

Full Length Research Paper

Effects of electrolyte total dissolved solids (TDS) on performance and anodic microbes of microbial fuel cells

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Effects of total dissolved solids (TDS) of electrolyte on performance and anodic microbes were studied in double-chamber microbial fuel cells (MFC) with batch operation. Results showed that the optimum voltage output (355 mV, 1000 Ω) and power generation (578 mW/m²) were achieved at TDS 20 g/L and 5 g/L, respectively. There were positive correlations between electrolyte TDS and voltage outputs at TDS 10 g/L and 20 g/L, but negative ones at TDS 30 g/L. Operations of the MFCs at TDS 30 g/L were not stable. Chemical oxygen demand (COD) removal rates decreased while coulombic efficiencies (CEs) increased with increased TDS. Anodic microbe species varied a lot at different TDS conditions. Main anodic microbes (*Alcaligenes*, *Gordonia* and *Syntrophaceae*) at lower TDS disappeared in MFCs at TDS 30 g/L. TDS as high as 30 g/L was harmful to the MFC system.

Key words: Microbial fuel cell, total dissolved solids (TDS), anodic microbe.

INTRODUCTION

Salt concentration is a common factor that affects the performance of bioreactors in wastewater treatment. Saline wastewaters are difficult to treat with biological methods for the negative effects of salinity on aerobic and anaerobic biological treatment (Zhou et al., 2010; Zou et al., 2009). On one hand, the microbial community may be changed by salinity (Yogalakshmi and Joseph, 2010) and on the other hand, high salinity is toxic to bacteria and inhibits their activity; besides, salinity may also affect the cellular infiltration pressure, leading to plasmolysis or even cell breakup (Ji et al., 2009). The new-developed

microbial fuel cells (MFC) technology provides a new way to saline wastewater treatment. MFCs are bio-electrochemical reactors which are different from traditional anaerobic biological treatment. In MFCs, addition of salts increases the conductivity and reduces the internal resistance, thus is beneficial to electricity generation (Mohan and Das, 2009). Mohan et al. (2009) treated composite chemical wastewater in a double-chamber MFC and approached 51% total dissolved solids (TDS) removal (initial TDS 56.8 g/L). It showed possibility of electricity generation of MFCs at high-salt conditions. MFC is regarded as promising biological treatment technology for its potential of simultaneous electricity-generation and waste removal. Recent development of MFC is microbial desalination cells (MDC), which is capable of removing salt using the current generated (Cao et al., 2009; Jacobson et al., 2011). MDCs are promising as an economical desalination process. Nevertheless, the salts in the MDC are merely relocated, and the effluent need further disposal. During the desalination process, the anodic bacteria are still faced with high-salinity shock. Although operations of MFCs at high salt conditions were put forward (Mohanakrishna et

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Abbreviations: BLAST, Basic Local Alignment Search Tool; CE, coulombic efficiency; COD, chemical oxygen demand; DGGE, denaturing gradient gel electrophoresis; DNA, deoxyribonucleic acid; MFC, microbial fuel cell; PCR, polymerase chain reaction; TDS, total dissolved solid; MDC, microbial desalination cells; rRNA, ribosomal ribonucleic acid; NCBI, national center for biotechnology information.

al., 2010), the correlations between salinity changes and voltages were not quantified. Meanwhile changes of anodic microbes in high TDS MFCs were not clear. In this study, double-chamber MFCs were operated at different salt concentrations (TDS 5 g/L, 10 g/L, 20 g/L and 30 g/L), with their performances evaluated and correlated to TDS. The anodic bacteria at different salinity were analyzed and compared with molecular biological methods (polymerase chain reactions and denaturing gradient gel electrophoresis).

MATERIALS AND METHODS

MFC reactors and electrode materials

Double-chamber MFCs were used in this experiment. The MFC reactors were made of poly-acrylic plastic with rectangular geometry. The working volumes of anode and cathode chambers were 200 mL each. Anode and cathode materials of the MFCs were non-catalyzed graphite felt (Morgan, USA). The electrodes were washed in 1 mol/L HCl and 1 mol/L NaAc to remove contaminants. Proton exchange membrane (Nafion117, USA) was used to separate the anode and cathode chambers. Surface areas of the anodes, cathodes and PEMs were 50 cm² each.

