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Shaping of Bacterial Community Structure in Microbial Fuel Cells by Different Inocula

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Abstract

To understand shaping community structure of the anode biofilms is important for enhancing performance of microbial fuel cell (MFC). The activated sludge (AS), garden soil (GS), wastewater (WW) and river sediment (RS) were inoculated into single-chamber MFCs to assess the effects of inocula on the power outputs and microbial communities of MFCs. MFCs with different initial inocula showed the differences in acclimation time and power densities. MFC-RS (river sediment inoculum) obtained a maximum power density (744.8mW/m^2), followed by MFC-AS, MFC-GS and MFC-WW. Illumina Miseq sequencing of 16S rRNA gene and comparative analyses indicated that microbial community structure of established anode biofilms obviously differentiated from that in initial inocula. The principal component analysis (PCA) proved that MFC-AS and MFC-GS were closely clustered and were separated from MFC-WW and MFC-RS. The majority of dominant populations of MFC-RS was affiliated with *Azoarcus* (45.20%). The most dominant

genus belonged to *Flavobacterium* (14.18%) in MFC-AS, *Geobacter* (14.40%) in MFC-GS and *Azovibrio* (11.11%) in MFC-WW, respectively. This study implies that different inocula influenced substantially shaping community structure of the anode biofilms of MFCs.

Keywords: Microbial fuel cells; Inoculum; Microbial community structure; Exoelectrogen; High throughput sequencing

1. Introduction

The microbial fuel cell (MFC) is a promising technology due to simultaneously pollutant removal and energy production from wastewater or solid waste [1]. Exoelectrogenic microorganisms oxidize organic matters and transfer extracellularly electron to the electrode, this environmentally-friendly process produces electricity without the combustion of fossil fuels [2, 3]. Understanding electron transfer of exoelectrogenic microorganisms and optimizing MFC configurations are important for enhancing power generation of MFC. In the previous studies, researchers made effort to improve the power output at many aspects. Simplified MFC without proton exchange membrane had higher power generation due to a lower internal resistance [4]. Electrode materials (carbon felt, graphite fiber, carbon cloth et.al.), operational conditions include substrate, temperature, pH and external resistance etc. have been extensively studied [5-11]. In order to use the electrical current, different types of microbial electrochemical systems (MESs) derived from MFC such as microbial electrolysis cell (MEC), microbial desalinization cell (MDC) and microbial reverse-electrodialysis cell (MRC) were developed [12-15]. However, the capacity of extracellular electron transfer of microbial biofim is key for improving performance of MESs [16].

Extracellular electron transfer of fixe-configuration microbial electrochemical systems (MES) depends upon community composition of biofilm. Niche-based deterministic factors such as temperature, pH and light conditions play significant role in shaping microbial community structure of MESs [17-19]. A recent study demonstrated that stochastic assembly plays a dominant role in determining community structure in MECs [20]. Both deterministic and stochastic factors play important role in shaping microbial community structure of biofilm in MES. Understanding relationship between ecosystem function and community structure is important for improving MES configuration and enhancing electron transfer.

Although some exoelectrogenic bacteria have been isolated from MFCs, understanding microorganism capable of extracellular electron transfer in natural environment is still deficient [2]. Community analysis proves that more diverse populations exist in the electrode biofilm. Exploring unknown exoelectrogenic microbes in natural environmental or engineered system will facilitate an illuminating insight into electron transfer. Some recent studies showed that interspecific interaction between exoelectrogens and non-exoelectrogens in MFCs for soil bioremediation [21-23]. Wastewater or activated sludge is used frequently as inoculum while mixed culture MFC has been developed as novel wastewater treatment technology [1]. Almost all previous studies on MFC inoculum were performed using two-chamber MFCs. Inocula (wastewater, waste sludge, defined mixed- or pure culture) influenced obviously power production and internal resistance of two-chamber MFCs [10, 24, 25]. In order to enhance phenanthrene degradation in MFCs, community composition of electrode was shaped by supplementing *Pseudomonas aeruginosa* into mixed cultures [26].

