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Syntrophic Processes Drive the Conversion of Glucose in Microbial Fuel Cell Anodes

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Microbial fuel cell (MFC) anodes are anaerobic bioreactors. Processes such as fermentations and methanogenesis are likely competitors to electricity generation. This work studied the pathway of glucose conversion in continuous microbial fuel cell anodes with an adapted bacterial community. The study revealed that the majority of glucose is first fermented to hydrogen and acetate. Both are then used as substrates for bacterial electricity generation. When methanogens are present, methane production occurs at a rate that slightly increases with the current. Methanogenesis and electricity generation compete for hydrogen, causing increased fermentation rates. In a rather young anodic biofilm on granular graphite, methanogenesis can be suppressed by aerating the anode compartment for one hour. Only short-term inhibition can be achieved applying the same technique on a well established biofilm on granular graphite. This study shows that fermentative processes are not detrimental to current generation, and that direct oxidation of glucose does not play a major role in mixed population conversions in a MFC anode.

Introduction

Microbial fuel cells (MFCs) have emerged as a technology that can turn energy intensive wastewater treatment into a clean, energy yielding process. The soluble organic matter of wastewaters can be metabolized by anodophilic bacteria which use an anode as electron acceptor (*1*). In a fuel cell setup, the electrons are transferred to a cathode via electrical circuitry, with the chemical energy of the organic substrate harnessed as clean electrical power. Sugars and volatile fatty acids (VFAs) have been shown to be ideal substrates for anodophilic bacteria (*2, 3*). If glucose is the electron donor, the electrogenic reaction at the MFC anode is given by reaction 9 in Table 1. The only products of the anodic process are a greenhouse "neutral" carbon dioxide (wastewater organics are generated from atmospheric CO_2 in the biological carbon cycle), protons that can be subsequently neutralized at the cathode (*4*), electricity, and biomass in far smaller amounts than conventional aerobic treatment (*5*).

However, the reactions are generally not as straightforward as depicted. As MFC anodes do not contain significant amounts of dissolved oxygen, other anaerobic processes may take place. In particular, as most sugars and higher VFAs are fermentable, fermenters are likely to grow alongside anodophiles. There are several types of fermentations that can occur. In the fermentative metabolism, the substrate is first oxidized to an intermediate compound and/or CO_2 with generation of reducing power (NADH). The reducing power thus generated has to be utilized to close the redox balance, and fermentative organisms do so by producing reduced byproducts. These include hydrogen, lactate, ethanol, propionate, butyrate, and formate. The stoichiometry and Gibbs free energy of some common fermentations are detailed in Table 1. Many fermentations produce gaseous hydrogen (H_2) to some extent. The production of H_2 is energetically favorable (reactions 3−5), but hydrogen accumulation can drastically slow down or even stop these bioreactions if hydrogen production proceeds via the NADH-hydrogenase pathway. This pathway generates 4 moles of ATP per mole of glucose. Much higher partial pressures of hydrogen are attainable if the bacteria resort to the ferredoxin pathway (*6*), with ethanol or butyrate as sinks of reducing power. However, this pathway can only generate 2−3 moles of ATP per mole of glucose. Therefore, it is generally considered that this is not the preferred or commonly used pathway if the hydrogen concentration in the biofilm is maintained at very low levels by hydrogen-consuming reactions. The most ubiquitous of these reactions (in the absence of alternative electron acceptors such as sulfate and nitrate) are methanogenesis and homoacetogenesis (reactions 7 and 8, respectively). The combinations of hydrogen producing fermentation and hydrogen consuming reactions (e.g., reaction 12) are energetically more favorable and thus better geared to compete for glucose degradation. Acetogenesis (reaction 3) is the fermentation that produces the most H_2 per mole of glucose and is the most energetically favorable (and thus the most likely to happen) when a sink of H_2 is present. Electricity generating reactions (reactions 9, 10, 11) are thermodynamically much more favorable than fermentations. The Gibbs free energies reported here refer to a typical operating anodic potential of +0.2 V versus standard hydrogen electrode (SHE). However, these reactions are subject to high electrochemical losses due to activation overpotentials (*7*), which severely reduce the actual energy gain to the bacteria. Moreover, the factors that determine which bacteria are more likely to take up the supplied glucose are not just energy related, but there are also accessibility issues: in the anodic biofilm, fermenters and methanogens are advantaged over anodophiles as they can grow at any distance from the electrode, whereas anodophiles are constrained to grow in a small layer close to the anode surface, because of the need of electrical contact via outer membrane cytochromes, nanowires (*8*), or diffusion of excreted mediators (*9*).

