Environ Sci Pollut Res (2017) 24:23226–23235
DOI 10.1007/s11356-017-9959-4

RESEARCH ARTICLE

Microbial community composition and electricity generation in cattle manure slurry treatment using microbial fuel cells: effects of inoculum addition

Binghan Xie[1] · Weijia Gong[2] · An Ding[1] · Huarong Yu[1] · Fangshu Qu[1] · Xiaobin Tang[1] · Zhongsen Yan[1] · Guibai Li[1] · Heng Liang[1]

Received: 4 April 2017 / Accepted: 11 August 2017 / Published online: 22 August 2017

Abstract Microbial fuel cell (MFC) is a sustainable technology to treat cattle manure slurry (CMS) for converting chemical energy to bioelectricity. In this work, two types of allochthonous inoculum including activated sludge (AS) and domestic sewage (DS) were added into the MFC systems to enhance anode biofilm formation and electricity generation. Results indicated that MFCs (AS + CMS) obtained the maximum electricity output with voltage approaching 577 ± 7 mV (~ 196 h), followed by MFCs (DS + CMS) (520 ± 21 mV, ~ 236 h) and then MFCs with autochthonous inoculum (429 ± 62 mV, ~ 263.5 h). Though the raw cattle manure slurry (RCMS) could facilitate electricity production in MFCs, the addition of allochthonous inoculum (AS/DS) significantly reduced the startup time and enhanced the output voltage. Moreover, the maximum power (1.259 ± 0.015 W/m^2) and the highest COD removal (84.72 ± 0.48%) were obtained in MFCs (AS + CMS). With regard to microbial community, Illumina HiSeq of the 16S rRNA gene was employed in this work and the exoelectrogens (*Geobacter* and *Shewanella*) were identified as the dominant members on all anode biofilms in MFCs. For anode microbial diversity, the MFCs (AS + CMS) outperformed MFCs (DS + CMS) and MFCs (RCMS), allowing the occurrence of the fermentative (e.g., *Bacteroides*) and nitrogen fixation bacteria (e.g., *Azoarcus* and *Sterolibacterium*) which enabled the efficient degradation of the slurry. This study provided a feasible strategy to analyze the anode biofilm formation by adding allochthonous inoculum and some implications for quick startup of MFC reactors for CMS treatment.

Keywords Microbial fuel cell (MFC) · Cattle manure slurry (CMS) · Microbial community structure · Inoculum · Startup time

Highlights 1. Addition of allochthonous inocula (AS/DS) shortened the startup period in MFCs.
2. The MFCs with AS as allochthonous inocula exhibited the best performance.
3. Co-addition of AS enhanced the anode biofilm formation and diversity in MFCs.
4. The finding contributed to a quick startup of MFCs for CMS treatment.

Responsible editor: Diane Purchase

✉ Weijia Gong
gongweijia@126.com

✉ Heng Liang
hitliangheng@163.com

[1] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, 73 Huanghe Road, Nangang District, Harbin 150090, China

[2] School of Engineering, Northeast Agriculture University, 59 Mucai Street, Xiangfang District, Harbin 150030, China

Introduction

Considerable amounts of high-strength animal manure slurries are produced each year in China that require extensive treatment (Saidu et al. 2013). The slurries, which produced from the intensive livestock industry and the biogas engineering, impose great burden to the society. Meanwhile, the animal manure slurries without appropriate treatment may cause serious environmental pollution, such as water contamination, nutrients loss, odors, and deteriorated system performance (Fangueiro et al. 2015). Conventional methods for slurry disposal include anaerobic digestion, wetland construction, farmland utilization, and feed additives (Toumi et al. 2015). Although anaerobic digestion was intensively investigated for slurry treatment in previous studies (Alsouleman et al. 2016; Ledda et al. 2013; Masse and Saady 2015; Usack and

Angenent 2015), the complicated composition and high-concentration biogas slurry bring troubles to the subsequent treatment. In addition, the anaerobic digestion is often constricted by the detrimental acidic condition associated with accumulation of volatile fatty acids (Li et al. 2013). Therefore, new strategies should be put forward to enhance the effective utilization of slurries.

Microbial fuel cells (MFCs) are promising electrochemical devices which use bacteria as catalyst to generate electricity and simultaneously treat wastewater (Jia et al. 2013; Min et al. 2005). A variety of studies has been performed regarding treatment of animal manure slurries using bioelectrochemical systems (BES). Cerrillo et al. had compared the operation performance between microbial fuel cell (MFC) and microbial electrolysis cell (MEC) in treating raw and anaerobically digested pig slurries (Cerrillo et al. 2016). Ayyaru et al. obtained the maximum power density of 5.7 W/m^3 in a dairy wastewater treatment by MFCs (Ayyaru and Dharmalingam 2011). Furthermore, the cassette-electrode microbial fuel cells (CE-MFCs) were constructed to analyze the electricity generation from cattle manure suspended in water (Inoue et al. 2013). To date, effects of inocula on the power production by MFCs were investigated with different types of wastewater, such as palm oil mill effluent, synthetic wastewater, and acidic food waste leachate (Baranitharan et al. 2015; Commault et al. 2015; Li et al. 2013; Lin et al. 2013; Tanikkul and Pisutpaisal 2015). Meanwhile, Joline et al. compared the electricity generation and microbial community structure with different inocula (wastewater sludge and cattle manure) in the air-cathode MFCs (El-Chakhtoura et al. 2014). It is known that electricity can be generated from slurries itself using MFCs; however, the mechanism for slurry degradation in MFCs with specific inocula is still unclear.

Anodic biofilm enrichment and microbial selection are important for improving MFC performance. Various molecular biology techniques were investigated to understand the microbial community structure in MFC systems. The bacterial communities exhibited high diversity bacterial communities including α-, β-, γ- or δ-*Proteobacteria*, *Firmicutes* as well as other uncharacterized microorganism in MFCs (Kiely et al. 2011; Logan and Regan 2006). Microbial community composition in MFCs was affected by substrate types for treating synthetic wastewater and pig slurry (Sotres et al. 2016). It was demonstrated that the system transfer from MFC to MEC resulted in an enrichment of eubacterial and archaeal groups in treating pig slurry (Sotres et al. 2015). So far, the conventional molecular biology techniques such as DGGE and clone libraries of the 16S ribosomal RNA (rRNA) gene were limited with respect to incomplete throughput and low resolution (Mei et al. 2015). And in the MFC system, a rare microbial population often plays a critical role in an entire community. Moreover, few studies explored the mechanism of the allochthonous and autochthonous inoculum effects on the anode biofilm formation for the cattle manure slurry treatment in MFCs.

