Storing of exoelectrogenic anolyte for efficient microbial fuel cell recovery

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Abstract

Starting up a microbial fuel cell (MFC) requires often a long-term culture enrichment period, which is a challenge after process upsets. The purpose of this study was to develop low cost storage for microbial fuel cell enrichment culture to enable prompt process recovery after upsets. Anolyte of an operating xylose-fed MFC was stored at different temperatures and for different time periods. Storing the anolyte for one week or one month at +4 °C did not significantly affect power production, but lag time for power production was increased from 2 days to 3 or 5 days, respectively. One month storing at -20 °C increased the lag time to 7 days. The average power density in these MFCs varied between 1.2 and 1.7 W/m^3. The share of dead cells (measured by live/dead staining) increased with storing time. After six-month storage the power production was insignificant. However, xylose removal remained similar in all cultures (99-100%) whilst volatile fatty acids production varied. The results indicate that fermentative organisms tolerated the long storage better than the exoelectrogens. As storing at +4 °C is less energy intensive compared to freezing, anolyte storage at +4 °C for maximum of one month is recommended as start-up seed for MFC after process failure to enable efficient process recovery.

Keywords

Exoelectrogenic culture; mixed culture storage; freezing; refridgerating; process recovery

1. Introduction

Microbial fuel cells (MFC) can be used for treating industrial wastewaters and producing electricity simultaneously [1]. Previous research with MFCs has shown promising results for treating wastewaters from very different industrial operations such as brewery [2], vegetable oil industry [3], dairy production [4], chocolate factory [5], cassava mill [6], corn stover biorefinery [7], pharmaceutical production [8], textile colour industry [9] and paper recycling [10]. Industrial wastewaters are often characterized by variations in water flow and compositions. For example in brewery wastewater, high substrate concentrations are typically present at the end of a brewing batch, and brewing is usually directly followed by the use of tank-washing chemicals [11]. Pulp and paper mills exploit continuous processes, but the chemical compositions of wastewaters from debarking, wood chipping, pulp manufacturing, bleaching, paper making and recycling processes are very different [12] and some wood extractives cause antimicrobial effects [13]. Also, interruptions in industrial processes can make the wastewater treatment process challenging. For example, shutdowns caused by maintenance work can disturb the microbial community of a MFC [14].

After disturbances, prompt wastewater treatment process recovery and start-up are required for interminable environmental protection. Depending on the wastewater, starting up a MFC can require very long time [15]. In our previous experiment with a xylose-fed up-flow MFC, the start-up time for stable electricity production was 44 days with anaerobic municipal wastewater sludge as a seed (data not shown). The start-up time can be shortened by using seed culture from an operating MFC maintained at similar conditions [16]. This can also increase the power density of the MFC [17,18]. However, continuous MFC operation just for maintaining enrichment cultures is not practical. For these reasons, means for enabling fast and low-cost process start-up and recovery of enriched exoelectrogenic cultures are needed.

Pure cultures of microroganisms are often stored by freezing with 10% glycerol as cryoprotective agent at -80 °C or colder [19]. Also pure culture of exoelectrogenic *Geobacter sulfurreducens* has been successfully stored by freezing [20] whereas the recovering electricity production from frozen mixed cultures has been difficult [21]. Other storage methods include freezing with other or without any cryoprotective agents, refrigerating (+4 °C), encapsulation [22] and drying e.g. with acetone [23] or by freeze-drying [22].

Wastewater treatment is always based on open microbial cultures, because they are able to degrade complex mixtures of organic substrates [24] and aceptic techniques are not needed as would be the case with pure culture operations. Storing of mixed microbial cultures has been studied at different temperatures, in different solutions and with different pretreatments such as drying or seeding with pellets. For example, Yükselen [25] studied preservation of UASB sludge at -18 °C, +4 °C, room temperature, and +37 °C for one year achieving highest methanogenic activity after storing at +37 °C. Li et al. [26] stored anaerobic sludge by drying for 4 months with insignificant loss in methane yield. Adav et al. [27] and Xu et al. [28] stored aerobic granules successfully for 3 months in different solutions and for 3 weeks as seeded with pellets (dewatered aerobic granules), respectively. However, different bacterial species have different survival rates during the storing and sometimes the most effective storage as measured by cell viability test does not result in the most active culture [29]. The survival of mixed cultures enriched for anodic electricity production can differ significantly from other anaerobic cultures. Only one previous study [21] reports on electrochemically active enrichment culture storage. Alam et al. [21] stored an exoelectrogenic biofilm on anode electrode by refrigerating, freezing, or dehydrating, but original current production was not reached after storing. They suggested that dead cells in the biofilm prevented contact between exoelectrogens and the electrode. Storing exoelectrogenic enrichment culture suspension instead of biofilm would overcome this problem.