Reactor inoculation and operation

Anaerobic sludge from a pharmacy factory was used as the inoculums for the MFCs. Electrolytes were consisted of NH₄Cl (0.31 g/L), NaH₂PO₄·2H₂O (5.6 g/L), Na₂HPO₄·12H₂O (6.07 g/L), KCl and mineral elements (Liu and Logan, 2004). Salt concentrations were evaluated by TDS of the electrolytes. Different levels of TDS were controlled by addition of KCl (Cheng et al., 2007). Sodium acetate was used as the sole carbon source in the anodic electrolyte. The MFCs were operated in batch mode, and the initial COD was 1000 mg/L. Anodic and cathodic electrolyte was replaced at the end of every electricity-generation cycle (when the voltage outputs were lower than 50 mV). The external resistance was 1000 Ω. The reactors were operated at 30 °C controlled by water bath.

Electrical and chemical analysis

Voltage outputs (*V*) at the 1000 Ω external resistance were measured using a multimeter (Victor, VC9804A+, China). Current (*I*), Power ($P = V \times I$), and CE were calculated as described in previous study (Logan and Regan, 2006). COD was measured according to APHA standard method (APHA, 1998). Polarization curves were plotted by varying the external resistance (10 Ω ~100000 Ω) and measuring the voltage outputs after stabilization of the MFCs for 1 h. Power density (mW/m²) and current density (mA/m²) were calculated by dividing the power and current with the surface area (m²) of the anode. TDS was monitored with a conductivity meter (Sanxin, SX751, China). The correlations between TDS and voltage outputs were analyzed in SPSS package 13.0, using Pearson correlation analysis (2- tailed test).

Bacterial community analysis

Deoxyribonucleic acid DNA extraction

A piece of anodic electrode was cut off with sterile razor after MFC performance tests and chemical analysis. Total genomic DNA was

extracted from the samples of anode electrode with Ultra Clean™ soil DNA Isolation Kit (MO BIO Laboratories, USA) following the instructions.

PCR (polymerase chain reactions) amplification of 16S ribosomal ribonucleic acid (rRNA)

The extracted DNA was amplified by PCR with the forward primer (GC-341f: 5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCC CCCGCCCCCTACGGGAGGCGACAG-3') and the reverse primer (534r:5'-ATTACCGCGGCTG CTG-3'). The PCR amplification was performed using the Veriti 96 Well Thermal Cycler (Applied Biosystems, Foster City, CA) as follows: initial denaturation at 94 °C for 10 min followed by 35 cycles of denaturation performed at 94 °C for 45 s, annealing at 60 °C for 45 s and extension at 72 °C for 45 s before the final extension at 72 °C for 10 min.

Denaturing gradient gel electrophoresis (DGGE) analysis

DGGE analysis was performed using a Dcode Universal Mutation Detection system (Bio-Rad Laboratories, Hercules, CA). The PCR products were loaded onto 10% polyacrylamide gel with a denaturing gradient ranging from 40 to 60% consisting of urea and formamide. The gel was stained with ethidium bromide (EB) for 20 min before DNA bands were observed using a Gel Doc™ XR Gel documentation system (Bio-Rad Laboratories, Hercules, CA) under ultraviolet illumination. Prominent bands (bands with high relative abundance or unique in a lane) were cut off from the gel and kept in ultra-pure water at 4 °C over night. These bands were further amplified by PCR using the forward primer (341f: 5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCGCCCGCCGCC CCTACGGGAGGCGACAG-3') and the reverse primer (534r:5'-ATTACCGCGGCTGCTG-3'). The second PCR products were sent for sequencing (Genscript Corporation, Nanjing, China). Sequencing results were subjected to the basic local alignment search tool (BLAST) search at the National Center for Biotechnology Information (NCBI) site to identify sequences with the highest similarity and were submitted to the GenBank (Accession Nos. JF451852 - JF451855).

RESULTS AND DISCUSSION

Voltage generation

After start-up period (a month), the initial TDSs of electrolytes in the MFCs were adjusted to different levels: 5, 10, 20 and 30 g/L. The MFCs at four TDS levels were operated for several cycles. Voltage outputs (1000 Ω external resistance) of the MFCs at different TDSs during one electricity-generation cycle are shown in Figure 1. The maximum voltage outputs were 342 mV (5 g/L), 355 mV (10 g/L), 335 mV (20 g/L) and 302 mV (30 g/L). Lengths of an electricity generation cycle were 10 days (5 g/L), 12 days (10 g/L), 15 days (20 g/L) and 17 days (30 g/L). With increasing electrolyte TDS, the electricity generation period prolonged, as a result of the decreasing decomposition capability of the microbes (Kargi and Dincer, 1996). At TDS 5, 10 and 20 g/L, there was stable electricity generation cycle of the MFCs. However, at TDS 30 g/L, the voltage output fluctuated