In this work, the effects between the allochthonous inoculum and the autochthonous inoculum present in the cattle manure slurry (CMS) on the electricity generation and CMS degradation were compared in MFCs: (i) CMS itself as MFCs feedstock and inoculum, (ii) CMS with activated sludge (AS) as the co-inocula, and (iii) CMS with domestic sewage (DS) as the co-inocula. Furthermore, the anode microbial communities were analyzed through the Illumina HiSeq technology of 16S rRNA gene. After that, the results of this systematic study were considered very useful as reference for improving the anodic biofilms diversities and power production in MFCs, while understanding the mechanism of slurries degradation by allochthonous inocula.

Materials and methods

Cattle manure slurry and inoculum

CMS (supernatant liquid) was collected from an anaerobic fermentation pool of a biogas project in Harbin. Two types of inocula, AS and DS, were added into MFCs as allochthonous inocula before initiating the MFCs. AS was collected in a secondary sedimentation tank of Wenchang Wastewater Treatment Plant in Harbin. DS was taken from a sewage pipe in the campus of the Harbin Institute of Technology. Subsequently, CMS and inocula were all filtrated by a 200-mesh sieve to remove large particles immediately and stored at 4 °C beforehand (Sun et al. 2015). Total COD concentrations of CMS, AS, and DS were 1500, 12,760 , and 456 mg/L, respectively. The MFCs were initially fed with substrate (CMS), inocula (CMS itself, CMS with AS, CMS with DS), mineral elements (12.5 mL/L), and trace elements (5 mL/L).

MFCs setup

Single-chamber air-cathode MFCs were constructed as previously described, which consist of cylindrical chamber with length of 4 cm and diameter of 3 cm (Cheng et al. 2011; Mei et al. 2015). Anode brushes were made of graphite fibers around a titanium core (2.5-cm length 2.5-cm outer diameter) and were heat treated at 450 °C for 30 min (Lu et al. 2012a, b). Cathodes (surface area = 7 cm^2) were made by the "rolling-press" method using activated carbon and PTFE (Dong et al. 2015). In order to form a closed loop, the cathode and anode electrodes were connected to a 1000 Ω external resistance using titanium wires.

Nine MFCs were randomly divided into three groups. The MFCs inoculated by allochthonous AS and DS were denoted as MFCs (AS + CMS)/MFCs (DS + CMS), respectively. The MFCs using autochthonous microorganism in the raw cattle manure slurry were named as MFCs (RCMS). The three groups of MFCs were respectively initiated with different inocula (5 mL/reactor) and then operated with CMS (chemical oxygen demand (COD) = 1500 mg/L) as the substrate, trace minerals (12.5 mL/

L), and a vitamin solution (5 mL/L) (Bond and Lovley 2003). All MFCs were started at room temperature (25 ± 5 °C) and were replenished fresh feedstock (COD = 1500 mg/L) while leaving 20% of the original medium before replacing fresh feedstock. The addition of inoculum was terminated as the output voltage exceeding 400 mV (~ 255 h) in MFC systems.

Calculations and electrochemical analysis

Electrochemical analysis was conducted after operating at least three stable cycles. Voltage was recorded by a multichannel data acquisition system connected to a personal computer via PCI interface (PISO-813U, Hongge Company, China). Power density curves were obtained via decreased external resistances from 5000 to 75 Ω. Current density (I_d, A/m^2) was calculated as $I_d = U/R \times S$, and power density (P_d, W/m^2) was calculated as $P_d = U^2/R \times S$, where U (V) is the voltage, R (Ω) is the resistor, and S (cm^2) is the normalized projected surface area of the cathode (7 cm^2) (Ren et al. 2014; Zhang et al. 2013). COD and coulombic efficiency (CE) were calculated as previously described (Cheng et al. 2015). Cyclic voltammetry (CV) was measured by an electrochemical workstation (Autolab PGSTAT 128N, Metrohm Autolab, Netherlands). A three-electrode included of working electrode (carbon brush anode), counter electrode (rolling-press cathode) and Hg/HgCl reference electrode was investigated. To make sure that the substrate was present at a significant concentration in solution, the medium in MFCs was refreshed before CV tests. CV tests were carried out with a scan rate of 10 mV/s, ranging from -0.8 to 0.8 V.

Fluorescence determination

A three-dimensional excitation emission matrix (EEM) fluorescence spectrum was applied to distinguish composition of the organic matter (Qu et al. 2012). Firstly, the samples were diluted and filtered through a 0.45-μm filter (Taoyuan, China) until ultraviolet absorbance at 254 nm (UV_{254}) of each sample is less than 0.3 cm^{-1}. The UV_{254} was determined using an UV/Vis spectrophotometer (T6, Puxi, China). Then, fluorescence spectra of feed water and effluents of MFCs were obtained using a fluorescence spectrophotometer (F7000, Hitachi, Japan). EEMs were obtained by scanning excitation wavelengths of 200–450 nm at an interval of 5 nm and emission wavelengths of 250–550 nm at an interval of 1 nm (Ding et al. 2014). Excitation and emission slit widths were both set at 5 nm. Photomultiplier tube (PMT) voltage at 700 V and scanning speed at 2400 nm/min were adopted (Yu et al. 2014).

DNA extraction, PCR amplification, and Illumina HiSeq

Samples were collected from the anode graphite fiber brushes in MFCs that were operated steadily for at least 60 days. The top, middle, and bottom fractions of graphite fiber brush were cut and fragmented by sterile scissors for DNA extraction (CTAB method). V4–V5 region (average length of ~ 373 bp) of the bacterial 16S rRNA gene was amplified using the universal primers 515F (5'-GTG CCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCCTTTAGTTT-3'), using TruSeq® PCR-Free DNA sample preparation kit, for building a kit for library construction, and using HiSeq 2500 PE250 to measure the sequence of anodic biofilms with Illumina HiSeq. The operational taxonomic unit (OTU) analysis was determined at 97% similarity levels using the UPARSE software (version 7.0.1001). Meanwhile, the principal component (PCA) analysis was based on OTUs and drawn by the QIIME software (version 1.7.0) and the R software (version 2.15.3).