A recent study analyzed microbial community of MFC with two kinds of wastewater inoculum using denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene [9]. Community analysis by conventional molecular tool such as DGGE and clone libraries of 16S rRNA gene provides incomplete information due to limited throughput and resolution. Although previous studies showed that inoculum influenced power generation of MFC, how the initial inoculum influence shaping community structure is still yet unknown based on high throughput sequencing. Community structure of microbial biofilm may be shaped by different initial inoculum, which will result in different electricity generation of MFC.

In this study, the effect of different inocula from natural consortia and wastewater on performance of microbial fuel cell was investigated. The bacterial communities of anode biofilm of MFC and initial inocula were assessed by sequencing 16S ribosomal RNA (rRNA) gene amplicons with the Illumina MiSeq technology.

2 Materials and methods

2.1 MFC configuration and operation

Cubic single-chamber MFC was made from polymethylmethacrylate (PMMA) (cylindrical chamber volume of 25 ml) as previous description [6]. Graphite fiber brush was used as anode [26]. The air-cathode was made from carbon cloth (7 cm^2 projected area) with a layer of platinum catalyst and three polytetrafluoroethylene (PDFE) diffusion layers [5].

Four types of initial inocula were used in MFCs. Natural consortia of river sediment (RS) were obtained from the Songhua River in Harbin. Garden soil (GS) was obtained from shrubs in Harbin. Wastewater (WW) and activated sludge (AS) were obtained from primary clarifier and secondary sedimentation tank of Wenchang Wastewater Treatment Plant of Harbin, respectively. The MFC reactors were fed a nutrient medium containing 1 g/L sodium acetate as substrate in a 50 mM phosphate buffer solution (PBS)(11.55 g $Na_2HPO_4{\cdot}12H_2O$, 2.77 g $NaH_2PO_4{\cdot}2H_2O$, 0.31 g NH_4Cl and 0.13 g KCl) [28] amended with 1.25 ml/L mineral solution and 0.5 ml/L vitamin solution. All MFC Reactors were operated in fed-batch mode, and every batch cycle was regarded as a period. The solution was replaced when the voltage decreased lower than 0.05 V. All MFC reactors were operated in a constant temperature room set at 30 ^{o}C.

2.2 Calculations and analyses

The cell voltage across an external resistor of 1000 Ω in MFC was collected automatically every 30 min by a multichannel data acquisition system (Model 2700 with 7702 module, Keithley Instruments Inc., USA) and then connected to a personal computer via PCI interface. The polarization curve was measured by changing the external resistance from 3000 to 50Ω. Power density and coulombic efficiency (CE) were calculated as previously described [6].

2.3 Illumina sequencing analysis of 16S rRNA gene amplicons

After the MFC steadily operated more than 2 month, brush anodes were cut and fragmented by sterile scissors [17]. Genomic DNA of anode biofilm and initial inocula was extracted using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. Bacterial 16S rRNA genes targeting the hypervariable regions of V4~V5 were amplified using a pair of universal primers as follows: 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTGAGTTT-3'). Individual samples were barcoded for pooling multiple samples in one run of an Illumina Miseq (Illumina, San Diego, CA)

2.4 Analysis of 16S rRNA sequencing data

The resultant sequencing reads were analyzed using using Quantitative Insights into Microbial Ecology (QIIME) software (http://qiime.org) [29]. Operational taxonomic units (OTUs) were determined at 97% similarity levels using UPARSE software (http://drive5.com/uparse/). A representative sequence from each OTU was selected for taxonomic identification using the Silva database (http://www.arb-silva.de) and Ribosomal Database Project (RDP) classifier (http://rdp.cme.msu.edu) with a 0.80 confidence threshold. Diversity index, the species richness of the Chao1 estimator and the ACE estimator of each sample was generated in the MOTHUR (http://www.mothur.org). The heat map of genus level was generated using a hierarchical clustering algorithm. Principal component analysis (PCA) and hierarchical cluster analysis were performed to visualize and interpret difference in microbial community structure between data set.