The goal of this study was to elucidate the pathways of glucose conversion in microbial fuel cell anodes, by determining which of the reactions listed in Table 1 do occur at a glucose-fed MFC anode and to what extent. This is highly relevant to the application of microbial fuel cells for wastewater treatment, where the objectives are (i) to maximize the conversion to current and (ii) to minimize methane generation, as not only does CH_4 constitute a Coulombic loss to the fuel cell, but it is also a potent greenhouse gas, which should not be freely released to the atmosphere.

Materials and Methods

Microbial Fuel Cell and Electrochemical Monitoring. A microbial fuel cell (MFC) in rectangular geometry was built as previously described (*5*) and filled with granular graphite as anode and cathode materials (2−6 mm diameter, available surface area 7.11×10^4 m^2/m^3, El Carb 100, Graphite Sales

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TABLE 1. Summary of Some of the Bioreactions That Are Likely to Occur at a Microbial Fuel Cell Anode Fed with Glucose

		$\Delta G^{\circ\prime}$ [a] (kJ/mol)
	fermentations	
(1) homolactic	glucose → 2lactate$^-$ + 2H$^+$	−197
(2) alcoholic	glucose → 2ethanol + 2CO$_2$	−235
(3) acetogenesis	glucose + 2H$_2$O → 2acetate$^-$ + 2CO$_2$ + 4H$_2$ + 2H$^+$	−216
(4) mixed acid	glucose → acetate$^-$ + propionate$^-$ + CO$_2$ + H$_2$ + 2H$^+$	−287
(5) butyric	glucose → butyrate$^-$ + 2CO$_2$ + 2H$_2$ + H$^+$	−264
(6) methanogenesis (acetoclastic)	acetate$^-$ + H$^+$ → CH$_4$ + CO$_2$	−36
	hydrogen consuming reactions	
(7) methanogenesis (hydrogenotrophic)	4H$_2$ + CO$_2$ → CH$_4$ + 2H$_2$O	−131
(8) homoacetogenesis	4H$_2$ + 2CO$_2$ → acetate$^-$ + H$^+$ + 2H$_2$O	−95
	electricity generating reactions[b]	
(9) glucose oxidation	glucose + 6H$_2$O → 6CO$_2$ + 24H$^+$ + 24e$^-$	−1438
(10) acetate oxidation	acetate$^-$ + 2H$_2$O → 2CO$_2$ + 7H$^+$ + 8e$^-$	−375
(11) hydrogen oxidation	H$_2$ → 2H$^+$ + 2e$^-$	−118
	combined reactions	
(12) = (3) + (7)	glucose → 2acetate$^-$ + CO$_2$ + CH$_4$ + 2H$^+$	−347
(13) = (3) + 4(11)	glucose + 2H$_2$O → 2acetate$^-$ + 2CO$_2$ + 10H$^+$ + 8e$^-$	−688

[a] Source of Gibbs free energies of formation: ref *10*. Data refer to pH 7 and 298 K. [b] The Gibbs free energy depends on the anodic potential; here we reported values for a near-optimum anodic potential of +200 mV SHE.

Inc., USA). The anode (160 mL net liquid volume, 50 mL headspace) was fed continuously with a modified M9 medium (*5*) at the feed rate of approximately 32 mL/hr, with glucose as electron donor at a concentration of 0.5 g/L chemical oxygen demand (COD). These feeding conditions led to an approximate organic loading rate of 2.4 $kg_{COD}m^{-3}d^{-1}$. The operating temperature was 22 ± 1 °C. The catholyte was a 150 mM potassium hexacyanoferrate ($K_3Fe(CN)_6$) solution and the cation exchange membrane was made of Ultrex (CMI-7000, Membranes International, USA). The choice of hexacyanoferrate as catholyte is dictated by the need to have a stable and not limiting cathode to study the activity of the anode alone. Recirculation was applied to both anode and cathode at the approximate rate of 100 mL/min. Reference electrodes (ref-201, Radiometer Analytical) were placed in both compartments. The external circuit was in some instances closed over a 20 Ω resistor; in other cases a potentiostat (VMP-3, Princeton Applied Research, USA) was used to control the anodic potential at a set point and record the current. When the circuit was closed with the resistor, a data acquisition unit (Agilient 34970A) was used to record voltage data; the current was then deduced from the voltage via Ohm's law.