Results and discussion

Electricity production and electrochemical analysis in MFCs by allochthonous inocula

Startup and electricity production

Figure 1a shows the output voltages after acclimation in MFCs. A prominent distinction was observed in output voltages due to adding allochthonous inocula. MFCs (AS + CMS) displayed the shortest startup time and the output voltage approached to 430 mV within 145.6 h (2 cycles), whereas the voltages of MFCs (DS + CMS) and MFCs (RCMS) were less than 350 mV at the second cycle. MFCs (AS + CMS) took only ~ 196 h (at the third cycle) to get the maximum power production. Cell voltages over subsequent cycles were then reproducible in terms of the maximum peak voltage, with an average of 577 ± 7 mV. By contrast, MFCs (DS + CMS) produced the first maximum peak voltage of 520 ± 21 mV at the third cycle (236 h). For MFCs (RCMS), a much longer startup time (~ 263.5 h) was demanded and the maximum peak voltage (429 ± 62 mV) was much less than those by MFCs with allochthonous inocula.

The maximum peak voltage obtained in MFCs (AS + CMS) with 577 ± 7 mV of MFCs in this study was much higher than that achieved in treating dairy wastewater (400 ± 15 mV) in a previous work (Ayyaru and Dharmalingam 2011). Though MFCs (RCMS) achieved an appreciable voltage, the output voltage could not be maintained in comparison with those by MFCs inoculated by AS or DS. The results indicated that allochthonous inocula played a positive role in shortening the startup period in MFCs. MFCs (AS + CMS) exhibited the maximum voltage production and the shortest startup time, implying that mixed culture from activated sludge demonstrated a higher vitality (Mei et al. 2015). Hongjian Lin et al. had demonstrated that activated sludge (AC) and anaerobic sludge (AN) could accelerate enrichment of MFC anodic biofilm by 2–3 days than river sediment (RS). Because the raw cattle manure slurry itself contained some organics that can be utilized by microorganism, an appreciable output voltage was achieved in MFCs (RCMS).

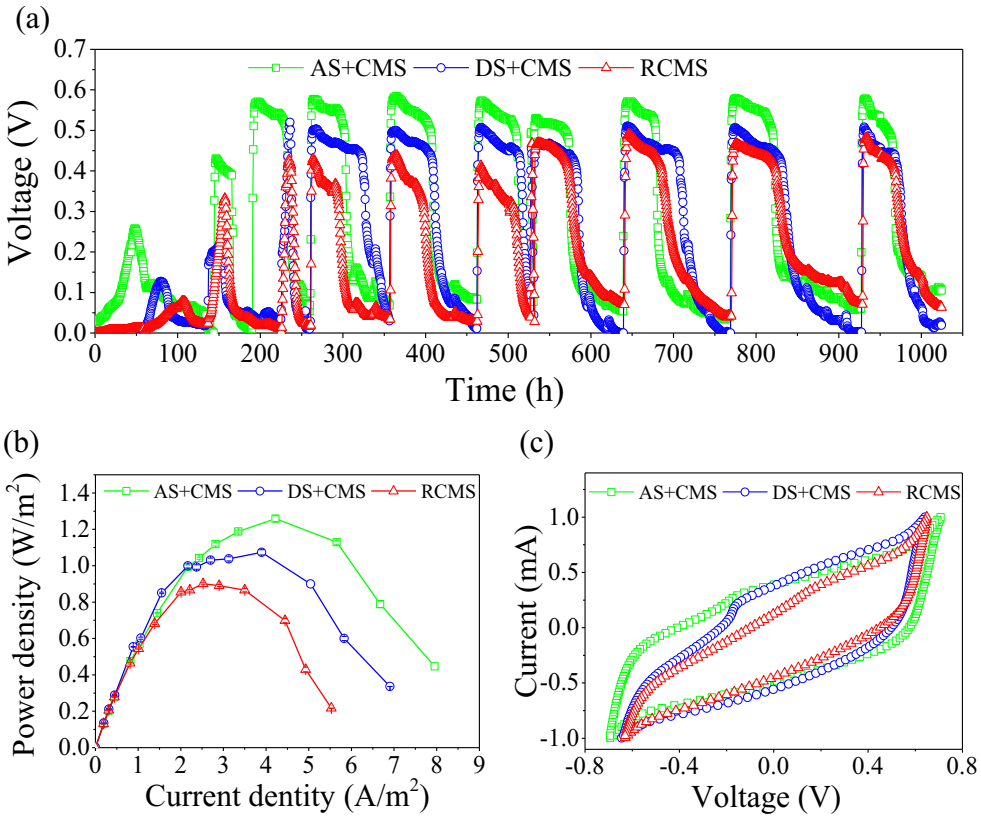

Fig. 1 **a** Voltage generation (external resistance R = 1000 Ω). **b** The power density. **c** Cyclic voltammetry (CV). (AS + CMS, DS + CMS, and RCMS were separately cattle manure slurry co-addition of activated sludge inoculum, domestic sewage inoculum, and the raw cattle manure slurry without inoculum)

However, compared with MFCs (RCMS), a much shorter startup time was demanded in MFCs with allochthonous inocula (AS or DS). The result was similar to a research that bioelectricity production from acidic food waste leachate was substantially affected by inoculum (Li et al. 2013).

Power generation

Figure 1b shows power density curves through decreasing the external circuit resistance (ranging from 5000 to 75 Ω) to evaluate MFC performance (Fig. 1b). When all MFC reactors produced reproducible current, MFCs (AS + CMS) could obtain the maximum stable power density with 1.259 ± 0.015 W/m^2. Meanwhile, the MFCs (DS + CMS) achieved the maximum power density with 1.073 ± 0.014 W/m^2 and MFCs (RCMS) got the maximum power density with 0.901 ± 0.005 W/m^2 at 100 Ω external resistances, respectively. More electron flow can be aggravated in MFCs at a low external resistance of which decreases the potential that can obtain higher power density (Mardanpour et al. 2012). Overall, MFCs (AS + CMS) obtained the maximum output power density.

Electrochemical analysis

As shown in Fig. 1c, MFCs (AS + CMS) displayed larger potential response (-0.7~0.7 V) than MFCs (DS + CMS) and MFCs (RCMS) of which the potential responses were both in the range from -0.65~0.65 V. The result indicates that using AS for inoculation resulted in a higher electrochemical activity. For MFCs (AS + CMS) and MFCs (DS + CMS), a peak current of 0.28 mA at -0.164 V and a peak current of 0.26 mA at -0.098 V in the oxidation scan were observed, respectively. Additionally, compared to the MFCs with allochthonous inoculum, the MFCs (RCMS) exhibited another peak intensity of oxidation with the value of 0.37 mA at 0.182 V. The results indicated that microbial communities were enriched on the anode of MFCs with AS or DS as co-inocula, significantly enhancing electron transfer efficiency from the bacteria to anode. By contrast, the raw cattle manure slurry showed minor impacts on the power output. Moreover, the biofilms on anode were necessary to enable oxidation reaction to occur, and AS facilitated electron transfer by achieving direct contact with the electrode.

