Using electrical signals of microbial fuel cells to detect copper stress on soil microorganisms

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Summary

A method based on microbial fuel cells (MFCs) was used to evaluate the effects of copper (Cu^{2+}) on soil microorganisms. Soil spiked with 50–400 mg kg^{-1} of Cu^{2+} as CuCl_2 was incubated for 24 hours before being packed into the MFC anode chambers and assayed for dehydrogenase activity (DHA), substrate-induced respiration (SIR) and microbial biomass carbon (C_{mic}). Soil was amended with 5% (w/w) glucose to accelerate 'start-up' and improve power generation, followed by 150 hours of operation. Anode biofilm and soil was extracted to recover total nucleic acids and the 16S rRNA gene was subjected to PCR-DGGE, sequencing and phylogenetic analysis. Results showed that increases in soil Cu^{2+} concentrations reduced voltage and postponed start-up. The quantity of generated electrons within 48 hours was 32.5 coulomb (C) in the without-Cu control and decreased with increasing Cu^{2+} concentrations (11.7, 7.7, 2.0 and 1.3 C under 50, 100, 200 and 400 mg kg^{-1} Cu^{2+} , respectively). Cyclic voltammetry identified decreased soil electrochemical activity with increasing Cu^{2+} concentrations. The results indicate that Cu^{2+} reduced electrical signals by inhibiting the electrochemical activity, metabolic activity and biomass of microorganisms. The 16S sequences of recovered anodic bacteria were assigned to *Firmicutes*, including *Bacillaceae*, *Acetobacteraceae*, *Clostridium*, *Bacillus* and *Sporolactobacillus*. In general, the DGGE band intensity of anodic bacteria decreased with increasing Cu^{2+} concentrations, except for bands assigned to *Firmicutes* and *Bacillus*, which increased with increasing Cu^{2+} concentrations. We suggest that the short-term electrical signals generated from MFCs with contaminated soil can be used to assess the toxic effect of heavy metal pollutants on soil microorganisms.

Introduction

A microbial fuel cell (MFC) is a device that converts chemical energy from organic compounds into electricity by using electrogenic bacteria as biocatalysts (Bond & Lovley, 2003). A dual chamber MFC usually consists of an anode chamber and a cathode chamber separated by a proton exchange membrane. In the anode chamber, electrogenic bacteria degrade organic compounds and transfer electrons to the anode. The electrons then flow through a conductor to the cathode, where they bind with an acceptor such as oxygen or ferricyanide and thus current is generated. Because the current is closely correlated with the metabolic activity of electrogenic bacteria, MFC-based biosensors have been developed to monitor the biological oxygen demand in wastewater (Di Lorenzo *et al.*, 2009), heavy metal toxicity (Stein *et al.*, 2010, 2011) and acid toxicity (Shen *et al.*, 2012). In MFC-based biosensors, a

culture of mixed electrogenic bacteria from other active MFCs (Stein *et al.*, 2010) or a pure culture of *Geobacter* or *Shewanella* have been used to generate a current with growth medium in the anode chamber (Dávila *et al.*, 2010). During the sensing process, wastewater or chemicals were added to the anode chamber after electrogenic bacteria enriched on anode and generated stable current (Shen *et al.*, 2013). The current decreased with increasing pollutant toxicity and increased with increasing BOD concentrations (Kim *et al.*, 2008).

It is known that soil can be used to generate electrical power in MFCs and a diversity of electrogenic bacteria have been detected. Ringelberg *et al.* (2011) found that a variety of soils had electrogenic activity and bacteria belonging to α - and β -*Proteobacteria* and *Firmicutes* dominated anodic biofilms in soil-based bio-electrochemical systems. Ishii *et al.* (2008) found that *Clostridiales*, *Chloroflexi*, *Rhizobiales* and *Methanobacterium* dominated the anode biofilm in MFCs inoculated with soil from a rice field. Kaku *et al.* (2008) operated plant-MFCs in which soil