Microbial Consortia. The reactor was first inoculated with effluent from microbial fuel cells that had been operating on acetate and/or glucose for over two years. The inoculum therefore contained a consortium already highly enriched in anodophiles. Some of the experiments reported in this study were run 6 months following inoculation (this biofilm will be called B1). The granules were subsequently removed from the reactor and replaced with new sterile granules, after the whole anode compartment was sterilized with a bleach solution. The residual bleach was subsequently removed by flushing with milli-Q water. The new anode was inoculated with effluent of an acetate-fed MFC and fed with acetate until a stable voltage developed. As acetate is normally nonfermentable, this start-up procedure was expected to enable the establishment of a biofilm with negligible presence of fermenters and hydrogenotrophic methanogens. Then the substrate was swapped to glucose and experiments were carried out immediately afterward (this biofilm will be called B2). The purpose of creating biofilm B2 was to study the glucose oxidation pathways before fermenters and methanogens would get a chance to colonize the anode.

Off-Gas Analysis and Liquid Analysis. The determination of methane and hydrogen production rates was done with the titration and off-gas analysis (TOGA) sensor, developed by Pratt et al. (*11*). The sensor works by stripping the gases produced in the reactor with helium and then sending the dried off-gas to a mass spectrometer (Omnistar Balzers AG, Liechtenstein), where its composition can be measured online. The TOGA was previously used to measure the composition of off-gases from microbial fuel cells fed with acetate and glucose (*5*), and detailed operational procedures can be found therein.

Samples from the anode liquid phase were taken at different times with a syringe through a septum, filtered with a 0.22 μm sterile filter, and analyzed for glucose, acetate, propionate, butyrate, valerate, hexanoate, lactate, formate, succinate, and ethanol by high-performance liquid chromatography (HPLC). In same cases the samples were also analyzed for chemical oxygen demand (COD) by the dichromate method (*12*).

Tests to Determine the Occurrence of Each Bioreaction. Experiments were designed to establish which of the reactions listed in Table 1 were carried out by the bacteria in the anodic biofilm. They were performed on the mature biofilm (B1).

Fermentation and Methanogenesis. During continuous feeding with glucose as electron donor, TOGA experiments were performed at anodic potentials of +200, 0, −200 mV SHE and open circuit. Each potential was maintained for 2 days before performing the respective test. As the anodic potential was gradually decreased from +200 mV to open circuit, the driving force for electricity generating reactions (9−11, Table 1) was reduced, causing fermentation products that would be substrates for anodophiles to accumulate in the liquid phase. This was used to qualitatively determine the types of fermentations occurring in the biofilm. Also, any methane detected in the off-gas would be a proof of methanogenesis (either hydrogenotrophic or acetoclastic). Each experiment was run over 3 h. The production of electrical current and gaseous products, namely hydrogen, methane, and carbon dioxide, were monitored continuously using methods described above. Liquid phase samples were taken hourly and analyzed for soluble fermentation products by HPLC. The hourly data were averaged to obtain statistical variability. At the end of the 3 h period, the circuit was opened for 1.5 h to reveal any intermediate fermentation products which could not be detected at closed circuit conditions due to their ongoing consumption by the anodophiles.

Acetoclastic Methanogenesis. To test for acetoclastic methanogenesis (reaction 6, Table 1), batch tests were

performed with acetate as electron donor. The feed to the anode was stopped and helium was used to strip the anode compartment until a steady endogenous methane production rate was reached. Acetate was then spiked into the reactor as a concentrated solution (10 mL of 1.55 g/L of sodium acetate, resulting in an initial acetate concentration of 70 mg/L), and the methane production rate was monitored online during current generation over a 20 Ω resistor. The batch was performed twice. Any increase in methane production from the endogenous level would reveal the occurrence of acetoclastic methanogenesis.

Hydrogen Oxidation and Homoacetogenesis. After depletion of previously added substrate, these hydrogen consuming reactions (reactions 11 and 8, Table 1) were investigated by rapidly flushing the anode compartment with 300 (or 200) mL of a gas containing 21.3% H_2, 19.9% CH_4, balance CO_2, and then placing the anode on gas recirculation (100 mL/min) with a peristaltic pump. Methane is not taken up by bacteria in anaerobic conditions, as it is the end product of all anaerobic reactions. Also, electrical current was not generated when pure methane was fed to another MFC anode in our laboratory (data not shown). Therefore, any electrical current would reveal H_2 oxidation, which could happen either directly or via homoacetogenesis followed by acetate oxidation (reaction 10). This test was done both with the MFC connected over a 20 Ω resistor and at open circuit. During the open circuit test liquid sampling for acetate would be able to reveal the occurrence of homoacetogenesis, as no means to oxidize acetate was available in that case.