In this work, the effect of simple and low cost MFC anolyte storage for recovering stable power density and lag time required for current production were studied. Anolyte from an operating xylose-fed MFC was freezed (-20 °C) or refrigerated (+4 °C) with different storing times (from 1 week to 6 months) and compared with fresh anolyte for MFC start-up. To our knowledge, this is the first study on the survival of exoelectrogenic cultures and their ability to regain current production by storing enriched MFC anolyte. Xylose was used as substrate, because forest industry wastewaters contain xylose from glucuronoxylan containing wood material [30,31].

2. Materials and methods

2.1 MFC construction and operation

Experiments were conducted in 3-chamber MFCs (one anode chamber and two cathode chambers) (Figure 1). The anode chamber (123 mL) was separated from the cathode chambers (62 mL each) on both sides with a 41 cm^2 cation exchange membrane (CME7000) coated with

PtNi (1:1) as described by Cetinkaya et al. [32] with an exception that they used air cathodes in place of cathode chambers. The total volume of anolyte was 500 mL from which the extra volume was circulated at a rate of 100 mL/min over a recirculation bottle placed in a 37 °C water bath. Two carbon brush electrodes with titanium wires as current collectors were used as anode electrodes. Reference electrode (BASi RE-5B Ag/AgCl) was positioned between the two carbon brush electrodes for anode potential measurements. The two anode electrodes were connected through 100 Ω external resistance to the two cathode electrodes forming a single circuit. Cathode chambers were equipped with air spargers (ouput 50 L/h) that provided dissolved oxygen as the terminal electron acceptor. Carbon cloth (one for each cathode chamber) located against the membrane was used as cathode electrode (projected area of 41 cm^2).

Catholyte solution (pH 7) contained 15.6 mM Na_2HPO_4, 34.4 mM NaH_2PO_4, and 150 mM NaCl in distilled water. Anolyte solution was prepared as described by Mäkinen et al. [33] without addition of EDTA and resazurin. In addition, the concentration of yeast extract was reduced to 0.03 g/L in the beginning of the experiment and to 0.003 g/L after the first feeding cycle. Xylose (1.0 g/L) was used as substrate and pH of the feeding solution was adjusted to 7.0 with NaOH. MFCs were fed with interval of 6-8 days by replacing 50 mL of anolyte solution with fresh feed. If the volume of the anolyte decreased during the feeding cycle, the volume was adjusted back to 500 mL with the removed anolyte. During the operation 5 M NaOH was added into the anolytes if needed after the feeding to ensure that pH did not decrease below 6.0.

MFCs were inoculated with anolyte from an operating fed-batch MFC using xylose as a substrate after five months of enrichment at similar conditions. This culture was originally enriched from an anaerobic digester of a municipal wastewater treatment plant (Viinikanlahti, Tampere, Finland). The anolyte solution to be used as inoculum for new MFCs (25 mL for each) was stored for the experiments in 60 mL batches in freezer (-20 °C) or fridge (+4 °C) under nitrogen atmosphere. Culture reactivation was tested after storing the anolyte for one week (+4 °C), one month (+4 °C and -20 °C), and six months (+4 °C and -20 °C). In addition to this, reference cultures were started straight after anolyte collection without storing the inoculum. Frozen anolyte batches were defrosted at room temperature. MFCs were washed with 1 M NaOH and 70 % ethanol between the experiments. All the experiments were conducted as duplicates.