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organic carbon and rice exudates served as energy resources, and found that *Natronocella acetinitrilica*, *Beijerinckiacae bacterium* and *Rhizobiales bacterium* were dominant on the anode. In recent studies, more than 30 isolates belonging to *Firmicutes* and α -, β -, γ - and δ -*Proteobacteria* were verified as electrogenic bacteria (da Rosa, 2011) and many more microorganisms detected on the anode by molecular methods were thought to have electrogenic activity. As electrogenic bacteria are widely distributed in soils and soil bacteria are sensitive to environmental changes, stress on soil microbial communities may inhibit electrogenic activity. For example, Deng *et al.* (2014) found that the voltage generated by a soil varied synchronously with incubation temperature. We hypothesize that the electrical signals produced by electrogenic bacteria in soil can be used to assess heavy metal toxicity in soil.

In the present study, we used soil with different amounts of Cu^{2+} added and subjected it to a series of measurements that included glucose dehydrogenase activity (DHA), substrate-induced respiration (SIR), microbial biomass carbon (C_{mic}) and the electrical signals of MFCs, including voltage and the quantity of electrons. The DHA, SIR and C_{mic} characteristics are sensitive to environmental changes and are conventional bioindicators of soil pollution (Wang *et al.*, 2007). In our study the monitoring process is different from that for wastewater. The pure culture electrogenic bacteria and medium are unnecessary. They increase cost and the medium, if it were to be discharged, may lead to pollution. Instead, we used the endogenous soil electrogenic bacteria and, because soil usually contains adequate nutrients, we only amended soil with glucose to minimize the 'start-up' time for power generation and to improve the voltage output (Kim *et al.*, 2000). A regression analysis was conducted to compare responses between the electrical signals, DHA, SIR and C_{mic} to Cu^{2+} toxicity. The effect of Cu^{2+} exposure on microbial community structure on the anode and in the soil was assessed by using DGGE, sequencing and phylogenetic analysis based on the 16S rRNA gene. The intent of this study was to develop a MFC-based method to detect heavy metal toxicity in soil microorganisms.

Materials and methods

Soil sampling and chemical analysis

Soil samples were collected in October 2012 at a depth of 0–20 cm from a broad-leaf forest at Nanjing Normal University, Nanjing City, China. The climate is sub-tropical and wet with an average annual precipitation of 1100 mm and a mean annual temperature of 15°C. After sampling, the soil was gently separated by hand to preserve soil structure, passed through a 2-mm mesh and thoroughly mixed. Part of the sieved soil was air-dried for physiochemical analysis. Soil texture was determined with the pipette method (Gee & Bauder, 1986). Total carbon and total nitrogen were measured with an elemental analyser (Vario EL III, Elementar, Hanau, Germany). Soil pH was measured at a soil:water ratio of 1:2.5 (w/v). Total Cu and total iron (Fe) in soil were determined using inductively coupled plasma (Prodigy, Leeman Labs Inc., Hudson, NY, USA). Soil electrical conductivity (EC) was determined with an electrical conductivity meter (DDSJ-308F, INESA Scientific Instrument Co.,

Ltd, Shanghai, China). The soil is categorized as an Alfisol (IUSS Working Group WRB, 2007). The soil physiochemical properties were as follows: soil texture, clay loam; total carbon, 18.1 mg g⁻¹; total nitrogen, 0.62 mg g⁻¹; soil pH, 7.57; total Cu, 41.1 µg g⁻¹; total Fe, 29.4 mg g⁻¹; EC, 76.6 µS cm⁻¹.

Copper stress experiment

The soil was divided into five aliquots, each with 600 g soil (dry mass). Thirty millilitres of CuCl_2 solution was added to each aliquot to yield 50, 100, 200 and 400 mg Cu^{2+} kg⁻¹ dry mass soil at a final soil moisture content of 18%. The remaining aliquot received 30 ml distilled water to serve as a control. The soil of each aliquot (600 g) was placed into a vial (1 litre) and incubated in the dark at 26°C for 24 hours. To help maintain the moisture content, a lid was placed loosely on each vial. After incubation, each soil aliquot was thoroughly mixed and equally divided into three sub-aliquots (200 g for each) as three replicates before the measurements of electrical signals, SIR, DHA and C_{mic} .