Effects of allochthonous inocula on pollutant removal performance in MFCs

COD removal and coulombic efficiency

After the successful startup of MFCs, the data in three experimental periods were randomly chosen to calculate the COD removal and CE. As shown in Fig. 2, MFCs (AS + CMS) and MFCs (DS + CMS) showed a similar COD removal and outperformed MFCs (RCMS). The maximum COD removal was obtained in MFCs (AS + CMS) (84.72 ± 0.48%), followed by MFCs (DS + CMS)

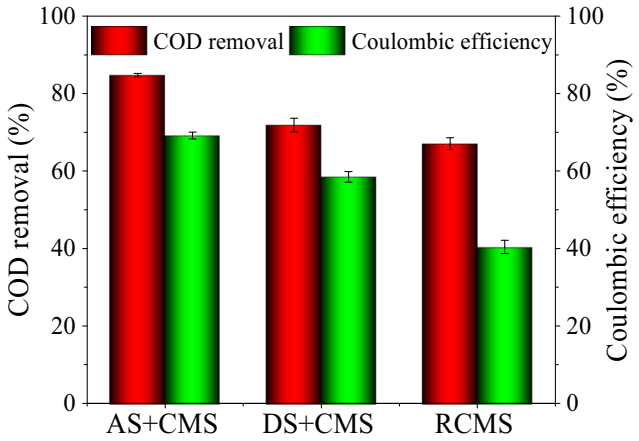

Fig. 2 The chemical oxygen demand (COD) removal and the coulombic efficiency (CE)

(71.6 ± 1.76%) and then MFCs (RCMS) (67.06 ± 0.52%). Furthermore, it can be observed that the maximum CE values were 69.15 ± 0.39, 58.49 ± 1.36, and 40.39 ± 0.7% in MFCs (AS + CMS), MFCs (DS + CMS), and MFCs (RCMS), respectively. The CE in this work was two to four times higher than that in a previous research (18 ± 1%). The probable reason is that it is easier to degrade the organics existing in the CMS with the allochthonous inocula and had higher CE than the MFCs with grass silage as a carbon source at the same COD (1500 mg/L) (Catal et al. 2011). Overall, MFCs (AS + CMS) achieved better performance in both COD removal and CE than MFCs (DS + CMS) and MFCs (RCMS). The results revealed that the addition of AS enhanced the electrochemical activity of bacteria on the anode biofilm, which improved the pollutant degradation.

EEM fluorescence spectra analysis

The three-dimensional EEM fluorescence spectra for influents and effluents of the MFCs are illustrated in Fig. 3. Two peaks were obviously observed (peak T1 and peak T2) in the fluorescent spectra. Peak T1, which is associated with proteins (tyrosine like) (Barker and Stuckey 1999; Chen et al. 2003), occurs at 225–230 nm/325–340 nm of the excitation/emission wavelengths, respectively. Peak T2 appeared at the excitation/emission wavelengths of 275–280/320–330 nm that is related to the tryptophan-like (Barker and Stuckey 1999; Chen et al. 2003). As shown in Fig. 3a, the strong intensities at peak T1 and T2 were observed, suggesting high concentrations of macromolecular organics in

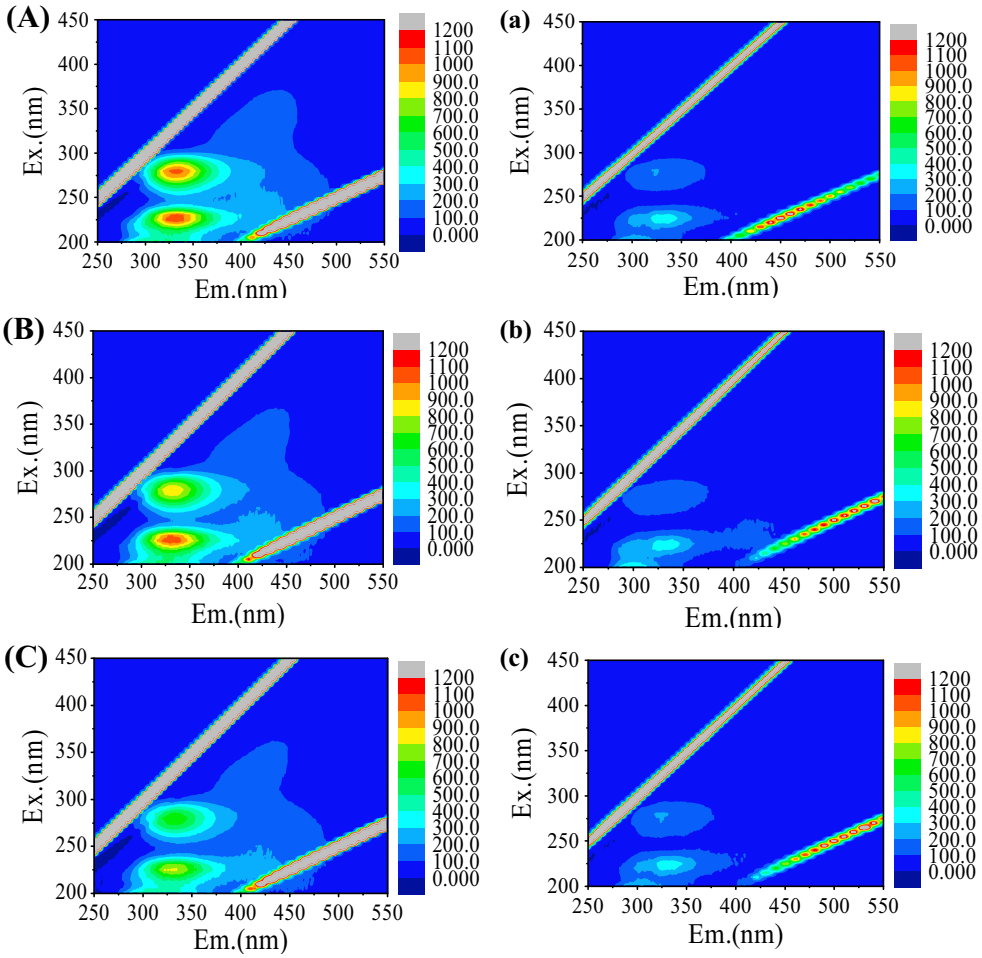

Fig. 3 The EEM fluorescence spectra in MFCs (A, B, and C are respectively the influent; a, b, and c are separately the effluent)

Table 1 Fluorescence spectral parameters of influent and effluent in MFCs by co-addition of inoculum