2.2 Analyses

2.2.1 Electrochemical measurements and calculations

Cell voltage and anode potential were recorded with 2 min intervals using Agilent 34970A data Acquisition/Switch Unit (Agilent, USA). Current and power densities were calculated against anode chamber volume using measured cell voltage data and external resistance according to Ohm’s law. Cell voltage data was used also for measuring lag time (d), which was determined as the time needed for achieving 100 mV cell voltage with 100 Ω external resistance.

Linear sweep voltammetry (LSV) was conducted using a potentiostat (Palmsens3, Netherlands) with the scan rate of 1 mV/s in the end of the experiment, 1-3 days after the last feeding. Analysis was conducted starting from 0-50 mV higher cell voltage values compared to open circuit voltage [34], which was measured after 30 min of stabilization. The measurement was continued by lowering the cell voltage from the starting value (150-550 mV) to the final value of 0.005 mV. Internal resistance was calculated from the LSV data ($R_{internal} = U/I$) by drawing a power curve against voltage to find the place of the power curve peak on voltage axis and using the data of this point for internal resistance calculations.

Coulombic efficiency (CE) was calculated from the xylose degradation and electrical current data over the last full feeding cycle with the Equation 1 (modified from [34])

$$C_E = \frac{M_s \int_0^{t_1} I dt}{F b_{es} \Delta m_{xylose}}, \qquad (1)$$

where Ms = molecular weight of xylose (g/mol), t_1 = length of feeding cycle (d), F = Faraday's constant (96 485 C/mol*e), b_{es} = number of electrons released per mol of xylose (20 e-), Δm_{xylose} (g). The mass of degraded xylose was calculated by subtracting the measured xylose in the end of the cycle from the concentration in the beginning of the same cycle.

2.2.2 Chemical analyses

Concentrations of xylose and fatty acids (VFAs) were measured from the anolyte samples taken before each feeding and in the end of the experiment. Also the anolyte pH was measured (WTW pH 330 meter) from the same samples. After pH measurement the solid particles were removed by centrifuging (10 min, 7500 x g) followed by filtering (0.2 µm polyester filter). Samples were stored at -20 °C. Xylose concentration was measured using phenol-sulphuric acid method [35] with the modifications described by Haavisto et al. [30]. VFA and alcohol (acetate, propionate, butyrate, isobutyrate, valeric acid, ethanol, and butanol) concentrations were measured with gas chromatograph as described by Haavisto et al. [30].

2.2.3 Microbial analyses

Microbial community samples were taken from the anode biofilms in the end of each experiment. The biofilm samples were obtained by sonicating the carbon brushes for 5 min in 0.9% NaCl. Then microbes were collected in a pellet with a centrifuge (10 min, 5000 x g) and by discarding the supernatant. The samples were stored at -20 °C and microbial communities were analyzed from defrosted samples as described by Haavisto et al. [30]. DNA was extracted with PowerSoil DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) and partial 16SrRNA genes were amplified with PCR using GC-BacV3f [36] and 907r [37] primers as described by Koskinen et al. [38]. After separating DNA sequences with DGGE according to Lakaniemi et al. [39] the sequences were reamplified according to Koskinen et al. [38] and sequenced at Macrogen Inc. (Seoul, Korea). Sequence data was analyzed with BioEdit software and compared to known sequences by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Two separate DGGE gels were prepared from which one contained biofilm samples from all the duplicate reactors while in the other gel the amount of samples was reduced by selecting only the communities with higher current density for easier comparison of different storing methods and times.

Microbial viability of the differently treated anolytes was estimated with LIVE/DEAD© BacLight™ Bacterial Viability Kit. Bacteria were stained with SYTO®9 and propidium iodide (pretreatment methods modified from [40]). Samples (1 mL) were mixed with 50 mL sterile filtered 0.9% NaCl followed by 1 min sonication (Finnsonic m03, Finland). Diluted samples (50 or 100 µL) were further diluted to 1 mL volume with 0.01 M $Na_4P_2O_7$ and 5 µL of the mixture (1:1) of fluorescent stains was mixed to samples by vortexing for 10 s. After incubating mixtures for 15 min in dark the samples were filtered with polycarbonate membrane filter followed by the examination with an epifluorescence microscope to determine the viability based on cell wall integrity.