3. Results

3.1 Power production of MFCs with different inocula

All MFCs showed voltage production after acclimation, there was obvious difference in lag phase after MFCs were inoculated with different inocula（Fig.1). A maximum peak voltage of MFC with active sludge inoculum (MFC-AS) reached more than ~540 mV after 4 day faster than that obtained by other MFCs, suggesting that mixed culture from sludge inoculum may have a high vitality. After 6 days MFC with river sediment inoculum (MFC-RS) and MFC with wastewater inoculum (MFC-WW) reached the maximum peak voltage except MFC with garden soil inoculum (MFC-GS). A small proportion of exoelectrogenic microorganism in the soil ecosystem may result in longer lag growth. When all MFC reactors produce reproducible current, the maximum power density of 744.8±11.7 mW/m^2 was obtained by MFC-RS, with 666.3±19.1 mW/m^2 of MFC-AS, 654.7±30.1 mW/m^2 of MFC-GS and 536.1±6.3 mW/m^2 of MFC-WW (Fig. 2).

3.2 COD removal and coulombic efficiency

All MFCs with different inocula had similar COD removal rates, and the maximum COD removal rate of 95.7% was obtained by MFC-AS, followed by MFC-WW (95.4%), MFCs-GS (94.3%) and MFC-.RS (93.6%) after operating for 20 days. Although the MFC-AS achieved the highest COD removal rate, but not the maximum power density, suggesting that some non-exoelectrogens in anode biofilm resulted in lost energy production. The coulombic efficiency (CE) of MFC-AS, MFC-RS, MFC-WW and MFC-GS were 27.4%, 28.6%, 29.8% and 31.2%, respectively.

3.3 Bacterial diversity of the anode biofilms and initial inocula

All high-quality reads of 98263 (average length of 402 bp) were obtained from the Illumina Miseq sequencing of 16S rRNA gene for identifying operational taxonomic units (OTUs) of eight individual samples (Table 1). A total 362 (MFC-AS), 169 (MFC-RS), 164 (MFC-GS) and 124 OTUs (MFC-WW) were determined at level of 97% similarity. Initial inocula showed higher relative abundance of OTUs, with 594 (GS), 478 of (AS) and 403 (RS) OTUs except 156 OTUs (WW). The species richness of MFC biofilms was lower than that of original inocula expect MFC-WW biofilm. GS inoculum had the greatest species richness than WW, RS and AS inocula. MFC-AS had the highest diversity (Shannon index, 4.04 and Simpson, 0.0429), followed by MFC-GS, MFC-WW, MFC-RS (Table 1).

3.4 Comparative analysis of microbial community structures

Hierarchical clustering and heatmap analysis were used to identify the differences of eight bacterial community structures (Fig. 3). The heatmap based on genus level showed clear distinctions of community structure between each anode biofilm and inoculum. Hierarchical cluster analysis of OTUs (on the top of heatmap) indicated that the anode biofilms of MFCs differentiated from initial inocula, suggesting obvious shaping community structure existed after enrichment of exoelectrogens in MFCs. The principal component analysis (PCA) showed that the anode biofilms and inocula were well separated, with 21.91% and 19.28% variation explained by PC1 and PC2, respectively (Fig. 4). MFC-AS and MFC-GS were closely clustered and were separated from MFC-WW and MFC-RS.

3.5 Community compositions of the anode biofilms and initial inocula

In terms of the assignment at the phylum level, *Proteobacteria* and *Bacteroidetes* were predominant in all communities, followed by *Firmicutes* except in GS and AS inocula. *Proteobacteria* apparently occupied the most composition of the sequences were 72% (MFC-RS), 69.33% (MFC-WW), 51.79% (MFC-GS), 34.42% (MFC-AS), 54.39% (WW), 22.05% (GS), 41.41% (RS) and 38.47% (AS), respectively (Fig. 5(a)). Among all phyla, *Bacteroidetes* accounted for 20.12% (MFC-RS), 11.29% (MFC-WW), 16.81% (MFC-GS), 35.99% (MFC-AS), 28.7% (WW), 15.05% (GS), 30.49% (RS) and 21% (AS), respectively.