Sample	Peak T1 (influent)		Peak T1 (effluent)		Peak T2 (influent)		Peak T2 (effluent)	
	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity
AS + CMS	225/330	1080.92	225/339	326.78	280/335	1037.27	275/323	202.06
DS + CMS	225/329	1091.17	225/336	363.05	280/336	867.63	–	–
RCMS	225/334	821.91	225/327	354.76	280/331	667.16	280/326	210.43

the influents of MFCs (AS + CMS). Meanwhile, as shown in Fig. 3a, no peak T1 was observed in the effluents of MFCs (AS + CMS), indicating that most of the proteins (tryptophan like) were removed. Hence, the allochthonous inocula (AS/DS) addition could help to degrade the macromolecular pollutants (like protein substances) in the electrochemical systems. As seen in Table 1, the peaks in the influent and effluent of MFCs by co-inocula were observed. Regarding the fluorescence peak intensity, the values of peak T1 and peak T2 of the influents were nearly three times higher than that of the effluents. Moreover, the fluorescent intensities of peak T1 and T2 in the effluent of MFCs (AS + CMS) and MFCs (DS + CMS) were both lower than those of MFC (RCMS) (Table 1). This result is well consistent with the COD removal in the electrochemical systems (Fig. 2) obtained by MFCs (AS + CMS). The EEM results indicated that the addition of allochthonous inocula, especially AS, could strengthen the degradation of organic pollutants during CMS treatment using MFCs. This may be attributed to the improvement in anode biofilm activity and diversity induced by allochthonous inocula (AS and DS). Moreover, the syntrophic interactions between exoelectrogens and fermentative bacteria obviously improved the degradation of macromolecular organics in the MFC systems (Lu et al. 2015).

Effects of allochthonous inocula on microbial communities in MFCs

Bacterial diversity of the anode biofilms

The Illumina HiSeq of 16S rRNA gene was employed to investigate the anode microbial communities in MFCs. The phylotypes in communities were classified in terms of OTUs. Over 40,000 effective sequences were obtained for each sample based on a threshold of 97%. Besides, the alpha diversity indexes, such as Shannon's diversity index, Simpson diversity index, Chao 1 richness index, and abundance-based coverage estimator (ACE) index, were summarized in Table 2. Based on the Chao 1 and ACE index, it can be noted that MFCs (AS + CMS) showed relatively higher diversity with Chao 1 and ACE of 975.94 and 1001.47, respectively. In terms of the Shannon and Simpson index, MFCs (DS + CMS) (4.92 and 0.86, respectively) displayed a better abundance than MFCs (AS + CMS) (3.83 and 0.75, respectively). It was notable that the raw CMS showed relatively higher biodiversity than all samples (Table 2). All the coverage values among the four samples varied from 0.95 to 0.97, indicating that the sequencing results were reliable to reflect characteristics of the microbial communities in the MFCs.

A Venn diagram was generated to determine the number of shared and unique OTUs among the different MFCs (Fig. 4). The total numbers of observed OTUs in the three samples were 1180, and 39.3% (464 OTUs) of the total OTUs were shared among them (Fig. 4). Lu et al. had demonstrated that the shared OTUs included some electrochemical active bacteria that affected the electricity production in MFC systems (Lu et al. 2015). Furthermore, the initial inoculum played an important in the relative abundance of unique OTUs on the different anode biofilms. MFCs (AS + CMS) accounted for 13.4% unique OTUs, which was higher than those in MFCs (DS + CMS) (1.3%) and MFCs (RCMS) (5.4%). Moreover, the existence of a variety of unique OTUs indicated that the addition of allochthonous inocula exhibited significant influence on the bacterial community structures and proportions.

Comparative analysis of the bacterial microbial community

Weighted UniFrac analysis for huge datasets was employed to identify the differences in bacterial community structures based on the principal component analysis (PCA), which

Table 2 Number of reads, operational taxonomic units (OTUs), abundance-based Shannon, Simpson, Chao1, and coverage estimator (ACE) were obtained from MFCs by co-addition of inoculum based on a threshold of 97%

Sample	Effective reads	Observed species	Shannon	Simpson	Chao 1	ACE	Good coverage
AS + CMS	48,145	839	3.833	0.751	975.935	1001.471	0.994
DS + CMS	41,966	837	4.918	0.855	928.415	950.271	0.995
RCMS	41,193	752	4.176	0.756	847.639	862.457	0.995
Control	45,727	1271	7.085	0.947	1344.845	1355.205	0.995

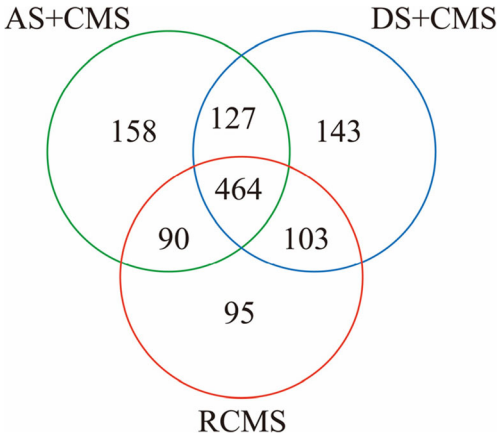

Fig. 4 Venn diagram showing shared OTUs between the different MFCs

explained by PC1 (10.12%) and PC2 (88.78%). Compared with AS, the composition of DS contained a smaller amount of microorganisms, which made MFCs (DS + CMS) and MFCs (RCMS) closely clustered and revealed a higher similarity in the microbial communities composition (Fig. 5). However, the group of the control (raw CMS) was obviously different from the samples in all MFCs. Overall, the anode microbial community structure composition was impacted by the allochthonous inocula in the electrochemical system.

Community compositions of the anode biofilms

To identify the phylogenetic diversity, qualified sequences were assigned to phyla, classes, and genera (Fig. 6). At the level of phylum, all OTUs belonged to some mainly core phyla, such as *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Chloroflexi*. In all anode biofilms, *Proteobacteria* was the most abundant phyla, MFCs (AS + CMS) accounted for 88.53%, followed by MFCs (RCMS) (85.66%), MFCs (DS + CMS) (80.87%), and the control (23.76%) (Fig. 6a). *Bacteroidetes* had relative abundances from 4.35 to 34.69% in all anode biofilms, and the control sample had the highest

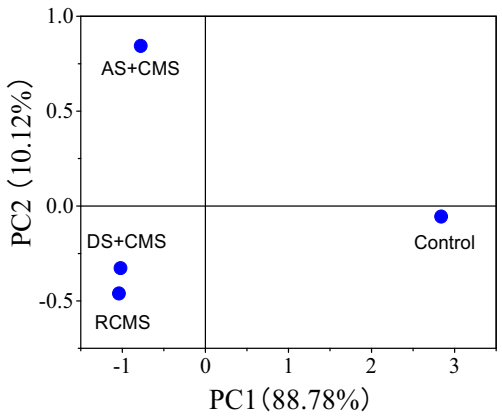

Fig. 5 The principal component analysis (PCA) in MFCs by co-addition of inoculum

proportion of *Firmicutes* (34.69%). *Firmicutes*, which belonged to obligate or facultative aerobes, were the major component in MFCs (Jung and Regan 2007). Besides, *Firmicutes* were sensitive to the electron donor types and enriched with glucose and propionate fed (Chae et al. 2009; Pasternak et al. 2016).