MFC set up

Fifteen dual chamber MFC reactors were constructed in oroglas®. An MFC reactor consisted of an anode chamber and a cathode chamber (each chamber with a dimension of 6 × 6 × 6 cm³ and a working volume of 170 ml) separated by a Nafion® cation exchange membrane (Figure 1). The anode and cathode were made of carbon felt with the same area of 16 cm² (4 × 4 cm²), fixed in parallel and separated by the cation exchange membrane. The distance between electrodes was 6 cm. The cathode chambers of the 15 MFC reactors were filled with 50 mM potassium ferricyanide (in 50 mM phosphate buffer solution (PBS)). The 15 reactors were randomly divided into five groups (three MFCs per group) according to Cu^{2+} concentration (control, 50, 100, 200 and 400 mg kg⁻¹). One hundred and fifty grams (dry mass) of contaminated or control soil was amended with 5% (w/w) glucose and packed into one anode chamber. Distilled water was added to the anode chamber to keep soil saturated. The anode and cathode were connected to a 1000 Ω external load by titanium wire. The 15 MFCs were operated at 26°C in a constant temperature incubator. The voltage was recorded with a data acquisition module every 10 minutes for 150 hours. To determine whether power originated from the microbial process or chemical processes, the voltage of an MFC reactor with uncontaminated soil sterilized by the chloroform fumigation method (Wolf *et al.*, 1989) was recorded.

Electrochemical activity

The electrochemical activity of soil from the anode chambers was examined by cyclic voltammetry (CV) following 150 hours of operation. Briefly, 50 g of Cu^{2+} -spiked soil from the MFC anode chamber and soil sterilized by CHCl_3 fumigation were collected and centrifuged at 1400 g for 10 minutes. The extracted soil solution was filtered through 0.45-µm pore size membrane and aerated with nitrogen gas for 10 minutes. Cyclic voltammetry

Figure 1 Diagram of MFC to detect Cu^{2+} stress on soil microorganisms. The anodic bacteria from soil degrade carbon substrate to produce electrons and protons. The electrons are transmitted to the cathode through the wire and reduce Fe(III) to Fe(II) in the cathode chamber. The produced voltage is recorded with a data acquisition module.

measurement of the extracted soil solution was conducted with a potentiostat (CHI 1040 C, Shanghai Chenhua, Shanghai, China) with a three-electrode configuration consisting of the working electrode (glassy carbon), a counter electrode (platinum wire) and an Ag/AgCl reference electrode. The scanned potential between -1 and $+1$ V (against standard hydrogen electrode (SHE)) was performed at a scan rate of 50 mV s^{-1} under quiescent conditions with a working volume of 5 ml.

DHA, SIR and C_{mic}

Values of DHA were determined according to Casida *et al.* (1964). Briefly, 1 g soil (dry mass equivalent) was combined with 2 ml 1% (w/v) 2, 3, 5-triphenyltetrazolium chloride (TTC) as a substrate. The triphenylformazane (TPF) produced was determined by spectrophotometry at 485 nm. Measurement of SIR was according to Lipson *et al.* (1999). Glucose was added to 10 g of soil (dry mass equivalent) in a 250-ml vial to give a final concentration of 30 mg g^{-1} dry soil. The vials were sealed and incubated for 12 hours at a constant 26°C . The CO_2 was trapped in 0.2 M NaOH solution in a 15-ml flask placed inside the 250-ml vial and determined by titration with 0.05 M HCl following the addition of 1 M BaCl_2 . C_{mic} was determined using the protocol described by Vance *et al.* (1987). Ten grams of soil (dry mass equivalent) were fumigated with ethanol-free CHCl_3 and extracted in 0.5 M K_2SO_4 . The organic carbon in the soil extract was measured with an automated TOC analyser (TOC-VCSN, Shimadzu, Kyoto, Japan).

DNA extraction and PCR

After the 150-hour operation of MFCs, a piece ($1.5 \times 1.5 \text{ cm}^2$) of the anode and 0.5 g of soil from the anode chamber were collected.