Tests on the New Biofilm (B2). The new biofilm was grown to repeat some of the experiments described above in the absence of methanogenesis and thus study the interactions between electricity generation and fermentation alone. However, the inoculum already contained methanogens as revealed by methane production during TOGA tests carried out at 20 Ω external resistance shortly after start-up. As methanogens are obligate anaerobes, aeration was attempted as a means to eliminate methanogenesis. This was done by aerating the anode compartment with the aid of an aquarium pump for 1 h. Tests with off-gas analysis and electrochemical monitoring were done immediately before and 1 and 4 days following the aeration period during continuous feeding, according to the same procedure as outlined for biofilm B1. Batch tests with glucose as electron donor were also carried out. The continuous feed was first stopped and after all the pre-existing substrate was depleted (indicated by the attainment of a low and steady endogenous current) glucose was injected into the anode compartment as a concentrated solution (10 mL of 1.14 g/L glucose, resulting in an initial glucose concentration of 70 mg/L). Hydrogen evolution was monitored by TOGA. Liquid samples were analyzed by HPLC to reveal any temporary accumulation of soluble fermentation products.

Results

Occurrence of Anaerobic Bioreactions on a Mature Biofilm. *Fermentations and Methanogenesis.* The results of the anodic potential controlled tests are reported in Table 2. The data include a complete electron balance in which all assumed electron sinks are quantified. The Coulombic efficiency as measured ranged from 28% to 45%. Methane production comprised 13% to 19% of the COD removed.

Methane production occurred at all potentials. The fact that methane was produced implies that fermentations were also occurring, as their products (H_2 and/or acetate) are necessary substrates for methanogens. Indeed traces of hydrogen were detected in the off-gas in all cases. No accumulation of fermentation byproduct was observed at higher currents (in the cases of 0 and +200 mV). After opening the circuit immediately at the end of these two potential

TABLE 2. Summary of Outputs of Glucose Fed Anodes (Loading Rate 2.4 $kg_{COD}m^{-3}d^{-1}$, Influent Strength 500 mg_{COD}/L) at Controlled Anodic Potential or Open Circuit (All Values Are Averages ± Standard Deviations (n = 3))

		closed circuit phase						open circuit phase[b]			
		net output rates ($kg_{COD}m^{-3}d^{-1}$)						net output rates ($kg_{COD}m^{-3}d^{-1}$)			
E_{an} (mV SHE)	pH	current	CH_4	H_2	acetate	prop	soluble COD	CE[a]	acetate	prop	CH_4	soluble COD increase[c]
+200	6.2	0.91 ± 0.01	0.31 ± 0.03	0.006 ± 0.001	BDL	BDL	NA	NA	0.28	BDL	0.27 ± 0.02	NA
0	6.2	1.00 ± 0.01	0.27 ± 0.03	0.011 ± 0.001	BDL	BDL	0.20 ± 0.02	45% ± 1%	0.35	BDL	0.24 ± 0.02	0.35
−200	6.6	0.51 ± 0.001	0.27 ± 0.03	0.008 ± 0.001	0.21 ± 0.001	0.23 ± 0.11	0.56 ± 0.12	28% ± 1%	0.42	0.09	0.26 ± 0.02	0.04
opencircuit (−220 mV)	6.4	0.00	0.24 ± 0.01	0.010 ± 0.004	0.41 ± 0.03	0.14 ± 0.01	NA	NA				

[a] Coulombic efficiency. [b] These measurements were done immediately after opening the circuit at the end of the poised potential tests and for the following 90 min. [c] Difference between the nonremoved soluble COD rates at open and closed circuit; BDL: below detection limit; NA: data not available (COD measurements not done for these runs).

TABLE 3. Summary of Outputs from a Newly Developed Anode, Fed with Glucose at 2.4 $kg_{COD}m^{-3}d^{-1}$ (Influent Strength 500 mg_{COD}/L; External Resistance = 20 Ω; Values are Expressed in Units of $kg_{COD}m^{-3}d^{-1}$ and are Averages ± Standard Deviations (n = 3))

	current	CH_4	H_2	acetate	propionate
before aeration	0.96 ± 0.02	0.53 ± 0.01	0.02 ± 0.002	0.87 ± 0.01	BDL
1 day after aeration	0.79 ± 0.02	0.03 ± 0.02	0.01 ± 0.002	BDL[a]	BDL
4 days after aeration	0.88 ± 0.02	0.01 ± 0.02	0.02 ± 0.001	BDL	BDL

[a] BDL: below detection limit.

controlled tests, only acetate accumulated in the reactor, while there was no significant difference in the methane production. The acetate accumulation rate matched well the rate of soluble COD accumulation: this revealed that no other fermentation pathways were active at these conditions. Acetate accumulation at open circuit should be equivalent to twice the COD amount of methane (from hydrogen), based on reaction 3 (Table 1). However, the acetate net production was lower, which may indicate storage of the excess acetate as described previously (*5*).