3. Results and discussion

3.1 Electricity production

The MFCs containing reference cultures without anolyte storing and cultures with different storing methods were compared. Reference cultures reached an average power density of 1.6 W/m^3 (141 mV as cell voltage, Table 1). After storing at +4 °C or at -20 °C for one week and one month, the average power densities of the last full feeding cycles were 1.2 – 1.7 W/m^3 whilst storing for six months in either temperature decreased power density to 0.004-0.06 W/m^3. The corresponding current densities were 10 - 12 A/m^3 and 0.6 - 2 A/m^3, respectively. Average anode potentials were also more than 200 mV less negative after six months storing compared to the shorter storage times (Table 1). Alam et al. [21] reported 75% of the original current density after 5 weeks storage of biofilm containing anode electrode at +4 °C, representing higher activity reduction than obtained after one month in this study (87% of the current density remaining after the storage). Also freezing with 10% glycerol at -70 °C for 5 weeks decreased current density about 75% in the study of Alam et al. [21]. Our MFCs did not show decrease in the average current density (based on the last full feeding cycle, days 14-21) after one month storing at -20 °C. However, the standard deviation between the duplicate MFCs after one month storing at -20 °C in this study was 20% of the average current density. In our study, internal resistance was over 700 Ω after six months storing at +4 and -20 °C, but only 40-47 Ω in all the other reactors. Massive increase in internal resistance during 6 month storage was likely due to changes in microbial community [41].

3.2 Lag time and cell viability

Lag time for reaching 100 mV cell voltage (i.e. power density of 0.8 W/m^3) was 1.9 d without storing and storing increased it by at least 0.8 days. Lag time increased with increasing storing time and was longest with storing at -20 °C for one month (Table 1). In all MFCs the power density increased close to the highest stable values in 1 ± 1 d after reaching the set point value of 0.8 W/m^3 (example power density curves in shown Figure S1). Average power densities (see section 3.1) and anode potentials (Table 1) obtained during the stable MFC operation (last full feeding cycle) were similar in MFCs started up with anolytes stored for one week and one month. After storing the anolyte for six months (at +4 °C or -20 °C) electrochemical activity did not recover. The observed lag times are well in accordance with Alam et al. [21] reporting faster re-activation in electrochemical activity after storing the anode biofilm at +4 °C compared to freezing (at -70 °C) with glycerol. The lag times (2-7 days) observed in this study were also significantly shorter than the start-up time (44 days) of our xylose-fed up-flow reactor seeded with anaerobic sludge from municipal wastewater treatment plant.

Cell viability after anolyte storing was calculated as live/dead stained cells by fluorescence microscopy. Without storing, approximately half of the cells stained as alive. After one-week storage at +4 °C, share of dead cells was 60%, while the longer storing times decreased viability more. After one month storing at -20 °C or +4 °C, the share of dead cells were 80% and 85-95%, respectively. After six months at +4 °C and -20 °C, the shares of dead cells were 95% and >98%, respectively. The cell viability measurements were disturbed by background noise. In addition, some of the cells may stain red with propidium iodide although being viable, as the method actually assays membrane integrity and not directly cell viability [42,43]. However, the results show that the relative share of cells stained as dead increased with increasing storing time (Figure 2). Interestingly the share of dead cells was lower at -20 °C compared to +4 °C at the same storing time, whilst the lag time for power production was longer for the anolyte stored at -20 °C. This shows that total number of microorganisms that survived storing (i.e. retained their membrane integrity) does not directly correlate with activity of stored exoelectrogenic microorganisms. This is in accordance with the observations of Balfour-Cunningham et al. [29], who reported that the most effective storage based on cell viability measurement does not always result in the most active culture.

3.3 Metabolic activity

Xylose removal (99-100%) during the last full feeding cycle after all tested storing times indicated high activity of xylose-utilizing microorganisms. However, the CEs were relatively low with the highest calculated values being 14 ± 3% (Table 2). After six months storing the CEs were only 0.7 - 2.8%, but a CE of 10% or higher was obtained in all the other MFCs. Measured CEs were low compared to the other published results for xylose-fed MFCs [44-46]. However, in this study, the CE values were calculated against fed xylose as compared to Huang et al. [44], Sun et al. [45], and Huang & Angelidaki [46], reporting values against removed COD.