The majority of classes or subclasses belonged to *Betaproteobacteria* in MFC-RS (55.80%), MFC-GS (20.77%), MFC-WW (33.66%), AS (27.53%) and RS (19.45%) (Fig. 5(b)). The predominant classes were affiliated with *Flavobacteria* (19.22%) in MFC-AS, *Acidobacteria* in GS (11.63%), *Epsilonproteobacteria* (29.26%) and *Bacteroidia* (27.59%) in WW, respectively.

The difference in dominant populations was more distinct at genus level (Fig. 6). The taxonomic assignments of genera were significantly shifted after the anode biofilms were enriched from different initial inocula. The populations accounted for less than 4% of relative abundance were designated as “others”. The majority of dominant populations in MFC-RS were affiliated with *Azoarcus* (45.20%). The dominant populations in MFC-AS belonged to *Flavobacterium* (14.18%), *Stenotrophomonas* (11.96%) and *Geobacter* (6.73%), compared to *Geobacter*

(14.40%) and *Victivallis* (8.62%) in MFC-GS, and *Azovibrio* (11.11%), *Alishewanella* (9.43%) and *Pseudomonas* (7.97%) in MFC-WW.

4. Discussion

The effect of inocula (natural consortia and mixed culture) on performances of single-chamber air-cathode MFCs was investigated. Our results were similar with previous reports that showed effect of inoculum types on the power density of MFCs [30]. At the beginning of start-up, MFCs inoculated by activated sludge and wastewater inocula showed higher peak voltages, compared to MFCs with RS and GS inocula (Fig. 1). After several cycles, the peak voltage of MFC-RS gradually increased. Compared to other MFCs, MFC-RS obtained a maximum power density. Our results implied that exoelectrogens inhabited widely in natural habitat and wastewater treatment bioreactor result in to obtain easily inoculum for MFC.

Community analyses based on Illumina Miseq sequencing of 16S rRNA gene indicated that different inocula resulted in the difference in community composition in the anode biofilms [31]. PCA analysis proved that MFC-AS and MFC-GS were closely clustered and were separated from MFC-WW and MFC-RS, which also obtained similar maximum power densities (Fig. 3). These results implies that the difference in power densities may arise from different community structure of anode biofilms. Microbial community structures of biofilms in MFCs were shaped by inocula, however stochastic factors may also play a role in the biofilm establishment as previous description [20].

Although MFC-RS obtained the maximum power density, the population diversity of anode biofilm was low (Table 1), presumably exoelectrogens were predominant in the community. The relative abundance of *Geobacter* (well-known exoelectrogen) in MFC-RS only accounted for 1.46%, compared with 6.73% (MFC-AS) and 14.40% (MFC-GS)(Fig. 6). A nitrogen fixing bacterium *Azoarcus* (45.20%) was predominant genus in MFC-RS, which was found as the dominant population in the anode biofilm in previous report [32]. The capacity for extracellular electron transfer of *Azoarcus* should be further tested in the future. The functions of uncultured and low abundance bacteria are unclear in anode biofilms, suggesting the large quantities of unkown exoelectrogens may be present in the natural environment.

Conclusions

The effect of different inocula including river sediment, activated sludge, garden soil and wastewater on microbial communities and performances MFCs was investigated. MFC with river sediment inoculum (MFC-RS) achieved the maximum power density, followed by MFC-AS, MFC-GS and MFC-WW. Community analyses indicated that different inocula resulted in the difference in community composition in the anode biofilms. The dominant populations of anode biofilms of differentiated obviously from that in the initial inocula. The principal component analysis (PCA) of OTUs showed that MFC-AS and MFC-GS were closely clustered and were separated from MFC-WW and MFC-RS. The results confirmed that the different initial inocula influenced community structures and power generation of MFCs.

Acknowledgements

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Table 1 Number of reads, operational taxonomic units (OTUs), Abundance-based Coverage Estimator (ACE), estimator Chao 1 and Shannon index obtained from different samples at 97% nucleotide identity.