Accumulation of both acetate and propionate was detected at −200 mV and at open circuit. The accumulation of acetate and propionate was further confirmed after the circuit was opened in the case of −200 mV. COD data confirmed that in this case no reduced soluble fermentation products other than acetate and propionate were excreted after opening the circuit. These results indicate that fermentation was limited to acetogenesis at high currents, whereas it comprised acetogenesis and mixed acid fermentation at low current. No other fermentative metabolisms were active in this biofilm as no other reduced fermentation products were detected.

The long-term open circuit test (last row) revealed that the stoichiometry of fermentation closely reflects the combination of reactions 3 and 4 in Table 1: the methane production rate calculated from the rates of accumulation of acetate and propionate based on reactions 3 and 4 is lower than the measured methane production rate by 22%. This error could be due to growth and storage. This is a further proof that no other fermentative pathways had significant activity in this biofilm.

Acetoclastic Methanogenesis. The test for acetoclastic methane production revealed that no methane was generated from acetate, as shown in Figure S1 in the Supporting Information. While anodophiles were able to readily take up acetate for current generation, CH_4 evolution never exceeded the endogenous level. This confirmed the earlier finding that methane is not produced from acetate in an anodic acetate-adapted biofilm (*5*). In light of this result, Table 2 can provide further information on the bioreactions occurring at the anode: if the only fermentative metabolism is the acetogenic one (as shown in the previous paragraph) and no conversion of acetate to methane occurs, then for each electron equivalent going to methane, two electron equivalents must go to acetate. The acetate thus produced may be washed out or used by anodophiles to generate current. For example, the 0 mV potential test in Table 2 reveals that 0.27 kg_{COD}-$m^{-3}d^{-1}$ of methane are produced, which implies that 0.54 $kg_{COD}m^{-3}d^{-1}$ of acetate (or even more if we account for fermenters growth) are excreted as fermentation byproduct. As no acetate is detected in the liquid during closed-circuit, this acetate must be entirely used to generate current. As the total current is 1.0 $kg_{COD}m^{-3}d^{-1}$, at least 54% of the current must be generated via acetate in a syntrophic interaction between fermenters and anodophiles. Syntrophic processes are therefore the leading pathways to current generation in these mixed communities.

Homoacetogenesis. As described above, homoacetogenesis was tested by flushing the anode compartment with hydrogen containing gas at open circuit. No acetate was detected in the liquid samples taken in the 90 min following initial flushing. This indicates that homoacetogenesis was not occurring in this system at an appreciable rate.

Hydrogen Oxidation. After flushing with hydrogen-containing gas, an electrical current developed over the external resistor of 20 Ω (Figure S2, Supporting Information). Currents up to 14 mA indicate that H_2 is a good substrate for anodophiles and that the existing biofilm is geared with the necessary enzymes to take up H_2. This is a strong indication that H_2 is routinely used by anodophiles to produce current. A control test was performed on an abiotic anode. No current was generated following H_2 flushing, demonstrating that the process is catalyzed by bacteria and that electrochemical discharge of H_2 on a graphite anode does not occur.

Results on a New Biofilm. The development of a stable voltage following inoculation took approximately a week under continuous feeding with acetate. As soon as the substrate was swapped to glucose, a test was run to determine the extent of fermentation and methanogenesis. Apparently the inoculum already contained fermenters and hydrogenotrophic methanogens (acetoclastic methanogens were never active in these biofilms, as shown by acetate spiking in the specific test described above), as these reactions were already happening at rates even higher than those observed in biofilm B1 (see Table 3). Methanogenesis was successfully suppressed by the 1 h aeration period, which also stopped the accumulation of acetate at open circuit (open circuit data not shown). If methanogenesis is removed and bacteria produce hydrogen via the NADH pathway (the direct ferredoxin pathway can be excluded by the lack of accumulation of more reduced products such as butyrate or ethanol), then they would be able to produce significant quantities of hydrogen and acetate only when a sink of hydrogen (in this case the anode) is available. In the absence of a current the whole metabolism would be stopped by the fact that hydrogen cannot be removed.