The anode was rinsed with sterilized deionized water to remove soil before DNA extraction (He *et al.*, 2009). The genomic DNA of the collected anode and soil was immediately extracted by using the Fast DNA SPIN kit for soil (BIO101, MP Biomedicals, Carlsbad, CA, USA) and following the manufacturer's instructions. The purity and the quantity of the DNA were determined by a nanodrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) at 230, 260 and 280 nm.

The bacterial 16S rRNA gene was amplified with the primer set 341f (5'-CCT ACG GGA GGC AGC AG-3') with a 40-bp GC clamp (5'-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC C-3') and 907r (5'-CCG TCA ATT CMT TTG AGT TT-3') (Muyzer *et al.*, 1993). The 50- μl reaction mixtures contained 1 μl template DNA, 1 μl of each 1 μM primer, 5 μl $10\times$ buffer (Mg^{2+} plus), 4 μl 10 mM dNTPs mixture (2.5 mM of each) and 2.5 units of Taq DNA polymerase (Sangon Biotech, Shanghai, China). Thermal cycling conditions were as follows: initial denaturation at 94°C for 4 minutes; 35 cycles consisting of denaturation at 94°C for 1 minute, primer annealing at 54°C for 30 s, and elongation at 72°C for 30 s.

DGGE, sequencing and phylogenetic analysis

The DGGE was performed with 8% polyacrylamide gel with a denaturing gradient of 40–80% (100% denaturant = 7 M urea, 40% (v/v) formamide) using the Bio-Rad Protean II System for 15 hours at a constant voltage of 100 V and a constant temperature of 60°C in $1\times$ TAE buffer. After electrophoresis, the gel was stained for 30 minutes with 1:10 000 dilution SYBRTM Green I nucleic acid gel stain (Invitrogen, Shanghai, China). The stained gel was immediately photographed under UV light with the Gel Doc XR+ gel imaging system and digitized with Quantity One

(version 4.4.0) software (Bio-Rad Laboratories, Hercules, CA, USA).

Eleven dominant bands were carefully excised from the DGGE gel and DNA was eluted by incubating each band in 40 µl sterilized distilled MilliQ water overnight at 4°C. Five microlitres of eluted DNA was used as a template for PCR amplification, using the same primers (341f/907r) and thermocycling programmes as described above. PCR products were cloned using the pEASY™-T3 Cloning Kit (TransGen Biotech, Beijing, China) according to the manufacturer's recommendations, and transformed into trans1-T1 phage-resistant chemically competent *E. coli* cells (TransGen). Transformed colonies were screened for inserts of the correct size by PCR amplification with the specific primers M13F (TGT AAA ACG ACG GCC AGT) and M13R (TCA CAC AGG AA ACA GCT ATG AC). Sequencing of correct colonies (five replicate colonies for each band) was carried out with vector primer set M13F/M13R (Invitrogen, Shanghai, China). Vector sequences were removed by using DNASTAR Lasergene 7.1. The gene sequences of the DGGE bands were subjected to taxonomic assignments with reference sequences using Blastn (<http://ncbi.nlm.nih.gov/blast>). Phylogenetic analysis was performed using a neighbour-joining algorithm and distance calculation by MEGA4. Sequences that were 97% or more identical were considered as a unique operational taxonomic unit (OTU). All the sequences determined in this study were deposited in the GenBank database and have been assigned accession numbers KF186478 to KF186493.

Statistical analysis

All statistical tests were performed using SPSS software (version 14.0). Significant differences between means ($n=3$) were determined using one-way ANOVA followed by the least significant difference test (LSD) at a level of $P < 0.05$. Exponential regression analysis was conducted to relate DHA, SIR and C_{mic} to the quantity of electrons present within 48 hours.

The quantity of electrons, Q (coulomb, C), produced was evaluated with the following formula:

$$Q = \sum_{n=1}^m \frac{(I_n + I_{n+1})}{2} \times 600, \quad (1)$$

$$I = U/R, \quad (2)$$

where U is the voltage (V), I is the current (A), R is the external loading 1000 Ω, n is the number of data recorded by a data acquisition module with a time interval of 600 s, and m is the total number of voltage data within 48 hours.