Samples	Reads	OTUs	Ace	Chao1	Shannon	Simpson
WW	11608	156	182	189	3.21	0.0894
GS	14896	594	605	607	5.58	0.0068
RS	9291	403	474	492	4.48	0.0313
AS	11876	478	512	510	4.75	0.0278
MFC-WW	10860	124	185	200	3.63	0.043
MFC-GS	11230	164	275	217	3.63	0.0453
MFC-RS	15927	169	293	238	2.5	0.2276
MFC-AS	12575	362	443	476	4.04	0.0429

Figure Captions

Fig. 1. Voltage generation (external resistance of 1000 Ω) of MFCs with different inocula. MFC-AS, MFC-RS, MFC-WW and MFC-GS were inoculated using active sludge, river sediment, wastewater and garden soil, respectively.

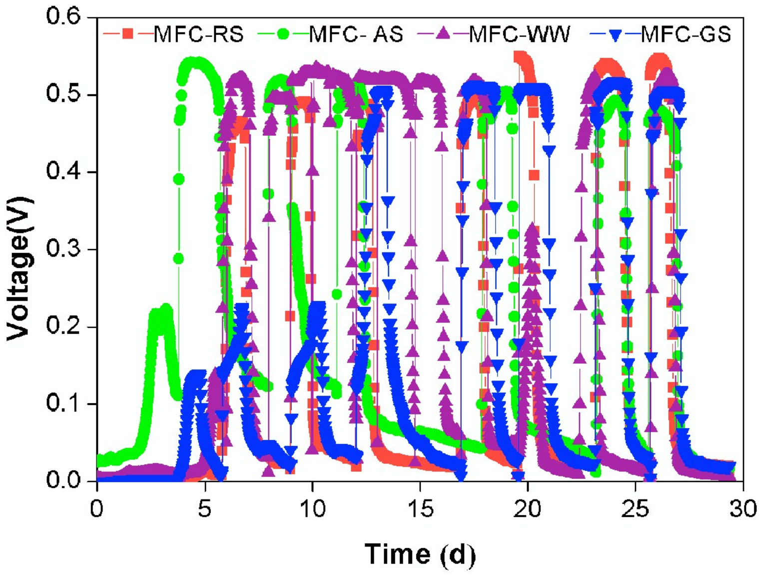
Fig. 2. Polarization curves of MFCs with different inocula. Error bars represent standard deviation based on measurements from duplicate reactors in three cycles.

Fig. 3. Principal component analysis (PCA) based on operational taxonomic units of anode biofilms of MFCs and initial inocula. Inocula and MFCs were clustered in the parallelogram and ellipse, respectively.

Fig. 4. Hierarchical clustering and heatmap analysis of eight bacterial community structures based on Illumina sequencing of 16S rRNA gene. Hierarchical cluster analysis is based on the OTUs abundance on the top of the heatmap. The heatmap is plotted at genus level. The bar on the bottom represents scale of the relative abundance.

Fig. 5. Microbial community structures of the MFC anodes and inocula at the phylum (a) and class (b) level. Items with relative abundance lower than 1% of total composition were classified into group “Others”.

Fig. 6. Relative abundance of dominant genera in microbial communities of the MFC anode biofilms and inocula. Genera with relative abundance lower than 4 % of total composition were classified into group “Others”.



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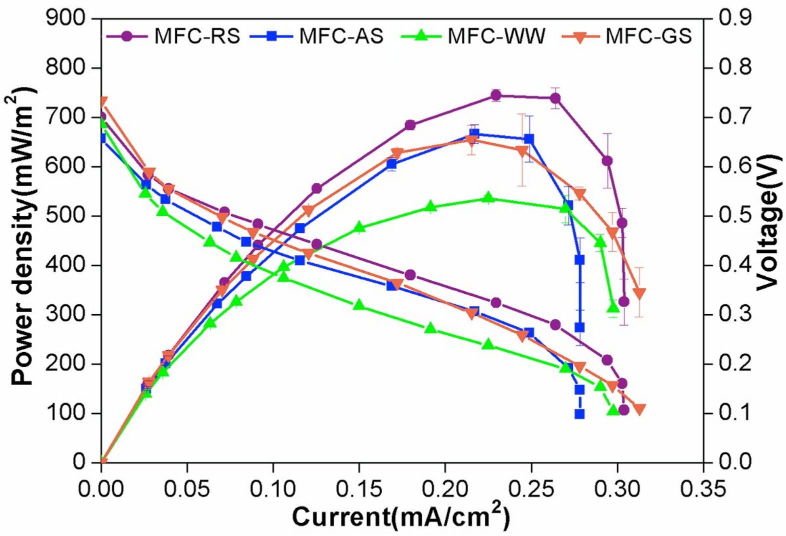

