Effect of pH on sulfate removal from wastewater using a bioelectrochemical system

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HIGHLIGHTS

► Effective sulfate removal at pH 4.5 using a bioelectrochemical system is obtained.
► *Paludibacter* sp. might play an important role in sulfate removal at pH 4.5.
► The results can help to reduce the operation cost for acidic wastewater treatment.

ARTICLE INFO

ABSTRACT

The effects of pH on sulfate pollutant removal, power generation and microbial community were investigated using a bioelectrochemical system, which was built on acclimatized sludge with sulfate at different pHs. For the experiment, ethanol was used as the electron donor for the reduction of sulfate pollutant. In the range of pHs between 2.5 and 10.5, the optimum condition for sulfate removal from wastewater is at pH 4.5 considering the capital cost, removal efficiency, chemical oxygen demand and coulombic efficiency. The results were different from previous studies that neutral condition is suitable for sulfate-reducing bacteria to treat pollutants. According to microbial community analysis, *Paludibacter* sp. might play the most important role in sulfate removal at pH 4.5. *Desulfuromonadaceae* sp., *Desulfobulbaceae* sp. and *Desulfovibrio* sp. were inferred to make major contributions to power generation. These results could help to reduce capital cost in treating acidic sulfate-rich wastewaters.

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1. Introduction

Sulfate pollutants present commonly in wastewaters, which are produced in many processes such as mining, animal husbandry, food processing, pulp and paper wastewaters, dye and detergent manufacture [1]. Many of adverse effects have been generated, e.g. the wastewaters negatively affect the aquatic ecosystem; the reduced products volatilize into the atmosphere and contribute to acid rain; the generated toxic acidic gas raises serious health risks to living beings and is corrosive to materials [2]. To date, quite a lot of efforts have been made to treat the sulfate-rich wastewater. The techniques generally include precipitation [3], membrane separation [4] and biological methods [5,6]. At present, biological method is the most commonly used technique for sulfate-rich wastewater treatment because of the relatively low cost and energy consumption compared to physicochemical methods [7].

Bioelectrochemical system (BES), coupled electrochemical and biological treatment, has been considered as an effective method for sulfate removal. Habermann and Pommer set a microbial fuel cell for sulfate removal [8], even though the proposed mechanism still remains contentious; many efforts have been made to developing this field [9–12]. Compared with conventional biological techniques for sulfate-rich wastewater treatment, the BES has some outstanding advantages as follows: the generated toxic sulfide was used for the production of power and value-added elemental sulfur [13].

In the BES, sulfate-reducing bacteria play important roles in sulfate reduction and power generation. Sulfate is reduced to sulfide by employing sulfate-reducing bacteria, and then sulfide is oxidized to elemental sulfur deposited in the surface of electrode along with the power generation. Several species of sulfate-reducing bacteria, e.g. *Desulfovibrio desulfuricans* [11], *Desulfuromonas acetoxidans*, *Desulfobulbus propionicus* [14], had been confirmed to produce electricity with concomitant sulfate reduction. It has been reported that sulfate-reducing bacteria were generally suitable for growth in the neutral conditions of pH 6–8 and sensitive to pH changes [15]. In addition, the optimum pH for sulfate-reducing bacteria removing sulfate was neutral [16]. However, pH value of sulfate-rich wastewater largely derived from acidic wastewater is

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usually around 3–4 [17,18]. Therefore, pH adjustment for wastewater is necessary in pretreatment process to increase treatment efficiency. Moreover, organic carbon is needed in the process of sulfate reduction. However, industrial effluents of high sulfate usually contain trace organics. Therefore, adding organic matter, e.g. lactate [19], ethanol, glucose [20] as carbon source is essential for sulfate-rich wastewater treatment. The costs become an obvious disincentive for a mass of sulfate-rich wastewater produced daily from these industries [18]. Hence, a better understanding of sulfate-rich wastewater treatment using BES is required.

With the aim to optimize the sulfate-rich wastewater treatment and reduce the cost in practical application, we investigated the performance of acclimatized sludge with sulfate at different pHs; the performance of the BES was evaluated by analyzing sulfate removal, carbon source consumption, power generation and microbial community.

2. Methods

2.1. Anaerobic sludge and culture medium

The anaerobic sludge was collected from Jimei Wastewater Treatment Plant, located in Fujian Province, southeast China. The pH of the sludge was 6.85. The raw sludge was filtered through a 2 mm sieve, then was fed with the following culture medium composition (per liter of deionized water): 1.0 g Na_2SO_4 (7.0 mmol), 0.06 g $MgSO_4·7H_2O$, 0.5 g KH_2PO_4, 1.0 g NH_4Cl, 0.03 g $CaCl_2$, 0.3 g sodium citrate, 0.1 g ascorbic acid, 1 ml absolute ethyl alcohol (51.3 mmol), and 1 mL trace elements. The trace elements composed of (per liter of deionized water): 5.0 g EDTA [21], 5.0 g $FeSO_4·7H_2O$, 0.99 g $MnCl_2·4H_2O$, 0.19 g $NiCl_2·6H_2O$, 0.011 g H_3BO_4, 0.2 g $ZnCl_2$, 0.12 g $Na_2MoO_4·2H_2O$, 0.24 g $CoCl_2·6H_2O$. The sludge and culture medium were mixed with the ratio of 1:1 in a blue cap reagent bottle. The pH of mixture was adjusted with 1 M sodium hydroxide or sulfuric acid prior to the bottle being placed in a shaker with 32 °C.

2.2. BES setup

2.2.1. Preparatory stage

The sludge was acclimatized at different pHs of 2.5, 4.5, 6.5, 8.5 and 10.5 in the preparatory stage. The process for sludge acclimation was batch mode. Two-thirds of the supernatant was replaced by fresh medium every 2 weeks. The performance of sludge was analyzed every 2 days. When the sulfate reduction rate remained almost unchanged, the sludge was deemed to have been acclimatized. The preparatory stage lasted for about 3 months.

2.2.2. BES operation

Five BES with different pHs were constructed after the sludge acclimation. The BES was a three-electrode system, which consisted of a working electrode, a counter electrode and a reference electrode. The three electrodes were in one chamber and no membrane was used. Both the working and counter electrodes were activated carbon cloth (Fresh Co., Ltd., China) with a surface area of 10 × 10 cm. Titanium wires were knitted into the electrode to provide connect terminal. A Ag/AgCl reference electrode (CHI111, Chenhua, China) was inserted into the system to record the electrode potentials.

A mixture of acclimatized sludge and culture medium of 500 mL were placed in the system. The potential of the BES was held at +0.2 V vs Ag/AgCl and no external load was used. A Series 4300 Battery Tested System (Maccor, Inc., USA) was connected to the BESs to monitor the current. