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~~Acclimatization~~ Acclimatisation of microbial consortia to ~~-~~ alkaline conditions ~~it~~and~~y~~ enhanced ~~es~~ electricity generation ~~in microbial fuel cells under strong alkaline conditions~~

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Air-cathode microbial fuel cells (MFCs), obtained by inoculating~~d~~ with ~~the~~ an aerobic activated sludge, ~~sampled~~ from a brewery waste treatment, were activated over ~~about~~ a one month period, at pH 10.0, to obtain ~~the~~ alkaline MFCs. The alkaline MFCs produced stable power of 118 mW m^{-2} (or 23.6 W m^{-3}) and ~~the~~ a maximum power density of 213 mW m^{-2} at pH 10.0. The performance of the MFCs was ~~further~~ enhanced to produce a stable power of 140 mW m^{-2} and ~~the~~ a maximum power density of 235 mW m^{-2} by increasing pH to 11.0. This is the highest ~~optimal~~ pH for stably operating MFCs reported in the literature. Power production was found to be suppressed at higher pH (12.0) and lower pH (9.0). Microbial analysis with high-throughput sequencing indicated that Firmicutes phylum was largely enriched in the anodic biofilms (88~~.14~~%), within which *Eremococcus* genus was the dominant group (4~~76.75~~%). To the best of our knowledge, it is the first time that *Eremococcus* genus was described in bio-electrochemical systems. Some alkaliphilic genera, including *Bacillus* (2.14%), *Alkalibacter* (5.14%), *Anoxynatronum* (0.548%), *Alkaliphilus* (0.09%), *Alkaliflexus* (2.1~~07~~%), *Nitrincola* (1.1~~07~~%) and *Corynebacterium* (0.55%) were also enriched in the present alkaline MFCs.

Keywords Microbial fuel cells; alkaline conditions; anodic biofilms; alkaliphilic genera

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1. Introduction

Microbial fuel cells (MFCs) aimed at the generation of electricity and the removal of waste compounds have received significant attention over the past decade. Currently, the generation of practicall~~y~~, usable power from MFCs remains a challenge~~s~~ for real world applications [1, 2], a~~s~~nd the current and energy densities obtained ~~will~~ have so far been~~always be~~ of limited magnitude relative to batteries and chemical fuel cells. However, MFCs have ~~other~~ certain advantages notably, f~~. F~~or example, ~~the most remarkable advantages are that~~ the electrochemically active microorganisms in MFCs are self-sustaining, and that various types of wastes, such as municipal wastewaters[3], organic matter~~s~~ in environmental sediments [4, 5], can be directly used as fuels. In MFCs, electro-active microorganisms accelerate the rate of electrochemical oxidation of complex organic substrates, mainly in the form of anodic biofilms[6], and the electroactivity of anodic biofilms play a key role in MFC power output.

Overall, microorganisms c~~a~~oul~~n~~d adapt to a broad ambient pH range, but most bacteria favour neutral pH conditions for their optimal growth. Thus far, most reported MFCs were operated in an ~~moderate~~ approximately neutral pH range ~~except~~ although~~for~~ a few studies showed that microorganisms exhibited electrochemical activity under extreme pH conditions. Acidophile species were recently ~~evidenced~~ reported to catalys~~z~~e the electrochemical oxidation of tetrathionate in the pH range of 1.2-2.5[7]. L~~, and l~~ow pH distillery wastewater (pH<4) was also demonstrated to be effective~~sufficient~~ly used as fuel~~s~~ for electricity generation[8]. In contrast, selected strains of alkalophilic *bacillus* organisms were reported to produce ~~the~~ a maximum current, through external redox mediators, at pH 10.5, nearly 30 years ago [9].

Recent reports ~~support~~ indicate that relatively ~~–~~higher pH (pH range of 6 to 9) was~~ more~~ favourable to increase the performance of MFCs ~~in pH range of 6 to 9~~ [10-14]. The positive effects of pH ~~enhancement~~ increase, on power production in MFCs, observed under conditions close to neutral pH can be attributed to several reasons. Firstly, higher pH would shift the anode potential to more negative values which consequently improve~~d~~ the ~~voltage of~~ MFC~~s~~ voltage [15]. Secondly, the internal resistance of MFCs was found to be reduced in high pH operation [16, 17]. ~~Finally~~Thirdly, the more alkaline condition was also ~~demonstrated~~ shown to facilitate improved~~the~~ electrochemical kinetics in microbial anodes through producing higher electroactive moieties~~y~~ in anodic biofilms [10], or through synthesizing soluble electron mediators at higher pH [18]. However, pH higher than 9 was generally found to significantly reduce the performance of MFCs [19].

It has been reported that a double-chamber MFC, using post methanation distillery effluent, could tolerate~~d~~ initial feed solution pH up to 10. However, the power density decreased by 60% at pH 10 as compared to that at pH 7 [20]. He et al. ~~has~~ reported an air-cathode MFC that can tolerate an electrolyte pH as high as 10, with optimal conditions between pH 8 and 10, and produced a~~that the~~ peak current at pH 10 ~~was~~ 7.2~~3~~% higher than that at pH 7. However, the impedance spectra of the anode and cathode of the MFC revealed that the anodic microbial process preferred a neutral pH and microbial activities decreased at higher or lower pH. Thus, the observed high current production at pH 9 and 10 could mainly be ascribed to the improved cathodic oxygen reduction reaction with increasing pH [17].

In an air-~~chamber~~ chathode MFC, inoculated with alkalophilic Gram-positive bacterium (*Corynebacterium* sp. strain MFC03), the optimal pH for current production wa~~i~~s reported as 9.0 ~~while~~ although the MFC exhibited considerable high current production at pH 10.0 [21].

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Interestingly, a~~n~~ strain of *Pseudomonas* sp. (*Pseudomonas alcaliphila*) was recently show~~ed~~n to generate the maximum power at pH 9.5, through excreting phenazine-1-carboxylic acid to transfer electron [22], while some other *Pseudomonas* sp. generally exhibit high electroactivity under neutral pH conditions [23, 24].

In the present study, the objective was to improve the adaptation of MFCs to high pH conditions. The experimental MFCs were designed~~ly~~ to ~~run~~ operate at pH higher than most reported optimal pH. The results demonstrated that ac~~c~~climi~~a~~tis~~z~~ation to alkalin~~ei~~t conditions~~y~~ ~~, indeed~~ enhanced the electroactivity of microbial consortium under quite high pH~~strong,~~ alkaline conditions, and consequently increased the electricity production in MFCs. The optimal pH for MFC operation~~s~~ was 11.0, much higher than most reported pH for MFCs operation, implying that MFCs could be developed as potential bio-electrochemical systems directly treating strong alkaline wastewater.

2. Methods

2.1. MFC configuration and operation

Experiments were carried out in single-chamber MFCs with ~~the~~ an air-cathode. MFCs were made of plexiglass with an effective solution volume of 60 cm^3 (mL). Three pieces of graphite felt~~s~~ (radius 2.3 cm, thickness 1.0 cm) were assembled together using stainless steel (316L) bolt and nuts to form an anode. The air-cathode was prepared using active carbon (Xinshen Carbon, Fujian), PTFE microporous filtering film (0.45 μm) and stainless steel mesh (316L) as catalyst, air-diffusion layer and current collector, respectively. Details ~~about~~ of the MFC setup ~~were~~ are provided in Fig. 1S.

Initially, the MFCs were operated by recycling 1.0 litre (~~L~~) of artificial growth medium (AGM) of pH 10.0, inoculated with ~~the~~ an aerobic activated sludge (sampled from the waste treatment plant in Yangzhou brewery), at a rate of 2 ml min^{-1} through the MFC ~~and a reservoir~~. The ~~AGW~~ AGM was mixed with the aerobic activated sludge ~~by~~ bat a concentration of 50 g L^{-1}. The mixture was purged with pure N_2 for 20 minutes to remove dissolved oxygen, and the pH was adjusted to 10.0 before use.

The MFCs were operated under recycling ~~operation conditionswas performed~~ , until the measured voltage output (across an external resistance of 200 Ω) increased to 100 mV. Following the recycling operation, MFCs were switched to batch operation by completely replacing the solution in MFCs with freshly prepared AGW of pH 10.0 when the voltage ~~dropped~~ fell to below 50 mV. The AGM, of pH 10.0, was prepared with the following constituents: NH_4Cl (1.5 g L^{-1}), KCl (0.1 g L^{-1}), K_2HPO_4 (0.13 g L^{-1}), Na_2CO_3 (7.4 g L^{-1}), $NaHCO_3$ (2.5 g L^{-1}), glucose (2.0 g L^{-1}), yeast extract (0.2 g L^{-1}) and mineral stock solution (12.5 ml L^{-1}). The initial pH of AGM was adjusted to 10.0 using HCl and NaOH solution. The AGMs for other pH tests were prepared with constituents identical to that of pH 10.0, except for the buffer constituents; Na_2CO_3 and $NaHCO_3$. The Na_2CO_3/$NaHCO_3$ ratio in AGMs of pH 9.0, 11.0 and 12.0 were 1:9 (Na_2CO_3 1g L^{-1}, $NaHCO_3$ 9g L^{-1}), 9:1 (Na_2CO_3 9 g L^{-1}, $NaHCO_3$ 1 g L^{-1}) and 10:0 (Na_2CO_3 10g L^{-1}, $NaHCO_3$ 0 g L^{-1}), respectively.

2.2. Measurements and calculations

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The voltage ~~outputs~~ produced by MFCs with an external load of 200 Ω was~~ere~~ continuously measured using a multiple-channel high-impedance voltmeter (Keithley 2700), and data were recorded ~~in~~ every minute. Electrode potential relative to a saturated calomel electrode (SCE) was also measured ~~by keithley 2700~~. Polarization curves of MFCs were measured using a battery testing system (Neware CT-3008W, Shenzhen, China) ~~by~~ in the mode of constant current discharge. Linear sweep voltammetry (LSV) ~~measurements~~ for bio-anodes was~~ere~~ performed using a potentiostat (CHI 660c, Shanghai, China) with ~~the anode,~~ a saturated calomel reference electrode (SCE) ~~–~~ and the air cathode ~~used as the working electrode, the reference electrode and~~as the counter electrode~~, respectively~~. For polarization and LSV measurements, MFCs were first disconnected from the circuit until the open circuit voltage plateaued before measurements. Current density and power density were normalized to the project area of anode surface or to the effective solution volume in the MFC chamber for comparison with literature~~s~~. Charge produced by the MFC for a cycled operation was determined ~~–~~by integration of the current~~ed~~ from the time of medium replacement to the time of cycle end~~ing~~, when that the voltage dropped below 50 mV. Medium pH was measured by a pH meter (PHS-3C, Leici, Shanghai).

2.3. Other analysis

Microbial communities of the inoculum sludge, suspended microorganisms formed in the anode chamber~~,~~ and anode-attached microorganisms were investigated using 16S rDNA gene amplicon sequencing (MiSeq system, Illumina, USA). Suspended microorganisms and anode-attached microorganisms were sampled after acclimati~~z~~ation of anode-attached microorganisms with AGW of pH 10.0 for 62 days. The DNA was directly extracted from samples using the PowerSoil DNA Isolation Kit (MIO-BIO), following the manufacturer's instructions. DNA concentration and purity were~~as~~ checked by running the samples on 1.0% agarose gels. PCR amplifications were conducted in triplicate with the primer set 515 Fin TTCCCTACACGACGCTCTTCCGATCTGTGCCAGC MGCCGCGGTAA and 926 Rin GAGTTCCTTGGCACCCGAGAATTCCACCGTCAATTCM TTTGAGTTT that amplifies the V4-V5 region of the 16S rDNA. The reverse primer contained a 6-bp error-correcting barcode unique to each sample [25]. DNA was amplified following the protocol described previously [26]. Sequencing was subsequently determined on an Illumina MiSeq platform by TinyGene (Shanghai, China).

The 16S rDNA gene sequences generated were analysed using the bioinformatic software package Mothur using the MiSeq SOP Pipeline to analyse a multiplexed set of samples on a single run. The paired reads were assembled using make.contigs that extract the sequences and quality score data from the fastq files, and creates the reverse complement of the reverse read and finally assembles the paired end reads into a contig. Screen.seqs that was used to remove low quality reads using the following filtering parameters, maxambig=0, minlength = 200 and maxlength =580, maxhomop= 8. The remained sequences were simplified using the unique.seqs command to generate a unique set of sequences, then aligned with the SILVA databases, version 119. The filter.seqs was used to remove empty columns from our alignment, which gives our shorter length of filtered alignment ~~shorter~~. Further de-noise sequences ~~is to pre-cluster~~ the sequences were pre-clustered using the pre.cluster command (http://www.mothur.org/wiki/Pre.cluster) allowing for up to 4 differences between sequences. Then reads were checked for chimeras using UCHIME algorithm and the

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chimeric sequences were removed by the chimera.uchime command with default parameters. To classify (classify.seqs) our sequences we used ~~a~~ Silva 119 reference sequence files and used the Wang method with a confidence threshold of 80%. The no-bacteria sequences were deleted. Then the distance matrix between the aligned sequences was generated by the dist.seqs command. Finally, these sequences were clustered to OTUs (operational taxonomic units) at 97% sequence identity (furthest neighbor method). A majority consensus taxonomy for each OUT was obtained by the classify.otu command with default parameters.

For scanning electronic microscopy (SEM), carbon cloth anodes were removed from the chamber, and biofilms were fixed with 1% gluteraldehyde _over ~~-~~night, followed by dehydration with a series of graded ethanol solutions (30, 50, 70, 80, 95, and 100%) . The anode surface was observed using scanning electronic microscopy (HITACHI S-4800 SEM). Chemical oxygen demand (COD) was measure~~dments were taken~~ by a fast digestion spectrophotometric method with a COD digester and photometer (Lianhua 5B-3C, China).

3 Results and Discussion

3.1. Electricity production of MFCs at pH 10

All MFCs fed with the AGW of pH 10.0 required~~showed~~ long activation periods. It took 27 to ~~-~~35 days for four independent MFCs to increase voltage across external resistance of 200 Ω from below 0.01 V to 0.1 V ~~in~~ during the cycling operation. However, when the activated MFCs were switched to batch operation with complete replacement of the medium, the voltage was ~~found to i~~ncreased quickly to 0.2 V. Typically, 8-10 batch-cycles could enhance the voltage of MFCs ~~over~~ to above 0.5 V, and stable electricity production could be obtained in subsequent batch operations (Fig. 1). ~~Measurements of electrode potentials showed that T~~the potential of the air-cathode was stable whil~~s~~te the anode potential varied significantly during batch cycles (Fig. 1B). The synchronization of anode potential variation with that of voltage indicated that power production was ~~almost~~ ascribed to the development of microbial anodes in the present air-cathode MFCs. This is in contrast to the previous study in which high current production was mainly attributed to the ~~high~~ improved performance of air-cathode rather than microbial anodes at high pH [17]. At the end of the batch cycles, the pH ~~was detected to drop~~had fallen to values in the range of 9.3 to 9.6, which were~~with~~ smaller pH changes compared with previous studies under high pH conditions [17, 18], indicating that the present MFCs were always maintained at pH range close to 10.

The stable power generation measured within batch cycles was 118±14 mW m^{-2} (or 23.6±2.8 W m^{-3}) based on measurements on four independent MFC replicates~~.~~: ~~-~~ _much lower than that in MFCs operated within the pH range of 6 to 9[10, 12], but higher than power output produced by MFCs inoculated with pure alkalophilic strains at pH 9 to 10 [21, 22]. To evaluate ~~the~~ COD removal and C~~e~~oulombic efficiency (CE) of the ~~present~~ MFCs, four batch cycles of stable electricity production were selected to determine the COD change. The results showed that ~~the~~ COD removal was 81±7% for the batch cycles fed with AGW containing COD of 2200 mg L^{-1}, similar to values of 70-90% in previous studies [12, 21]. The corresponding CE measured for selected batch cycles was 19.8±2.3%, much lower in compar~~ed~~ison with values of 60-70% in previous MFCs [12], but higher than the value of 5.9 % reported in the high COD removal MFCs under the conditions of pH 9 [21]. In air-cathode MFCs, several factors, including the electron consumption by methanogenesis,

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aerobic respiration of the cathode biofilm and the fermentation metabolism by microorganisms in MFC chamber, were believed to be responsible for low CE [12, 21, 27]. The methanogenesis was suggested to be completely suppressed when pH was over 10 [28]. The low CE obtained in the present alkaline air-cathode MFCs might be ascribed to aerobic respiration of the cathode biofilm and the fermentation metabolism by microorganisms in the MFC anode chamber.