Acetate accumulation at closed-circuit ceased after the aeration event. A possible explanation for this observation may be that this caused a reduction in the fermentation rate due to the removal of hydrogenotrophic methanogens as sink for the fermentation product hydrogen. Interestingly, the aeration event caused also a slight decrease in the current. This could be due to the fact that the hydrogen concentration in the biofilm increased due to the lack of methanogens, thus reducing the rate of fermentation and thus the current. Other plausible reasons for the current decrease would be that the aeration event could have killed or inhibited some fermenters or the obligate anaerobes among the anodophiles.

Glucose-fed batch tests at 5 Ω were performed after the aeration period to determine whether hydrogen or acetate accumulation would occur in the initial phase of the batch. This was done to clarify whether the electricity generation in the new biofilm proceeded as direct glucose oxidation or through acetogenesis followed by oxidation of acetate and hydrogen. Figure 1 reveals that the development of a current was accompanied by a (albeit small) release of hydrogen gas and accumulation of acetate. Fermentation of glucose to hydrogen and acetate was thereby occurring even in the

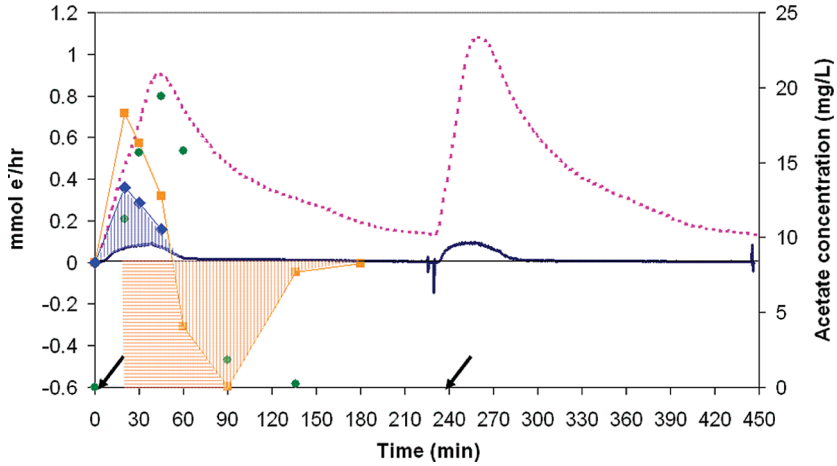

FIGURE 1. Batch tests with glucose as electron donor in a methanogenesis-free MFC anode (biofilm B2), with external resistance of 5 Ω. Current (---); hydrogen evolution (—); acetate accumulation rate (□); calculated minimum H_2 production (◆); acetate concentration (•). Acetate data were collected only for the first batch, the arrows on the time axis indicate injection times. Vertically shaded areas: minimum mmoles of electrons transferred to the anode via hydrogen and acetate; horizontally shaded area: additional mmoles of electrons transferred if the rate of acetate oxidation were constant from 20 to 90 min.

absence of methanogenesis and the electrical current was (at least partially) being generated by an anodophilic population feeding on hydrogen and acetate. By assuming that the stoichiometry of acetogenesis (reaction 3, Table 1) holds (this assumption was supported by the fact that no other reduced fermentation products were detected in the liquid), we could estimate a minimum hydrogen production rate (diamonds in the figure) which is the production that would occur if acetate were produced but not consumed. The H_2 production rate thus estimated is much larger than the hydrogen evolution rate (continuous solid line), indicating that most hydrogen must be consumed within the biofilm. Moreover, the calculated hydrogen production rate is a close match of the current (dashed line) in the first 15 min of the batch, suggesting that hydrogen is the primary substrate being oxidized in that phase. After 60 min the acetate consumption rate (squares) becomes the predominant route to current production. The calculation of the vertically shaded areas in the graph is a quantification of the minimum quantity of electrons transferred to the anode from hydrogen and acetate. The integration of these areas in Figure 1 revealed that at least 63% of the current was generated via these fermentation products. However, this percentage represents a lowest limit. If acetate oxidation were constant from time 20 min to time 90 min (this is a reasonable scenario based on the acetate concentration profile), the percentage of current generated via hydrogen and acetate would increase to 89%, as calculated by adding the horizontally shaded area in the figure. These observations imply that direct anodic oxidation of glucose is not a major route to current generation, at least at the concentrations and in the conditions applied here. Instead, electricity generation must be carried out primarily via the hydrogen and acetate produced by fermentative organisms.