Results

The response of MFC voltage to Cu exposure

Plots of MFC voltage against operation time are depicted in Figure 2. It took approximately 8 days for MFCs without glucose

Figure 2 The voltage of MFCs ($n=3$) with Cu-spiked soil and control soil (without Cu addition). Data were collected every 10 minutes.

to reach 'start-up' and another 3 days to reach a peak voltage of 52 mV (data not shown). Glucose addition accelerated the start-up and increased the peak voltage. For the control MFC with 5% glucose, start-up occurred after 15 hours of operation and reached a peak voltage (354 mV) after 25 hours of operation (Table 1). The copper additions at 50 and 100 mg kg⁻¹ delayed the start-up, reduced the peak voltage and decreased the quantity of electrons produced significantly ($P < 0.05$). The MFC amended with 200 and 400 mg kg⁻¹ Cu²⁺ did not show a peak voltage; instead, the voltage of these treatments increased throughout the operation time. The MFC with fumigated soil had voltage below 1 mV during the 150 hours of operation, indicating that the electrical power was produced by soil microorganisms.

Values of DHA, SIR and C_{mic} decreased significantly with increasing Cu²⁺ concentrations (Table 1) and were significantly correlated with the quantity of electrons produced within 48 hours, as indicated by an exponential regression (Figure 3). The transition from a steep to a flat slope of the exponential curve occurred with the decreasing Cu²⁺ concentration and greater numbers of electrons produced.

Electrochemical activity

The electrochemical activity of the soil solution recovered from anode chambers was determined with cyclic voltammetry (Figure 4). The fumigation treatment did not show any electrochemical activity. With the increase of Cu²⁺ concentration, the reduction peaks at around -550 mV became less negative. A pair of symmetric oxidation and reduction peaks at around -50 and 50 mV were from Cu²⁺.

Sequencing and phylogenetic analysis of the DGGE pattern

The DGGE profiles of the bacterial 16S rRNA gene showed three distinct bands 4, 10 and 11, which were dominant in the anode samples rather than in the soil samples (Figure 5). Bands 4 and 11 were mainly detected in the 400 mg kg⁻¹ Cu²⁺ treatment while band 10 was mainly in the control treatment. The other bands

Table 1 Mean and standard error (in parentheses) of quantity of electrons and peak voltage for microbial fuel cells amended with 5% glucose, and summary of the analyses of variance

Cu addition / mg kg ⁻¹	Quantity of electrons in 48 hours / C	Peak voltage / mV	Dehydrogenase activity / mg TPF kg ⁻¹ soil day ⁻¹	Substrate-induced respiration / mg CO ₂ kg ⁻¹ soil hour ⁻¹	Microbial biomass carbon / mg kg ⁻¹ soil
0 (control)	32.5 (6.00)	353 (39.2)	90.2 (2.1)	4.03 (0.17)	261 (9.3)
50	11.7 (2.60)	271 (12.1)	52.8 (3.1)	3.85 (0.08)	280 (25.7)
100	7.7 (2.15)	189 (5.9)	43.6 (2.9)	3.15 (0.05)	217 (12.0)
200	2.0 (0.20)	–	29.2 (6.9)	2.89 (0.16)	195 (11.9)
400	1.3 (0.06)	–	16.6 (3.8)	2.36 (0.11)	193 (19.4)
LSD _{0.05}	9.7	75.6	12.9	0.38	52.8
<i>ANOVA results</i>					
Between treatments sum of squares	1954.2	40 180.2	9464.7	5.70	18 731.0
Within treatments sum of squares	282.7	10 364.8	505.7	0.44	8439.2
Between treatments mean squares	488.6	20 090.1	2366.2	1.43	4682.8
Within treatments mean squares	28.3	1727.5	50.6	0.04	843.9
F value	17.3	11.6	46.8	32.25	5.5
P value	< 0.001	0.009	< 0.001	< 0.001	0.013

LSD_{0.05}, least significant difference at $P < 0.05$.

were largely shared between the anode and soil. Unlike many other bands, the intensity of band 6 did not decrease with increasing Cu²⁺ amount. The phylogenetic tree for 16S rRNA gene sequences (Figure 6) from the anode samples only clustered bands 4 and 11 with *Firmicutes* and *Bacillus*, respectively. Band 10 was clustered with *Acetobacteraceae* and *Bacillaceae*. Bands 1, 2, 3, 5 and 6 were assigned to *Clostridium*. Bands 7, 8 and 9 were assigned to *Sporolactobacillus*.