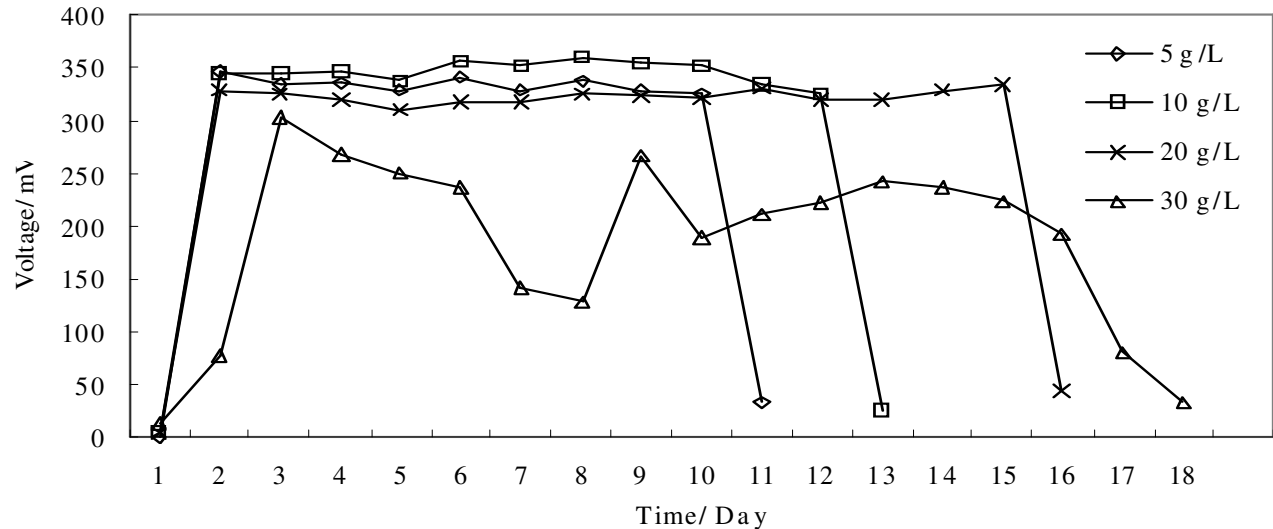


Figure 1. Voltage outputs of MFCs operated at different initial electrolyte TDS.

Table 1. Pearson correlation coefficients between maximum voltage outputs and TDS of the electrolytes.

TDS	Anolyte	Catholyte
TDS 5 g/L	0.186	0.033
TDS 10 g/L	0.498**	0.47**
TDS 20 g/L	0.43	0.734*
TDS 30 g/L	-0.383*	-0.354*

* P value < 0.05, significant correlation; ** P value < 0.01, very significant correlation.

greatly during an electricity-generation cycle. It implied the shock of high salt concentration on anodic microbes. During the electricity generation process, the voltage outputs (1000 Ω external resistance) of the MFCs fluctuated, meanwhile TDS of the electrolytes varied with time. Correlations between the two parameters were analyzed with Pearson correlate analysis (2-tailed test) to test their relevance. Pearson correlation coefficients between voltage outputs and TDS of the electrolytes at different TDS are listed in Table 1. The Pearson correlate coefficients were calculated in the following equation:

$$r = \frac{\sum XY - \frac{\sum X \sum Y}{N}}{\sqrt{\left(\sum X^2 - \frac{(\sum X)^2}{N}\right) \left(\sum Y^2 - \frac{(\sum Y)^2}{N}\right)}}$$

Where, X and Y are variants, and N is the number of samples. At TDS 5 g/L, there were no significant correlations (P value > 0.05) between TDS of the electrolytes and maximum voltage outputs. At TDS 10 g/L, there were significant correlations (P value < 0.01)

between TDS of the electrolytes (in both anolytes and catholytes) and voltage outputs of the MFCs. At TDS 20 g/L, significant correlations (P value < 0.05) were found only between TDS of the catholytes and voltage outputs, but not with TDS of the anolytes. As to the significant correlations between TDS of the electrolytes and voltage outputs of the MFCs, they were positive at TDS 10 and 20 g/L, but negative at TDS 30 g/L. The results above suggest that voltage outputs were influenced significantly by electrolyte TDS when TDS > 5 g/L. Nevertheless, the effects turned negative when the TDS was 30 g/L. The inhibition of high TDS on voltage generation (Figure 1) may also be proved in this way.