Regarding the class level, most of the sequences were affiliated to nine classes (Fig. 6b). The predominant classes in MFCs (AS + CMS), MFCs (DS + CMS), and MFCs (RCMS) were affiliated with *Betaproteobacteria*, *Deltaproteobacteria*, *Bacteroidetes*, and *Clostridia*, followed by a small proportion of *Gammaproteobacteria*, *Alphaproteobacteria*, *Epsilonproteobacteria*, *Anaerolinea*, and *Synergistia*. *Betaproteobacteria* was the most dominant class in the four samples, and *Deltaproteobacteria* was the second dominant class enriched in all MFCs with relative abundance increasing from 7.14% in raw water to 58.75% in MFCs (AS + CMS). The class of *Bacteroidia* accounted for the relative high abundance in the phyla of *Bacteroidetes*, while *Actinobacteria* was the dominant class in the phyla of *Actinobacteria*.

A more in-depth characterization of the microbial communities was performed at the genus level. Fifteen genera with relative abundance > 1% in the four microbial communities are shown in Fig. 6c. Regarding microbial population, *Geobacter* obviously dominated in all samples (relative abundance varying from 2.192 to 51.66%), well consistent with the results in relevant works (Bonmati et al. 2013; Dennis et al. 2013). MFCs (AS + CMS) had the largest proportion of *Geobacter* (51.66%), in comparison with MFCs (DS + CMS) (34.64%), MFCs (RCMS) (21.96%), and the controlled sample (2.192%). *Shewanella*, which belonged to exoelectrogens, was not observed in the controlled sample, and MFCs (DS + CMS) showed the highest relative abundance of *Shewanella* (5.8%), followed by MFCs (AS + CMS) (1.58%) and MFCs (RCMS) (1.32%). By contrast, *Desulfuromonas* (2.79%), whose members included exoelectrogenic bacteria, was enriched in MFC (DS + CMS).

Though there were some share populations on the anode biofilms, the bacterial growth and activity were greatly influenced by the allochthonous inocula. AS contained diverse organic matters (e.g., carbohydrate, protein, and lipid) that contributed to the rich diversity of the anode microbial community (Ghadge et al. 2015). In the MFC groups with allochthonous inocula, *Geobacter* took the largest proportions among all genera and played a key role in the power generation. *Bacteroides* belonged to the fermentative bacteria which were demonstrated to obviously hydrolyze complex organics (Rismani-Yazdi et al. 2013). *Azoarcus* and *Sterolibacterium*, which were the nitrogen-fixating bacteria (Scherr et al. 2016; Yu et al. 2016), occurred in both MFCs (DS + CMS) (5.95%) and raw cattle manure slurry (3.73%), respectively. Therefore, the macromolecular organics can be effectively degraded in the electrochemical systems. *Smithella* was the predominant

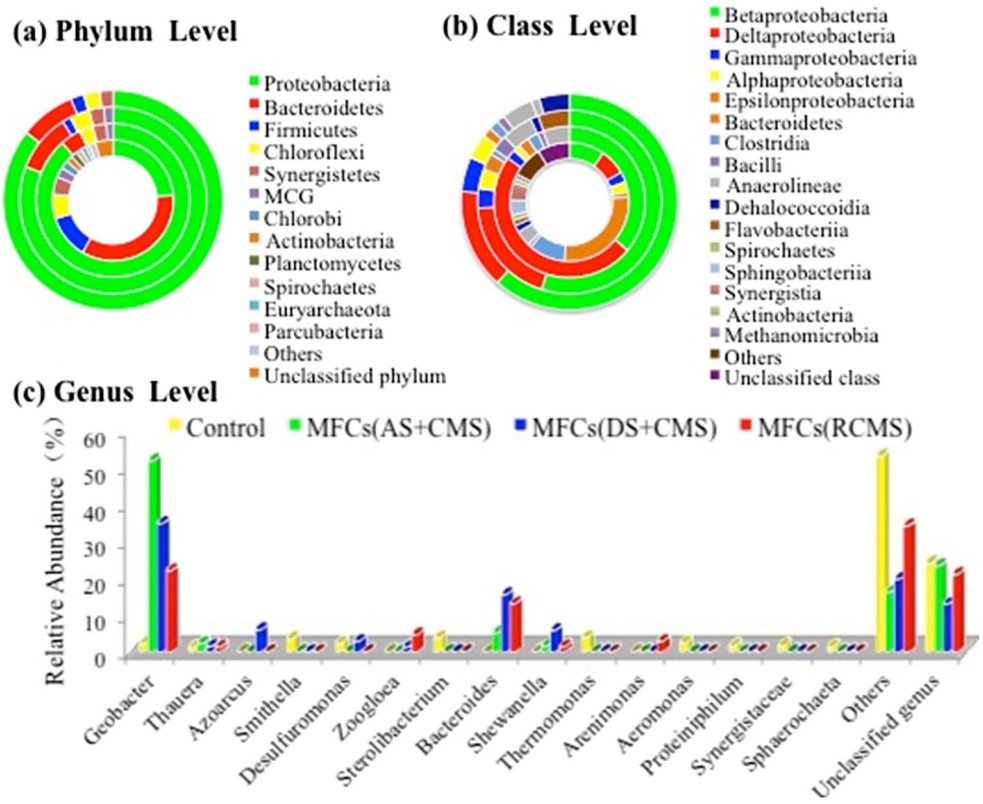

Fig. 6 Microbial community composition on the anode biofilms in MFCs by co-addition of inoculum based on Illumina HiSeq at **a** the phylum level, **b** class level, and (**c**) the genus level. Phyla, classes, and genera with relative abundance lower than 1% were classified into group "Others." In **a**, **b**, the inner circle represents the control followed by MFCs (AS + CMS), MFCs (DS + CMS), and MFCs (RCMS)

propionic acid oxidizing bacteria (POB) (Kim et al. 2015; Xiao et al. 2015), only existing in raw cattle manure. Moreover, the electrochemical active bacteria were selectively enriched by electric field and built the anode biofilms for continuously generating electrons to support cathode reaction (Cui et al. 2016). MFCs (RCMS) contained only the raw cattle manure slurry that might also serve as an inoculum for microbe colonizing on the cathodes. The reason is that anode biofilms could produce mediators that affected microorganism growth and electrochemical activity. These genera were enriched in the anode biofilms first and then migrated to the cathodes (Cui et al. 2016; Velvizhi and Mohan 2015). Moreover, the existence of unique microorganisms had lead to the discrepancy on anode biofilms formation in MFC systems. In addition, the syntrophic interactions between exoelectrogenic (e.g., *Geobacter* and *Shewanella*) and fermentative bacteria (e.g., *Bacteroides*) effectively enhanced the electricity generation and the CMS degradation in MFCs.