Discussion

The electrical signals from soil MFCs

In MFCs, the electrogenic bacteria require time to enrich the anode to start-up and to produce power (Borole *et al.*, 2010). The addition of easy-to-degrade carbon sources, such as glucose and acetate, accelerates the start-up of MFCs and improves power output (Deng *et al.*, 2012). In our study, soil was mixed with 5% glucose (w/w) to accelerate start-up effectively and improve voltage. We noted that peak voltage did not increase and start-up was not accelerated with the addition of more glucose (8%, w/w) (data not shown). This could be because the current was saturated with 5% added glucose (Lee *et al.*, 2008), although we cannot discard the possibility that the organic acids produced from the fermentation of excess glucose inhibited microbial activity (Sundberg & Jonsson, 2005).

Cyclic voltammetry was used to identify the electrochemical activity of soil in the anode chamber in response to Cu²⁺ addition. The electrochemical activity decreased with increasing soil copper concentrations and was absent in the sterilized soil. This result indicated that increasing soil Cu²⁺ concentrations had a negative effect on the redox activity of the anode system, and that the measured electrochemical activity was related to soil microbial activity, either by electrogenic bacterial growth or by the presence of electrochemically active metabolic products.

Figure 3 (a) Exponential regressions ($n = 15$) of dehydrogenase activity (DHA), (b) substrate-induced respiration (SIR) and (c) microbial biomass carbon (C_{mic}) to the quantity of electrons produced within 48 hours of MFCs operation. The quantity of electrons produced is expressed in coulombs (C).

The reliability of the MFC-based method

The quantity of electrons produced within 48 hours decreased significantly with increasing soil Cu²⁺ concentrations. This result demonstrates the potential use for MFCs in assessing heavy metal

Figure 4 Cyclic voltammograms of soil solution from the MFC anode chamber with 50, 100, 200 and 400 mg Cu²⁺ kg⁻¹ (b–e). (a,f) The without-Cu control and fumigation treatment were set as references.

Figure 5 DGGE profiles of 16S rRNA gene fragments from the anode surface and anode chamber soil under different Cu²⁺ concentrations. The numbers 0–400 denote Cu²⁺ concentrations of 0 (control), 50, 100, 200 and 400 mg kg⁻¹. The numbers 1–11 represent the dominant DGGE bands, which were excised for sequencing and phylogenetic analysis.

toxicity in soil microorganisms. To confirm the agreement of the MFC-based method with traditional methods, SIR, DHA and C_{mic} were also studied. Values from DHA provide one of the most sensitive indicators for soil microbial activity (Goyal *et al.*, 2008).

The value of SIR largely depends on the metabolically active component of microbial biomass and is thus an indicator of the viable microbial community activity (Deng *et al.*, 2009). The C_{mic} is a measurement of the abundance of living microorganisms. Results of our study indicated that SIR, DHA and C_{mic} decreased with increasing amount of Cu²⁺, which is consistent with a large number of previous studies. Large concentrations of heavy metals can adversely affect soil microorganisms, inhibiting enzyme activities and thereby reducing overall microbial activity. However, C_{mic} measurements include active, inhibited and dormant cells: therefore it could be less sensitive to stress than DHA and SIR (Guo *et al.*, 2013). The exponential regressions used to fit DHA, C_{mic} and SIR in relation to coulombs measured suggest that the quantity of electrons produced decreased to a greater extent than did SIR and C_{mic} in control and 50 mg kg⁻¹ Cu²⁺. Exocellular electron transfer by electrogenic bacteria is carried out by membrane-bound electron transport proteins, such as c-type cytochromes (Logan, 2009). In contrast, bacterial metabolic activities are controlled largely by endoenzymes. Copper toxicity is mitigated by a series of internal cellular mechanisms (Silver & Phung, 1996). In addition, as a specific function, electrogenic activity is guaranteed by less redundant metabolic pathways or species than general functions of SIR or C_{mic} . Therefore, the measure of power generation was more sensitive to the smaller Cu²⁺ concentrations than either SIR or C_{mic} . These results indicate that electrical signals may be useful in identifying the presence of small amounts of pollution in soil. We also suggest that soil be tested under controlled conditions