Power generation

Polarization and power density curves of the MFCs were depicted by varying the external resistance from 10 to 100000 Ω . As shown in Figure 2a, maximum voltages of the MFCs were 407 mV (TDS 5 g/L), 459 mV (TDS 10 g/L), 421 mV (TDS 20 g/L), and 343 mV (TDS 30 g/L), respectively. Meanwhile, the maximum power densities of the MFCs were 578 mW/m², 420 mW/m², 308 mW/m²

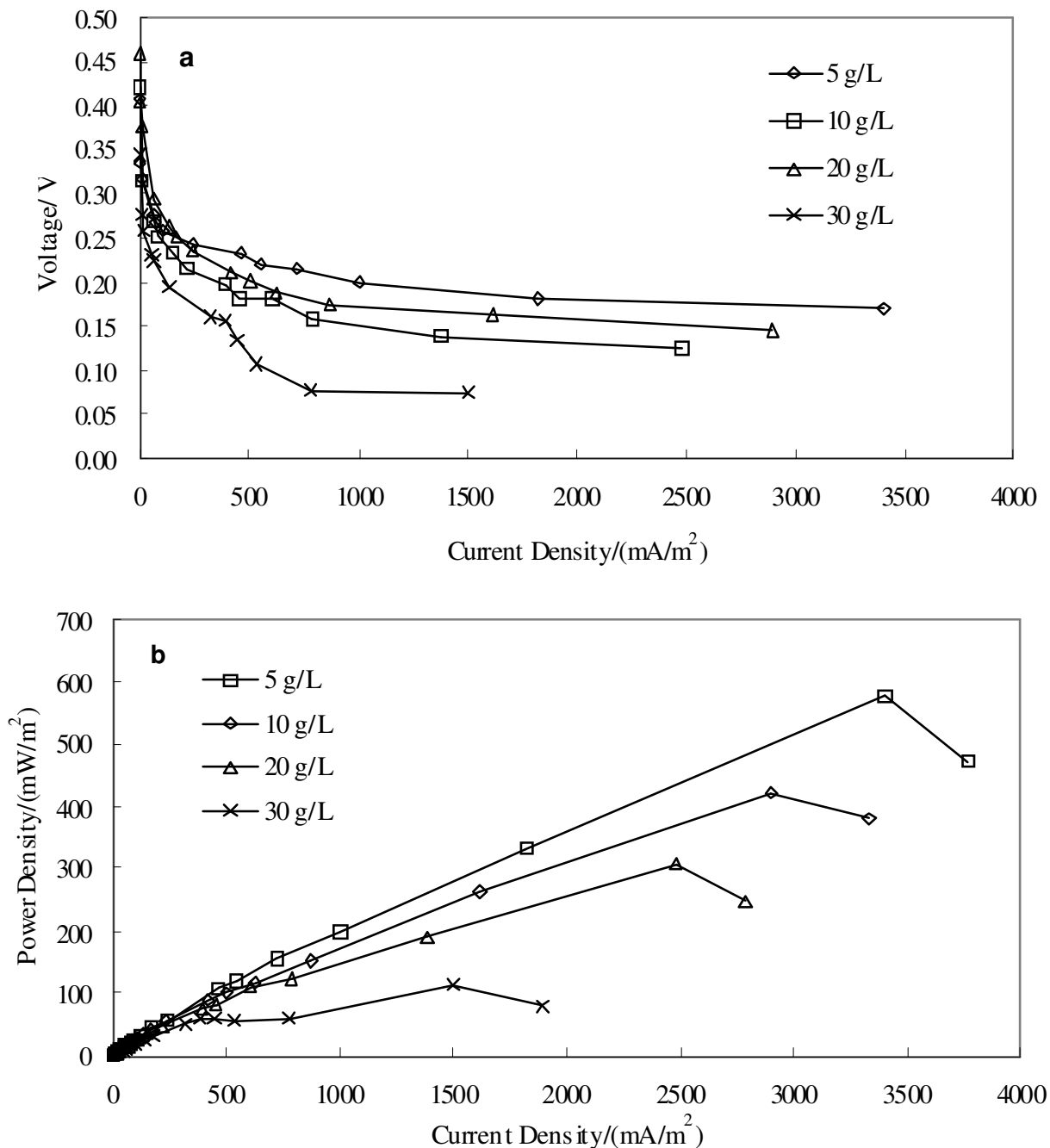


Figure 2. Polarization (a) and power density (b) curves of the MFCs at different initial electrolyte TDS.

and 112 mW/m^2 (Figure 2b). Internal resistances were changed at different TDSs (Aaron et al., 2010), thus performances of the MFCs were affected. Similar to voltage generation, power generation of MFCs operated at 30 g/L was not stable (Figure 2b). The high salt concentration also had negative effects on power generation of MFCs. The highest voltage and power densities were approached at TDS 5 g/L as shown in Figure 2. Combined with the voltage generation section, it could be concluded that operation of the MFCs were reversely

affected at TDS 30 g/L.

COD removal and coulombic efficiency (CE)

COD removal rates and CEs of the MFCs operated at different TDSs are shown in Figure 3. COD removal rates of the MFCs were 100% (5 g/L), 97.3% (10 g/L), 94.7% (20 g/L) and 92% (30 g/L) respectively, while the corresponding CEs were 3.1, 4.2, 5.6 and 5.3%. CEs of

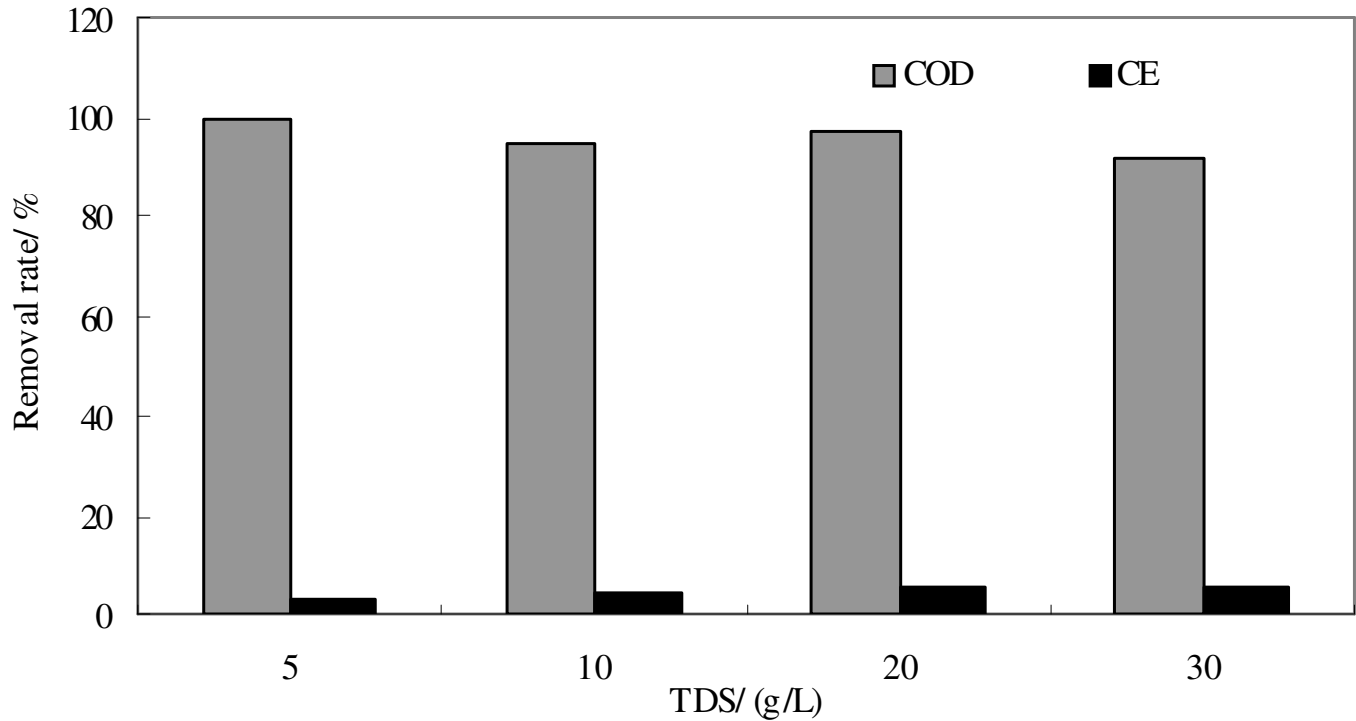


Figure 3. COD removal rates and CEs of the MFCs.

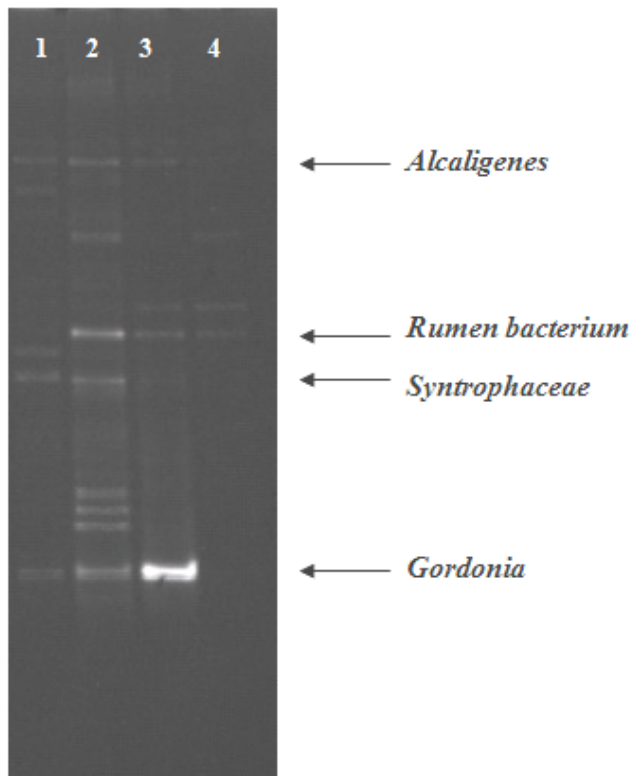


Figure 4. DGGE profiles of anodic microbes at different TDS. Lane 1, TDS 5 g/L; lane 2, TDS 10 g/L; lane 3-TDS 20 g/L; lane 4, TDS 30 g/L.

all the MFCs in this study were at relative low level. The low CEs were mainly caused by the non-catalyzed cathodes used in the experiment (Cheng et al., 2006). In Figure 3, COD removal rates at higher TDSs were lower compared with that of MFCs at lower TDS (Figure 3). The phenomenon resulted from inhibited activity of anodic microbes as discussed above. However, CEs were higher in MFCs operated at higher TDS. This may be due to the concurrent changes of conductivity with TDS. Conductivity of the electrolytes increased with increased TDSs, thus the proton transfer rate in the MFC was accelerated (Huang et al., 2010). As a result, the transformation from chemical to electrical energy was more efficiently at higher TDSs in this study.

Analysis of anodic microbes

Under saline condition, microbes may experience changes in and out cellular (Vyrides et al., 2010), which may influence performances of the MFCs. Anodic microbes in MFCs operated at different TDSs were analyzed with DGGE method. As shown in Figure 4, biodiversity of the bacteria varied greatly with changes of TDS. The microbial communities were versatile at TDS 5 g/L and 10 g/L, but were unique at TDS 20 and 30 g/L (especially at TDS 20 g/L). Analyzed in Quantity One software, the Dice Coefficients of the lanes were: 41.9% (lane 1 and 2), 42.2% (lane 2 and 3), and 1.3% (lane 3

and 4). The results suggest that the anodic microbes were influenced significantly by TDS of the electrolytes. Notably, anodic microbes of MFCs operated at TDS 30 g/L were sharply different from others. It suggests that the high TDS condition was not suitable for the stable operation of the MFCs. Sequencing of the prominent bands in the DGGE profile showed that *Alcaligenes* and *Gordonia* existed when TDS < 30 g/L, but disappeared in lane 4. As for *Syntrophaceae*, it even disappeared at both lanes of TDS and 30 g/L. Reversely, *Rumen bacterium* was not found at TDS 5 g/L, but it existed in other lanes with higher TDS. Compared to other lanes, the number and density of bands in lane 4 were the smallest. It suggests that microbial community in the MFCs was damaged at TDS 30 g/L. This result is consistent with the conclusion that biological treatment was inhibited when TDS > 25 g/L (Mohan et al., 2009). In lane 4, absence of the prominent bands of other lanes might have led to the decreased performance of MFCs at TDS 30 g/L.

Conclusions

The performance and anodic microbes of MFCs were studied at TDS 5, 10, 20 and 30 g/L. Highest voltage output was approached at TDS 20 g/L, while the highest power generation was obtained at TDS 5 g/L. The MFCs could not reach stable operation at TDS 30 g/L as a result of the negative effects of high TDS on electricity generation. COD removal decreased with increase of TDSs, but CEs were higher at higher TDS. Anodic microbes varied greatly at different TDSs and main species at other TDS conditions disappeared at TDS 30 g/L. Over high TDS may bring severe damage to the whole MFC system: both reactor performance and function microbes. In MFC operation, TDS condition should be controlled in suitable range to optimize MFC performance.

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