Residual VFA concentrations in the end of the last full feeding cycle increased with storing time indicating efficient recovery of VFA-producing fermentative microorganisms. VFAs included mainly acetate, propionate and butyrate as also other xylose-fed MFCs [47,48]. The highest concentrations of total VFAs were obtained after six months storing (Table 2). Residual propionate and butyrate increased with increasing storing time, whereas acetate concentrations were similar after one month and six months storing at -20 °C (Table 2). Propionate concentrations in MFCs with anolyte after one month storing were only 50% of the values obtained after six months storage. These results show that xylose fermenting microorganisms regained their activity after storage. Acetate and propionate are typically suitable substrates for exoelectrogens [49], but they were not efficiently utilized and rather accumulated to the anolyte. This indicates that long-term storage at +4 and -20 °C directly affected the exoelectrogens rather than other microorganisms involved in the anaerobic degradation of xylose.

3.4 Microbial community

Microbial community analysis (Figure 3) revealed the presence of well-known exoelectrogen *Geobacter sulfurreducens* [50] with 97.9-99.6% similarity in all the MFCs with considerable power production (reactors with anolyte storing time of one month or less). Alam et al. [21] also found *G. sulfurreducens* after 5 weeks storing at +4 °C and freezing at -70 °C with glycerol, but reported that the share of *G. sulfurreducens* in microbial community decreased from 70% in the original biofilm to 10-30%. Alam et al. [21] also reported that the storing of the anode biofilm increased the diversity of the microbial community. Similarly in this study, some microorganisms that were not detected in MFC inoculated with fresh cultures, became enriched and thus, detectable from MFCs inoculated with the stored anolytes. These included species having high similarity to *Lentimicrobium saccharophilum*, *Pluralibacter gergoviae* and *Citrobacter freundii* (Figure 3, Table 3). DGGE-profiling of mixed cultures is, at best, a semi-quantitative analysis. This method does not detect minor quantities of DNA and some of the microorganisms present in samples remain undetected [51,52]. This may be the case for some microorganisms in fresh, unstored samples. During storage, the microbial composition may change and re-cultivation may thus, result in enrichment of microorganisms that remained undetected in original samples.

After one month storage at -20 °C, the electricity production in the duplicate reactors was different. Therefore the microbial communities from both MFCs' anodes were characterized. The anode biofilm with lower power density did not contain *G. sulfurreducens*, but another exoelectrogen, *Citrobacter freundii* [53]. *C. freundii* was present also in the biofilm of other MFCs started with anolyte stored for one month either at +4 °C or -20 °C. The only bacterium with known exoelectogenic activity found after six months storing at +4 °C was *Escherichia coli* [54], but after six months storing at -20 °C no known exoelectrogenic bacteria were detected. According to sequencing results (Table 3), band 4 identified as *E. coli* could be also *Tumebacillus flagellatus*, but as an aerobe, it is unlikely that *T. flagellatus* would grow in anode biofilm [55]. *E. coli* was found also from the other biofilm samples after storing anolyte at +4 °C.

All the MFC biofilms contained known fermentative bacteria (*E. coli*, *Proteiniphilum acetatigenes*, *C. freundii*, or *Lentimicrobium saccharophilum*) [56-59] and facultative anaerobes (*E. coli*, *C. freundii*, or *Pluralibacter gergoviae*) [53,56,60]. The presence of facultative anaerobes is important for the strict anaerobes, because facultative anaerobes are able to consume oxygen, which is potentially penetrating to the anode chamber from the cathode. Among the identified bacteria, *E. coli* is known to be able to degrade xylose [56] and it was found from most of the samples. The results of microbial community analysis are in line with metabolic activity results and give further evidence that long-term storage had direct influence on exoelectrogenic bacteria.