After one month of continuous feeding with glucose, tests revealed that the methanogenic capacity was completely restored. The aeration experiment was repeated, but in this case methane production was only inhibited for a period of 2 h. This indicates that when the biofilm has grown and fully established itself, the methanogens are better protected against adverse environmental conditions.

Discussion

Pathways of Glucose Oxidation in Anodic Mixed Cultures. The results gathered in this study were used to generate an electron flux chart (Figure 2) where the fluxes (in kg_{COD}-$m^{-3}d^{-1}$) are for the experiments summarized in Table 2. Acetoclastic methanogenesis (acetate to methane) and homoacetogenesis (hydrogen to acetate) were not indicated in this figure because their occurrence was excluded by specific tests described above. Although in methanogenic reactors about two-thirds of methanogenesis occurs from acetate (*13*), it is not surprising that this reaction is not happening here, as it is characterized by a much lower energy gain than anodic oxidation (see Table 1). Anodic hydrogen oxidation was shown to be a major path of electricity generation, based on the results of the test with hydrogen flushing (Figure S1, Supporting Information). Also, the fact that fermentations switch from acetogenic to mixed acid at decreasing currents (Table 2) points to the fact that the anode is an important sink for fermentative hydrogen: when the current is reduced, the hydrogen concentration in the biofilm tends to rise and the bacteria switch to a fermentation that produces less H_2. To simplify this flux analysis, it was assumed that direct glucose oxidation by anodophiles did not occur, based on the results presented in Figure 1. Although direct anodic oxidation of glucose by pure cultures has been previously observed (*14*), this is not the most important pathway in a mixed culture based on the results of this work. Direct electron transfer from fermenters to other bacteria via bacterial nanowires was previously described (*15*) and could play a role here. However, the anodophilic community was shown to possess the right enzymes to take up H_2 efficiently (Figure S1, Supporting Information). This shows that they are able to routinely use H_2, which must be produced by fermenters. In light of this, the transfer of electrons is more likely to occur via hydrogen than via nanowires.

What the experiments do reveal is that to some extent glucose is taken up by fermenters, which mostly carry out acetogenesis or mixed acid fermentation under the loading conditions applied. The products of these fermentations (acetate, propionate, and hydrogen) are used as substrates by anodophiles and converted to electrical current. The overall process is the net conversion of glucose to electricity and carbon dioxide, with some energy and carbon retained for the growth of fermenters and anodophiles. To calculate the fluxes, the stoichiometry of fermentation was deduced from the intermediate species that were observed after opening the circuit. At +200 mV and 0 mV SHE, only acetate was detected, so reaction 3 fully describes the fermentation. At −200 mV SHE, the open circuit ratio of combined methane and hydrogen production rates to combined acetate and propionate production rates enabled the determination of the relative extents of acetic (reaction 3) and mixed acid (reaction 4) fermentations. A linear system of 4 equations