Conclusion

In this study, the additions of allochthonous inocula (AS/DS) on the CMS degradation and anode biofilm formation were evaluated in MFCs. Though CMS itself could serve as an inoculum for electricity generation, the addition of AS obtained the shortest startup time and the best pollutant removal performance in the electrochemical system. Moreover, adding allochthonous inocula to MFCs significantly enhanced the anode microbial community composition activity and diversity, which facilitated the favorable electricity generation and slurry degradation. In general, this work provided a method for quick anodic biofilm enrichment during cattle manure slurry treatment using MFCs and better understanding on the contribution of allochthonous inocula.

Acknowledgements The authors would like to specially thank the supports by the Scientific and Research Foundation for Returned Scholars of Heilongjiang Province (LC2016015), the Young Talents Project of Northeast Agricultural University (14QC35), the Specialized Fund Project for Harbin Innovative Talent in Science and Technology Research (2013RFQXJ094), and the HIT Environment and Ecology Innovation Special Funds (HSCJ201603)

References

Alsouleman K, Linke B, Klang J, Klocke M, Krakat N, Theuerl S (2016) Reorganisation of a mesophilic biogas microbiome as response to a stepwise increase of ammonium nitrogen induced by poultry manure supply. Bioresour Technol 208:200–204

Ayyaru S, Dharmalingam S (2011) Development of MFC using sulphonated polyether ether ketone (SPEEK) membrane for electricity generation from waste water. Bioresour Technol 102:11167–11171

Baranitharan E, Khan MR, Yousuf A, Teo WFA, Tan GYA, Cheng CK (2015) Enhanced power generation using controlled inoculum from palm oil mill effluent fed microbial fuel cell. Fuel 143:72–79

Barker DJ, Stuckey DC (1999) A review of soluble microbial products (SMP) in wastewater treatment systems. Water Res 33:3063–3082

Bond DR, Lovley DR (2003) Electricity production by Geobacter sulfurreducens attached to electrodes. Appl Environ Microbiol 69: 1548–1555

Bonmati A, Sotres A, Mu Y, Rozendal R, Rabaey K (2013) Oxalate degradation in a bioelectrochemical system: reactor performance and microbial community characterization. Bioresour Technol 143: 147–153

Catal T, Cysneiros D, O'Flaherty V, Leech D (2011) Electricity generation in single-chamber microbial fuel cells using a carbon source sampled from anaerobic reactors utilizing grass silage. Bioresour Technol 102:404–410

Cerrillo M, Oliveras J, Vinas M, Bonmati A (2016) Comparative assessment of raw and digested pig slurry treatment in bioelectrochemical systems. Bioelectrochemistry 110:69–78

Chae KJ, Choi MJ, Lee JW, Kim KY, Kim IS (2009) Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. Bioresour Technol 100:3518–3525

Chen W, Westerhoff P, ‡, JAL, §, Booksh‖ K (2003) Fluorescence excitation–emission matrix regional integration to quantify spectra for dissolved organic matter. Environ Sci Technol 37:5701–5710

Cheng S, Xing D, Logan BE (2011) Electricity generation of single-chamber microbial fuel cells at low temperatures. Biosens Bioelectron 26:1913–1917

Cheng HY, Liang B, Mu Y, Cui MH, Li K, Wu WM, Wang AJ (2015) Stimulation of oxygen to bioanode for energy recovery from recalcitrant organic matter aniline in microbial fuel cells (MFCs). Water Res 81:72–83

Commault AS, Barriere F, Lapinsonniere L, Lear G, Bouvier S, Weld RJ (2015) Influence of inoculum and anode surface properties on the selection of Geobacter-dominated biofilms. Bioresour Technol 195: 265–272

Cui M-H, Cui D, Gao L, Cheng H-Y, Wang A-J (2016) Analysis of electrode microbial communities in an up-flow bioelectrochemical system treating azo dye wastewater. Electrochim Acta 220:252–257

Dennis PG, Guo K, Imelfort M, Jensen P, Tyson GW, Rabaey K (2013) Spatial uniformity of microbial diversity in a continuous bioelectrochemical system. Bioresour Technol 129:599–605

Ding A, Liang H, Qu F, Bai L, Li G, Ngo HH, Guo W (2014) Effect of granular activated carbon addition on the effluent properties and fouling potentials of membrane-coupled expanded granular sludge bed process. Bioresour Technol 171:240–246

Dong Y, Qu Y, He W, Du Y, Liu J, Han X, Feng Y (2015) A 90-liter stackable baffled microbial fuel cell for brewery wastewater treatment based on energy self-sufficient mode. Bioresour Technol 195: 66–72

El-Chakhtoura J, El-Fadel M, Rao HA, Li D, Ghanimeh S, Saikaly PE (2014) Electricity generation and microbial community structure of air-cathode microbial fuel cells powered with the organic fraction of municipal solid waste and inoculated with different seeds. Biomass Bioenergy 67:24–31

Fangueiro D, Hjorth M, Gioelli F (2015) Acidification of animal slurry—a review. J Environ Manag 149:46–56

Ghadge AN, Jadhav DA, Pradhan H, Ghangrekar MM (2015) Enhancing waste activated sludge digestion and power production using hypochlorite as catholyte in clayware microbial fuel cell. Bioresour Technol 182:225–231

Inoue K, Ito T, Kawano Y, Iguchi A, Miyahara M, Suzuki Y, Watanabe K (2013) Electricity generation from cattle manure slurry by cassette-electrode microbial fuel cells. J Biosci Bioeng 116:610–615

Jia J, Tang Y, Liu B, Wu D, Ren N, Xing D (2013) Electricity generation from food wastes and microbial community structure in microbial fuel cells. Bioresour Technol 144:94–99