3.5. Implications

Based on the power production, xylose removal, and microbial community data, fermentative bacteria tolerated the storage better than exoelectrogenic bacteria both at -20 °C and +4 °C when the storing time was six months. Previous studies have shown that fermentative bacteria e.g. from cow rumen can be stored at least for two years at -20 °C with glycerol [61]. However, at +4 °C agar deep cultures of the same microbes lost viability already after 0.5-2 years [61]. Lower storing temperatures and use of cryoprotective chemicals such as glycerol generally result in higher stability and more successful preservation of microbial viability and activity [62]. In case of frozen cultures, the rate of temperature changes both during freezing and thawing is also important for the survival of the microorganisms [62, 63].Temperatures below -140 °C are typically recommended for most efficient culture storage, as such temperatures rule out the possibility of presence of even traces of liquid water that can cause cryoinjury especially if temperature fluctuates during the storage [62, 64]. However, temperatures below -140 °C would require specialized equipment and liquid nitrogen, while the focus of this study was on more commonly available simple and low-cost storing methods available in e.g. wastewater treatment facilities.

It has also been shown that subjection of microbial culture to certain adverse conditions before storing can increase the tolerance of the culture to temperature shocks caused by storing at low temperature [65]. No spore forming bacteria were identified in the biofilm samples of this study, but some exoelectrogens, such as *Bacillus subtilis* [66,67], can form endospores to survive harsh conditions, which could also significantly help storing exoelectrogenic cultures. However, although inducing of intentional stress on the culture could be possible under laboratory conditions, it would not be a viable option for real wastewater treatment applications, because it could cause unwanted deterioration in the quality of the treated wastewater.

Based on the results of this study, storing time clearly affected the survival of bacteria and the lag time for electricity production, when the anolyte what stored at +4 or -20 °C without any cryoprotective agents or induced stress condition before the sampling. Storing anolyte of an operating mixed culture MFC for one month at +4 or -20 °C can help to speed up the process recovery with minimal power density losses on clean anode electrode after process disturbances. In actual MFC treatment of wastewater, storage of effluent at +4 °C would serve as means to be prepared for process upsets and their recovery. The stored anolyte should be changed with fresh on monthly basis.

4. Conclusions

The results of this study demonstrated that storing anolyte from an operating MFC for one month or less at +4 °C or -20 °C resulted in similar power density (1.2-1.7 W/m^3) as was obtained in reference MFCs started with fresh anolyte. Further, both the lag time of process recovery to reach reasonable cell voltage and the percentage of dead cells in the stored anolyte (based on live/dead staining) increased with increased storing time. After six months storing of the anolyte solution at either temperature, the power production remained negligible. Xylose removal was not affected by the storing remaining at 99-100% in all MFCs. Similarly, VFA producing microorganisms remained active in all storage conditions and produced acetate and propionate for exoelectrogens. Decreased power production during long-term storage was directly associated with exoelectrogenic bacteria. Anolyte storage at +4 °C for maximum of one month is recommended as start-up seed for MFC after process failure to enable efficient process recovery. This suggests that effluent storing from continuous-flow MFCs would be a practical way of being prepared for process upsets.

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Table 1. Lag time for the start-up of MFCs, cell voltage and anode potential with 100 Ω resistance and internal resistance calculated from LSV data at the point of highest power density values. The standard deviation values show the difference between duplicate reactors. No lag time is reported after six months storing, because the cell voltage remained negligible.

	Lag time[a] (d)	Average cell voltage[b] (mV)	Average anode potential[b] (mV)	Internal resistance (Ω)
Without storing	1.9 ± 0.5	141 ± 14	-454 ± 7	41 ± 11
1 week at +4 °C	2.7 ± 0.3	146 ± 5	-456 ± 16	47 ± 4
1 month at +4 °C	5.0 ± 0.9	123 ± 11	-461 ± 7	44 ± 6
1 month at -20 °C	7 ± 3	150 ± 30	-451 ± 4	40 ± 20
6 months at +4 °C	-	27 ± 12	-240 ± 20	764 ± 13
6 months at -20 °C	-	7 ± 3	-170 ± 30	900 ± 300

[a]Before cell voltage reached 100 mV (0.1 mW); [b]Values from the last full feeding cycle

Table 2. Coulombic efficiency (CE), xylose degradation efficiency and VFA concentrations in the end of the last full feeding cycle. The standard deviation values show the differences between duplicate reactors. VFA concentrations were measured in the end of the feeding cycle.