3.2. The effect of pH

Polarization measurements were performed at different pH (9.0, 10.0, 11.0 and 12.0) ~~to evaluate the effect of pH~~ on MFCs that ~~have~~ were acclimitised~~ated~~ to pH 10 for stable power generation (Fig. 2). The ~~results showed that the~~ present MFCs exhibited the highest performance at pH 11.0, resulting in ~~the~~ a maximum power densi~~i~~isty of 235 mW m^{-2} (47 W m^{-3}), about 11% higher than that at pH 10.0. Further ~~enhancement~~ increase of pH to 12.0 reduced the maximum power density to 106 mW m^{-2}, about 53% lower than that at pH 11.0. Power output ~~was observed to~~ significantly decreased at pH 9.0; which was previously reported to be the optimal pH ~~condition~~ for ~~previously reported~~ MFCs [10, 17, 18, 21]. The maximum power density ~~measured a~~at pH 9.0 was 156 mW m^{-2}, about 27% lower than that at pH 10 (213 mW m^{-2}). Unlike ~~the~~ previous studies, in which the MFCs were generally acclimitised~~ated~~ in the pH range of 5 to 9 with mixed bacterial consortium [10, 17], the present alkaline MFCs were always operated under conditions close to pH 10. The different pH acclimitisaation of the MFCs might be one ~~of the~~ reason~~s~~ for the difference in optimal pH between these studies.

Measurements of the individual electrode potentials showed that ~~the~~ cathode potentials were almost identical in all cases, except ~~for~~ that measurements at pH 9.0 were 20~30 mV higher than that obtained at pH 10.0, 11.0 and 12.0, within the tested current range (Fig. 2C). In contrast, the individual anode potentials varied significantly under different pH conditions. The open circuit potentials ~~–~~(OCPs) of the anode were:~~as~~ -620±3 mV, -640±2 mV, -671±3 mV and 662±6 mV (vs. SCE;~~,~~ with standard deviation based on the measurements on three independent MFCs) for anodes at pH 9.0, 10.0, 11.0 and 12.0, respectively. These data indicate~~d~~ that higher pH generally resulted~~produced~~ in more negative OCP of anodes, except for the case of pH 12. However, the unit pH increase only produced -20 mV/pH to -30 mV mV/pH, lower than data previously reported in the pH range of 7 to 10 [11, 15, 29], which was probably due to different pH response of the bacterial metabolism between different pH range.

When the anodes were polarized by pass~~flow~~ing higher current, the anodes ~~that~~ operated at pH 11.0 had the most negative ~~individual~~ potentials within the tested current range, whereas the anode at pH 12.0 had the most positive individual potentials (Fig. 2C). The response of the polari~~s~~zed anode potentials to pH change followed the same order ~~with~~ as that observed for the voltage (Fig. 2A) and power density (Fig. 2B). The measurements of the electrode potentials demonstrated that the microbial anodes were the limiting factor in the present air-cathode MFCs, and the variation in power output observed at the four tested pH can be ascribed to the different performance of anodes ~~by~~ in the pH ~~change~~range. Thus, the high~~est~~ performance of MFCs at pH 11.0 ~~means~~ was linked to~~that~~ the anode in ~~MFCs~~ operated at pH 11.0 ~~had the maximum electroactivity.~~ which is~~This thought was~~ further supported by the LSV measurements on the anodes (Fig. 3). ~~Slow~~ The LSV measurements showed that ~~the~~ anodes operated at pH 11.0 produced the highest catalytic current, whil~~s~~te the electroactivity of anodes operated at pH 9.0 and 12.0 were suppressed, compared with

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that obtained at the originally acclimitisationating pH of 10.0, consistent with the polarization measurements (Fig. 2).