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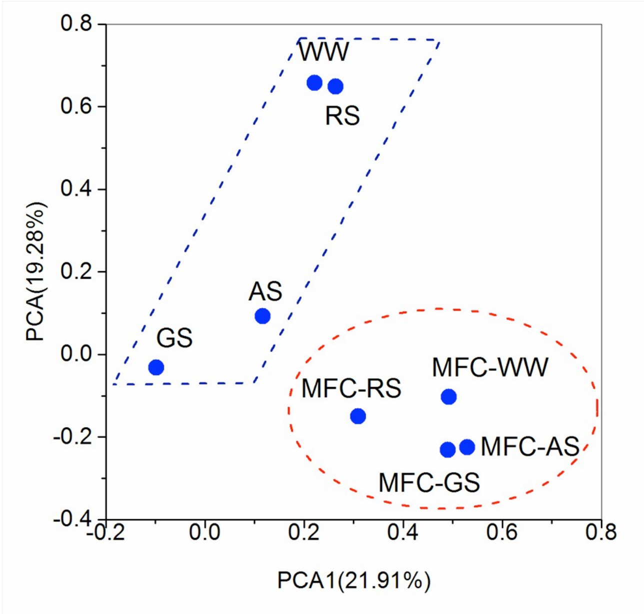

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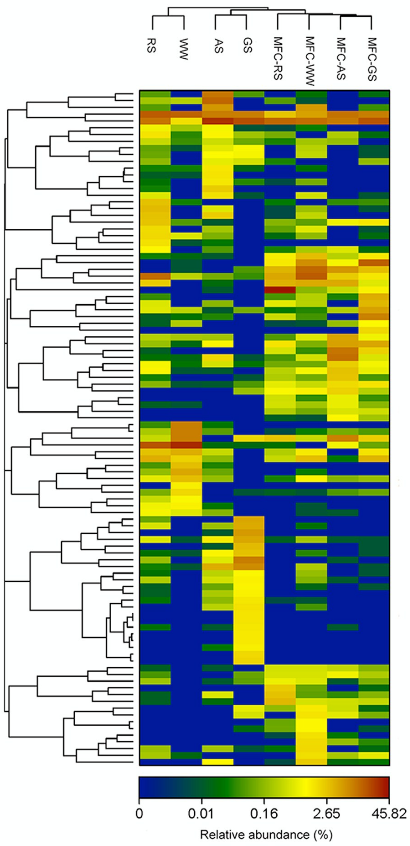
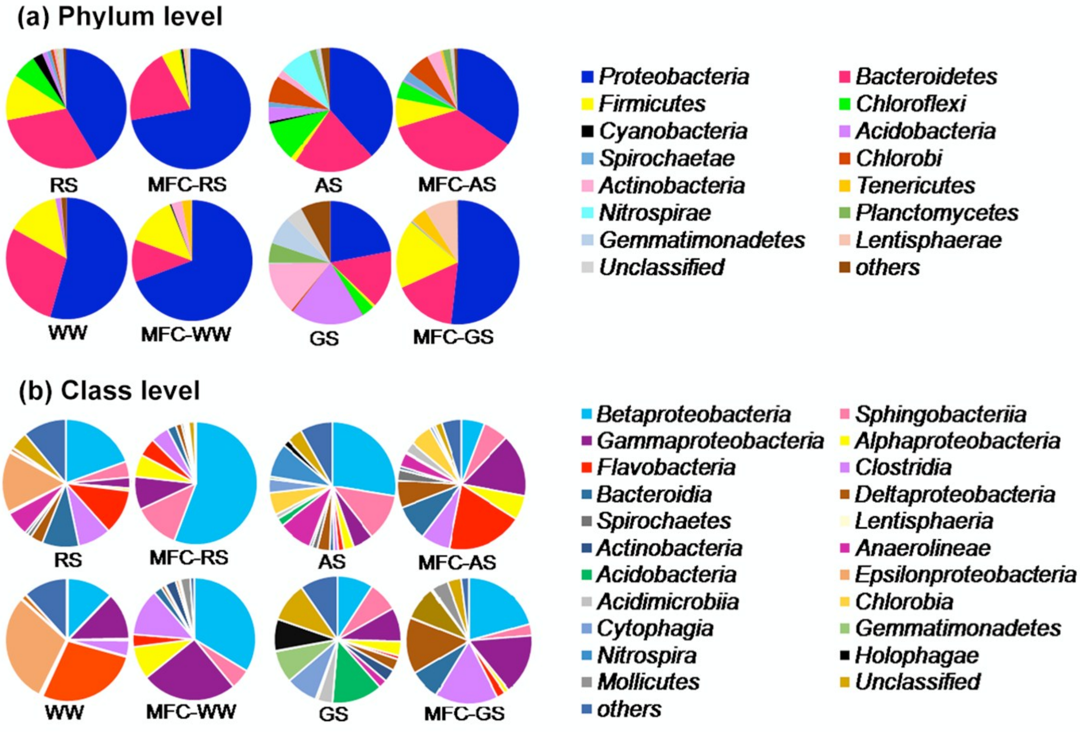


