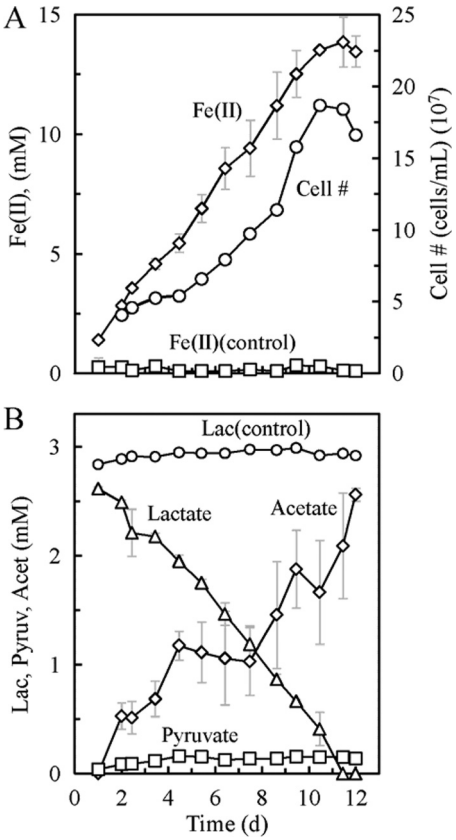

FIG. 2. Fe(II) concentrations and cell numbers (A) and lactate (Lac), acetate (Acet), and pyruvate (Pyruv) concentrations (B) of *G. sulfurreducens* cells oxidizing lactate as the sole electron donor under conditions of limited Fe(III). Experiments were performed in triplicate with two uninoculated controls. Error bars show standard deviations.

suggesting lactate oxidation via equation 2 with the transfer of two electrons, followed by pyruvate oxidation to acetate (equation 3). This result is consistent with pyruvate oxidation by *G. sulfurreducens* PCA, as described previously (26).

All cultures were tested for purity by extracting DNA, conducting PCR of the 16S rRNA genes using forward primer 530F (5′-GTCCCAGCMGCCGCGG-3′) and reverse primer 1490R (5′-GGTTACCTTGTTACGACTT-3′), and sequencing the purified PCR products. All sequences returned clean and were confirmed as pure using the BLASTn algorithm at the National Center for Biotechnology Information (NCBI) website.

Implications. These results provide the kinetics of lactate oxidation by *G. sulfurreducens* PCA coupled with either electrode or Fe(III) reduction along with the first direct comparison to a member of the *Shewanella* genus. Although sequences matching *G. sulfurreducens* in mixed communities supplied with lactate may be associated with strains capable of lactate oxidation, researchers have previously concluded that these *Geobacter* species relied on acetate production from incomplete lactate utilization by other members of the community (13–15), probably due to the previous conclusion by Caccavo et al. that *G. sulfurreducens* PCA did not utilize lactate (3). The results shown here expand upon more recent findings that strain PCA can grow using lactate and show, based on current generation in MECs, that electrode-reducing rates are substantially higher than those obtained with strain MR-1 under the same conditions. Elucidating the contribution of *G. sulfurreducens* to lactate oxidation within mixed communities warrants further investigation, along with examining the functional role of the genes (lactate permease and dehydrogenase) associated with lactate utilization.

Expanding the substrate diversity of *G. sulfurreducens* PCA is promising for the further development of MFCs. Bacterial respiration rates can be a limiting factor in MFCs and other BESs, as it is well known that certain substrates (e.g., acetate) result in higher current and power densities than others (e.g., butyrate and complex substrates, such as wastewaters) (8, 17). Therefore, methods to improve microbial kinetics in BESs can help to improve system performance. Adaptive evolution of *G. sulfurreducens* PCA for high-current-producing strains has been shown with acetate (33), but wastewaters and cellulosic fermentation effluents contain a diverse range of substrates. Although mixed communities can generate current from these waste streams (15), using strains of *G. sulfurreducens* that are highly adapted to acetate, lactate, pyruvate, and formate (7) may allow for increased current production compared to the levels obtained in mixed cultures.

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