Table 1

Performance indices of MFCs batch-operated at pH 10.0 and 11.0

pH	Batch cycle period/hour	Integrated charge/C	Maximum power density/ mW m^{-2}
10.0	29±3	218±15	118±14
11.0	37±4	246±22	140±11

Batch cycle period was the span from the time of medium replacement to the time at which the voltage dropped below 100 mV. CIntegrated charge was calculated by integrating the current over time within the batch cycle period. Each mean value is the average calculated based on measurements over four batch cycles on four independent MFC replicates.

Measurements shown above demonstrate that the present high pH acclimitiseated MFCs exhibited the highest MFC performance and the maximum electroactivity in microbial anodes at pH 11.0. To our knowledge, this is the highest optimal pH reported for microbial anodes up to now [9-13, 15, 17, 21, 22]. To avoid the potential of for adverseillusive response of anodic biofilms to pH "shock" in the transient polarization and LSV measurement process, MFCs were operated at pH 11.0 in batch mode to obtain the long term pH effect, and the results were compared with that measured at pH 10.0. The resultsData show showed that the present MFCs exhibited high stable electricity production at pH 11.0 (Fig. 4A). Variations in the individual electrode potentials during batch cycles (Fig. 4B) once againalso supported that the conclusion that the microbial anode was responsible for the variation in MFC performance. Compared with the operation at pH 10.0, the performance of MFCs at pH 11.0 was evidently enhanced in terms of the batch period, integrated charge and the maximum power density (Table 1), confirming that the optimal pH for the present alkaline-adaptive MFCs was as high as 11.0.

Thus far, the highest optimal pH for bioelectrochemical activity was reported to be 10.5 for selected strains of alkalophilic *bacillus* organisms [9]. The adaptation of anodic microorganisms to pH as high as 11.0, observed in the present MFCs, indicated that a mixed bacterial culture was more resistant to high pH than a pure culture. However, long acclimitisingating periods (27-35 days in the present MFCs) may be necessary for the mixed cultures to evolve into microbial consortia adaptive to strong alkalinity. In a previous study, an adapted microbial consortium in MFCs operated under the at an anolyte pH condition of 10.0, over long time periods was only used as inoculum in double-chamber MFCs for investigating the pH effect in the pH range of 7.0 to 10.0, and no data were provided for the performance of MFCs or microbial anodes at pH 11.0 in that study[15]. Thus, it is difficult to compare the present results with other studies in the present stage.

3.3. Microbial community composition

Bacterial morphology analysis of on the anodic biofilm observed bywith SEM, after the MFC was operated at pH 10.0 for over 80 days, indicated that coccus bacteria were in the majority in the anode-attached microbial communities.and a A small quantity of rod bacteria could was also observed presentin the anodic biofilms (Fig. 2S).

To analyze the microbial community composition in the MFC, DNA was extracted from the

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anodic biofilms and planktonic communities and analysed by~~in the MFC for~~ 16S rDNA based high throughput sequencing, and ~~the~~ results ~~we~~are compared with the inoculum of aerobic activated sludgein~~(~~ Fig. 5~~)~~. Significant ~~variations~~ differences in the microbial communities were observed with~~that~~ phyla Firmicutes ~~was~~ being largely enriched in the anodic biofilms and planktonic communities~~;~~, changing from 14.1~~2~~% in inoculum to 88.1~~4~~% in the anodic biofilm, and 71.8~~5~~% in the planktonic community. Th~~ese is~~ese changes ~~pattern was~~were quite different ~~from~~ to the previously reported results measured in MFCs, operated at neutral pH, which showed that Proteobacterias were the dominant groups in the anodic biofilms[25]. In the present alkaline MFCs, Phyla Proteobacteria content was ~~largely~~ reduced from 16.5~~3~~% in inoculum to 2.3~~28~~% in anodic biofilms, whil~~e~~ste it was enriched to 21.8~~76~~% in planktonic communities. To understand the contribution of specified populations to the MFC function, the composition and abundance of microbial communities ~~in~~ at the genus level were retrieved (Table 2). The most enriched

Table
Changes in relative abundance of the bacterial genera along as MFCs were operated at pH 10.0