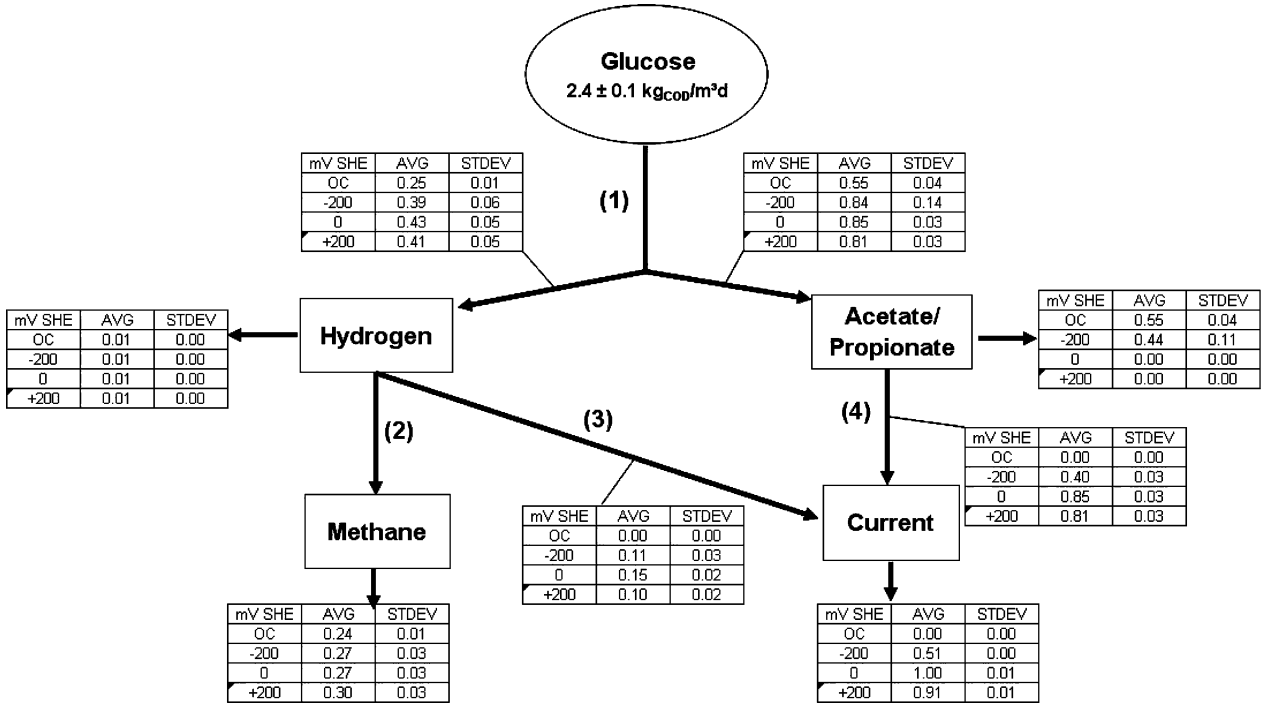

FIGURE 2. Simplified chart of electron fluxes in continuous glucose fed MFC anode with 6 month old biofilm (B1). All values in units of kg$_{COD}$m^{-3}d^{-1}. OC: open circuit. (1) fermentation; (2) methanogenesis; (3) hydrogen oxidation; (4) VFA oxidation.

(H_2 balance, CH_4 balance, VFA balance, and electrical current balance) and 4 unknowns (the fluxes indicated as 1 to 4 in Figure 2) was solved to reveal the fluxes.

The time frame for the experiments reported here was several days. Further research is needed to elucidate whether longer term operation would demonstrate the same behavior.

Syntrophy. Glucose conversion to electrical current and CO_2 in a microbial fuel cell anode is a complex process involving different populations of bacteria. In this picture, it appears that fermenters are not competing with anodophiles for the electron donor. Instead, a syntrophic interaction between the two populations must occur, with the hydrogen and acetate produced by the fermenters being rapidly consumed by the anodophiles. The latter thus make fermentation faster and more energetically favorable by continuously removing its products. For hydrogen, this situation is known as interspecies hydrogen transfer, where one population (the fermenters) needs a second population (the anodophiles) to maintain low concentrations in solution. In this view, fermentation is not detrimental to electricity generation. An exception would be during episodes of overfeeding. When too much glucose becomes available, fermentations switch from acetic to mixed acid and butyric (*16*). The excess of acids thus produced lowers the pH in the biofilm and eventually inhibits the anodophiles. As hydrogen is available in the biofilm, inevitably other hydrogen utilizing populations develop, primarily methanogens. Figure S3 (Supporting Information) depicts the three main populations of this biofilm and their interactions. The results of this study were obtained using glucose as a model fermentable substrate, but are likely to be extendable to other fermentable substrates as well, primarily carbohydrates.

Aeration to Suppress Methanogens. Methanogens are unwanted in a microbial fuel cell anode. This is not just because they cause a Coulombic loss, but also because methane is a potent greenhouse gas and its emission should be avoided. Anodic potential or electrical current control (Table 2) proved ineffective to reduce methanogenesis, showing that methanogens operate independently from the anode process. Exposing the anode to air was successful to suppress methanogenesis, but only in a newly forming biofilm. In a well established biofilm aeration could at most inhibit methane generation for a few hours.

The solution to the problem of methane formation in MFC anodes is not expected to be simple. Periodic aeration of a new biofilm or oxygen diffusion in the case of air cathodes may delay the colonization of an anode by methanogens. Periodic electrode replacement, mechanical cleaning, or sterilization may also be useful. However, it should be pointed out that the results of this study may not be valid for anode materials other than granular graphite. The use of less porous electrodes, such as carbon fibers, may make it more difficult for methanogens to find protection against aeration. Further studies are required to determine the efficacy of aeration to curb methanogenesis in anodic biofilms growing on different materials.

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Supporting Information

Additional figures as quoted in the text above. This material is available free of charge via the Internet at http://pubs.acs.org.

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