Fig. 4. Hierarchical clustering and heatmap analysis of eight bacterial community structures based on Illumina sequencing of 16S rRNA gene. Hierarchical cluster analysis is based on the OTUs abundance on the top of the heatmap. The heatmap is plotted at genus level. The bar on the bottom represents scale of the relative abundance.



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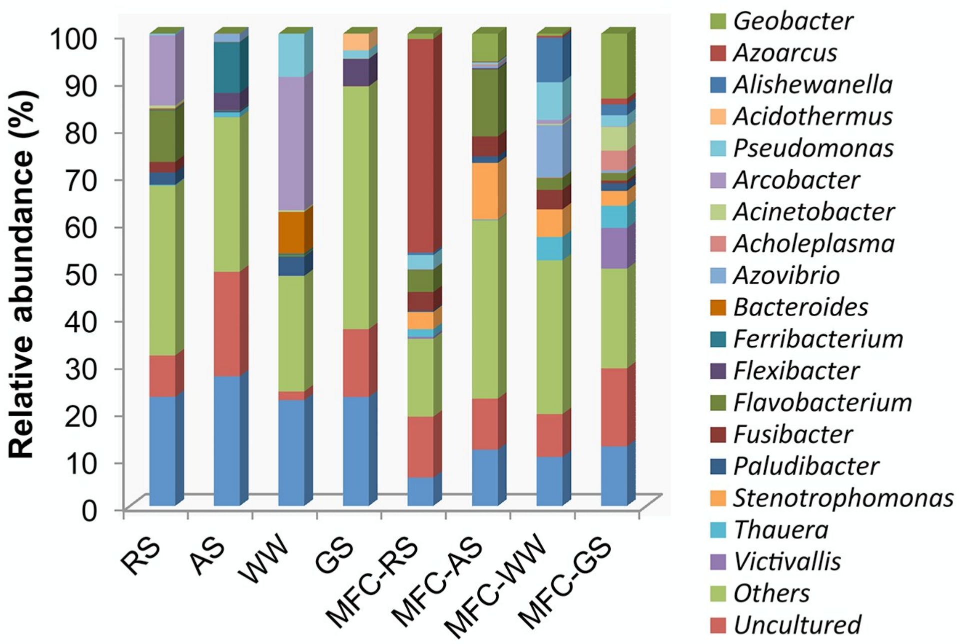

Fig. 6. Relative abundance of dominant genera in microbial communities of the MFC anode biofilms and inocula. Genera with relative abundance lower than 4 % of total composition were classified into group "Others".