Taxonomy		Abundance (%)		
Phyla	Genera*	Inoculum	Planktonic consortia	Anodic biofilm
Firmicutes	*Eremococcus*	0.01	8.16	46.75
	Alkalibacter	0.14	2.16	5.14
	Enterococcus	0.07	1.34	2.80
	Bacillus	0.01	7.30	2.14
	Atopostipes	0.00	29.82	0.96
	Proteiniclasticum	0.32	1.99	0.97
	Tissierella	0.00	4.85	0.65
	Anoxynatronum	0.00	0.44	0.48
	Hydrogenoanaerobacterium	0.01	0.01	0.11
	Alkaliphilus	0.01	0.30	0.09
Bacteroidetes	*Alkaliflexus*	0.07	0.07	2.07
	Proteiniphilum	0.23	0.75	0.77
	Petrimonas	1.14	0.70	0.39
	Paludibacter	0.06	0.03	0.11
	Empedobacter	0.02	0.04	0.07
Proteobacteria	*Nitrincola*	0.00	12.04	1.07
	Halomonas	0.00	0.09	0.25
	Alcaligenes	0.00	5.25	0.21
	Oceanobacter	0.00	0.02	0.09
Actinobacteria	*Corynebacterium*	0.00	0.01	0.55

*Only established genera were shown.
**The darker background indicating higher abundance (%).

bacterial genus in anodic biofilms was *Eremococcus* (46.75 %). *Eremococcus* was also enriched in planktonic communities (8.16%), whil~~s~~te this genus was only detected below 0.01% ~~in~~ in the inoculum. *Eremococcus* ~~were~~ are described as Gram-positive, facultatively anaerobic genus of

Bacilli class in Firmicutes phylum [30]. To the best of our knowledge, *Eremococcus* genus has not been described as electroactive bacteria in any bio-electrochemical reactors. Other classified genera of Bacilli class detected in anodic biofil were *Enterococcus* (2.80%), *Bacillus* (2.14%) and *Atopostipes* (0.96%) ~~in anodic biofilms~~. Species of the *Bacilus* were reported to generate electricity at an optimal pH 10.5 [9]. Many genera and species from Firmicutes phylum, often from Clostridiales order, have shown~~ed~~ exoelectrogenic activity [31, 32]. The classified genera of the Clostridiales order that were detected in the anodic biofilms were *Alkalibacter* sp. (5.14%), *Proteiniclasticum* sp. (0.97%), *Tissierella* sp. (0.65%), *Anoxynatronum* sp. (0.48%), *Hydrogenoanaerobacterium* sp. (0.11%) and *Alkaliphilus* sp. (0.09%). The species from *Alkalibacter*, *Anoxynatronum* and *Alkaliphilus* were known to be true alkaliphilic [33, 34]. *Alkalibacter* and *Anoxynatronum* were also the dominant bacterial genera in alkaline MFCs reported recently [13]. Among the Bacteroidetes phylum, *Alkaliflexus* genus, which was known to be alkaliphilic [35], was the dominant group (2.07%) in the anodic biofilms. *Nitrincola* genus from the Proteobacteria phylum, which was recognized as an alkaliphilic genus [36], was ~~detected to be~~present at 1.07% in the anodic biofilms, similar to the results in ~~the~~ a previous study[13]. However, *Geoalkalibacter*, an exoelectrogenic genus from Proteobacteria phylum which was reported to be largely enriched in the alkaline MFCs [13], was not detected in the present study. In Actinobacteria phylum, *Corynebacterium* genus, from which one species was previously isolated from a microbial fuel cell fed continuously with alkaline artificial wastewater,~~–~~ and was shown~~proved~~ to be alkaliphilic [37], was detected ~~to be~~at 0.55% in the present alkaline anodic biofilms.

4. Conclusion

The present study demonstrated that anodic biofilms, adaptive to fairly strong alkaline conditions in MFCs, could be activated by acclimitisingating microbial consortia to high ~~ambient~~ pH under ambient conditions. MFCs operated at pH 10.0 over several months produced the maximum power at an optimal pH as high as 11.0, which was higher than most reported optimal pH for MFC operations. Firmicutes phylum was largely enriched in the anodic biofilms (88.14%), within which a high percentage of *Eremococcus* genus was first detected in the alkaline anodic biofilms (46.75% of sequences), along with alkaliphilic genera within the phyla Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria.

Acknowledgments

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