	CE (%)	Xylose removal (%)	Acetate (mM)	Propionate (mM)	Butyrate (mM)
Without storing	11.2 ± 1.2	99.0 ± 0.2	10.6 ± 1.4	2.7 ± 0.5	n.d.[a]
1 week at +4 °C	11.6 ± 0.5	99.12 ± 0.04	18 ± 2	5.0 ± 0.8	n.d.
1 month at +4 °C	11.9 ± 1.1	99.5 ± 0.3	16 ± 4	6.3 ± 0.5	< 0.5[b]
1 month at -20 °C	14 ± 3	99.10 ± 0.09	24.4 ± 0.9	8.2 ± 1.0	0.8 ± 0.3
6 months at +4 °C	2.8 ± 1.2	99.5 ± 0.3	27 ± 3	16 ± 2	1.38 ± 0.03
6 months at -20 °C	0.7 ± 0.4	99.2 ± 0.5	22 ± 17	15 ± 10	1.5 ± 0.7

[a] n.d. = not detected; [b] below detection limit, which was 0.5 mM

Table 3. Identified organisms from DGGE gel shown in Figure 3. Variation in sequence length and similarity is caused by identification of multiple bands with similar affiliation.

Band label	SL	Sim (%)	Affiliation (acc number)	Class / Family	Origin of the sample
1	406	99.5	Proteiniphilum acetatigenes (NZ_KB905705.1)	Bacteroidia / Porphyromonadaceae	UASB reactor treating brewery wastewater
2	257	98.4	Lentimicrobium saccharophilum (NZ_DF968182.1)	Bacteroidia / Lentimicrobiaceae	Methanogenic Wastewater Treatment System
3	384-424	98.7-99.8	Pluralibacter gergoviae (NZ_CP009450.1)	Gammaproteobacteria / Enterobacteriaceae	Isolated from Packed Fish Paste
4	414-426	98.6-100	Tumebacillus flagellatus (NZ_JMIR01000093.1)	Bacilli / Alicyclobacillaceae	Cassava wastewater
	414-426	98.6-100	Escherichia coli (NC_011751.1)	Gammaproteobacteria / Enterobacteriaceae	Human urine
5	379-446	97.9-99.6	Geobacter sulfurreducens (NC_002939.5)	Deltaproteobacteria / Geobacteraceae	
6	432	96.7	Phascolarctobacterium sp. (NZ_GL830850.1)	Negativicutes / Acidaminococcaceae	Human gut
7	365	99.2	Citrobacter freundii (NZ_CP007557.1)	Gammaproteobacteria / Enterobacteriaceae	Sink aerator

SL = sequence length of the sample, Sim (%) = similarity (%), Affiliation (acc number) = closest species in database and its accession number, and Origin of the sample = Origin of the sample with the closest match. Band number 4 matched with two different organisms in a similar way.

Figure captions

Figure 1. Schematic diagram of a MFC showing the anode and cathode chambers and anolyte circulation. A) Carbon brush electrodes in anode chamber, B) Anolyte circulation tubes (arrows show the liquid flow direction), C) Anolyte circulation bottle, D) Aeration stones used in the cathode chambers.

Figure 2. Share of cells stained as dead after different MFC anolyte storing times. Square shaped markers stand for the storing at +4 °C, and the triangles the storing at -20 °C. (Here 1 month = 4.4 weeks, 6 months = 25.9 weeks)

Figure 3. Microbial community samples from anode electrode biofilm. Samples A-F show the microbial community from the duplicate MFC that resulted in the higher power density of the two parallel reactors operated after similar inoculum treatment: A) without storing, B) 1 week at +4 °C, C) 1 month at +4 °C, D) 1 month at -20 °C, E) 6 months at +4 °C, and F) 6 months at -20 °C. Sample G represents the parallel reactor for D (1 month at -20 °C) another DGGE gel to elucidate the difference of the microbial communities of these duplicate MFCs that enabled quite different power densities.