Jung S, Regan JM (2007) Comparison of anode bacterial communities and performance in microbial fuel cells with different electron donors. Appl Microbiol Biotechnol 77:393–402

Kiely PD, Cusick R, Call DF, Selembo PA, Regan JM, Logan BE (2011) Anode microbial communities produced by changing from microbial fuel cell to microbial electrolysis cell operation using two different wastewaters. Bioresour Technol 102:388–394

Kim W, Shin SG, Han G, Cho K, Hwang S (2015) Structures of microbial communities found in anaerobic batch runs that produce methane from propionic acid—seeded from full-scale anaerobic digesters above a certain threshold. J Biotechnol 214:192–198

Ledda C, Schievano A, Salati S, Adani F (2013) Nitrogen and water recovery from animal slurries by a new integrated ultrafiltration, reverse osmosis and cold stripping process: a case study. Water Res 47:6157–6166

Li XM, Cheng KY, Selvam A, Wong JW (2013) Bioelectricity production from acidic food waste leachate using microbial fuel cells: effect of microbial inocula. Process Biochem 48:283–288

Lin H, Wu X, Miller C, Zhu J (2013) Improved performance of microbial fuel cells enriched with natural microbial inocula and treated by electrical current. Biomass Bioenergy 54:170–180

Logan BE, Regan JM (2006) Electricity-producing bacterial communities in microbial fuel cells. Trends Microbiol 14:512–518

Lu L, Xing D, Ren N (2012a) Bioreactor performance and quantitative analysis of methanogenic and bacterial community dynamics in microbial electrolysis cells during large temperature fluctuations. Environ Sci Technol 46:6874–6881

Lu L, Xing D, Ren N (2012b) Pyrosequencing reveals highly diverse microbial communities in microbial electrolysis cells involved in enhanced H2 production from waste activated sludge. Water Res 46:2425–2434

Lu L, Xing D, Ren ZJ (2015) Microbial community structure accompanied with electricity production in a constructed wetland plant microbial fuel cell. Bioresour Technol 195:115–121

Mardanpour MM, Nasr Esfahany M, Behzad T, Sedaqatvand R (2012) Single chamber microbial fuel cell with spiral anode for dairy wastewater treatment. Biosens Bioelectron 38:264–269

Masse DI, Saady NM (2015) High rate psychrophilic anaerobic digestion of undiluted dairy cow feces. Bioresour Technol 187:128–135

Mei X, Guo C, Liu B, Tang Y, Xing D (2015) Shaping of bacterial community structure in microbial fuel cells by different inocula. RSC Adv 5:78136–78141

Min B, Kim J, Oh S, Regan JM, Logan BE (2005) Electricity generation from swine wastewater using microbial fuel cells. Water Res 39: 4961–4968

Pasternak G, Greenman J, Ieropoulos I (2016) Regeneration of the power performance of cathodes affected by biofouling. Appl Energy 173: 431–437

Qu F, Liang H, He J, Ma J, Wang Z, Yu H, Li G (2012) Characterization of dissolved extracellular organic matter (dEOM) and bound extracellular organic matter (bEOM) of Microcystis aeruginosa and their impacts on UF membrane fouling. Water Res 46:2881–2890

Ren L, Ahn Y, Logan BE (2014) A two-stage microbial fuel cell and anaerobic fluidized bed membrane bioreactor (MFC-AFMBR) system for effective domestic wastewater treatment. Environ Sci Technol 48:4199–4206

Rismani-Yazdi H, Carver SM, Christy AD, Yu Z, Bibby K, Peccia J, Tuovinen OH (2013) Suppression of methanogenesis in cellulose-fed microbial fuel cells in relation to performance, metabolite formation, and microbial population. Bioresour Technol 129:281–288

Saidu M, Yuzir A, Salim MR, Salmiati AS, Abdullah N (2013) Influence of palm oil mill effluent as inoculum on anaerobic digestion of cattle manure for biogas production. Bioresour Technol 141:174–176

Scherr KE, Backes D, Scarlett AG, Lantschbauer W, Nahold M (2016) Biogeochemical gradients above a coal tar DNAPL. Sci Total Environ 563-564:741–754

Sotres A, Cerrillo M, Vinas M, Bonmati A (2015) Nitrogen recovery from pig slurry in a two-chambered bioelectrochemical system. Bioresour Technol 194:373–382

Sotres A, Tey L, Bonmati A, Vinas M (2016) Microbial community dynamics in continuous microbial fuel cells fed with synthetic wastewater and pig slurry. Bioelectrochemistry 111:70–82

Sun R, Zhou A, Jia J, Liang Q, Liu Q, Xing D, Ren N (2015) Characterization of methane production and microbial community shifts during waste activated sludge degradation in microbial electrolysis cells. Bioresour Technol 175:68–74

Tanikkul P, Pisutpaisal N (2015) Influence of inoculum pretreatment on the performance of an air-cathode single-chamber microbial fuel cell. Energy Procedia 79:641–645

Toumi J, Miladi B, Farhat A, Nouira S, Hamdi M, Gtari M, Bouallagui H (2015) Microbial ecology overview during anaerobic codigestion of dairy wastewater and cattle manure and use in agriculture of obtained bio-fertilisers. Bioresour Technol 198:141–149

Usack JG, Angenent LT (2015) Comparing the inhibitory thresholds of dairy manure co-digesters after prolonged acclimation periods: part 1—performance and operating limits. Water Res 87:446–457

Velvizhi G, Mohan SV (2015) Bioelectrogenic role of anoxic microbial anode in the treatment of chemical wastewater: microbial dynamics with bioelectro-characterization. Water Res 70:52–63

Xiao K, Zhou Y, Guo C, Maspolim Y, Ng WJ (2015) Dynamics of propionic acid degradation in a two-phase anaerobic system. Chemosphere 140:47–53

Yu H, Qu F, Liang H, Z-s H, Ma J, Shao S, Chang H, Li G (2014) Understanding ultrafiltration membrane fouling by extracellular organic matter of Microcystis aeruginosa using fluorescence excitation–emission matrix coupled with parallel factor analysis. Desalination 337:67–75

Yu J, Fan C, Zhong J, Zhang Y, Wang C, Zhang L (2016) Evaluation of in situ simulated dredging to reduce internal nitrogen flux across the sediment-water interface in Lake Taihu, China. Environ Pollut 214: 866–877

Zhang J, Zheng P, Zhang M, Chen H, Chen T, Xie Z, Cai J, Abbas G (2013) Kinetics of substrate degradation and electricity generation in anodic denitrification microbial fuel cell (AD-MFC). Bioresour Technol 149:44–50