Figure 6 Phylogenetic relationships between DGGE band sequences and 16S rRNA gene sequences retrieved from the GenBank database. The tree represents the alignment of a 525-bp region in 16S rRNA gene sequences. Genebank accession numbers are KF186478 to KF186493. The scale bar represents dissimilarity among sequences.

with constant temperature to prevent power generation fluctuations (Deng *et al.*, 2014).

The DNA-based analysis of anodic bacteria

Phylogenetic analysis of the 16S rRNA gene from the anode demonstrated the composition of anodic bacteria. Three bands distinct to the anode samples were assigned to *Firmicutes*, *Bacillus*, *Acetobacteraceae* and *Bacillaceae*. Previous research has assigned electrogenic bacteria to the *Proteobacteria* and *Firmicutes* phyla (Kim *et al.*, 2008) and within the *Firmicutes*, *Acetobacterium*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Lactobacillus*, *Lactococcus* and *Streptococcus* were identified. A number of anodic bacteria have

been isolated and their electrogenic activities confirmed (Park *et al.*, 2001; Chung & Okabe, 2009; Xing *et al.*, 2010; Wu *et al.*, 2013). *Bacillus* sp. transfer electrons to the anode mainly through soluble redox-active mediators (Nimje *et al.*, 2009). In a study of forest soil, Kappler *et al.* (2004) found that iron (Fe(III)/Fe(II)) and humic acids acted as electron mediators. *Acetobacterium* plays a role in current generation by producing acetate, one of the most favourable substrate for electrogenic bacteria, through glucose fermentation or homo-acetogenic processes (Ziv-El *et al.*, 2012). In addition to the three distinct bands from the anode samples, bands shared by both anode and soil were identified and assigned to *Clostridium* and *Sporolactobacillus*. *Sporolactobacillus* ferment glucose

and produce D lactate (Wang *et al.*, 2011), but an electrogenic activity has not been reported. *Clostridium* organisms are electrogenically active. The *Firmicutes*, *Clostridium* sp. and *Bacillus* sp. represented by bands 4, 6 and 11, respectively, are possibly Cu-resistant. The *Bacillus* sp. dominate bacterial communities in heavy metal-polluted soils (Ellis *et al.*, 2003). However, in spite of the presence of *Firmicutes*, *Bacillus* and *Clostridium* in 200 and 400 mg kg⁻¹ Cu²⁺-amended soil, the measured voltage was very small with a slow start-up. The Cu²⁺ exposure severely inhibited the electrogenic activity and the start-up of voltage probably resulted from adsorption of Cu²⁺ on soil, and resilience of microbial activity (Deng *et al.*, 2009).

Conclusions

Our study is the first to use the electrical signals of soil-based MFCs to monitor the effects of Cu²⁺ on soil microorganisms as far as we are aware. We conclude that the electrical signals of MFCs with glucose-amended soil can be used to evaluate the ecotoxicity of soil pollutants. The electrical signals produced are very sensitive to small stresses. Nevertheless, there are several limitations that should be overcome to facilitate application, including (i) minimizing the volume of MFC reactors and the amount of soil for a test, (ii) using oxygen as a sustainable electron acceptor rather than ferricyanide, and (iii) reducing the cost and improving the performance of electrodes and the cation exchange membrane. Further research is needed to validate the technique and to assess the reliability of the method with different stressors and with different soil types.

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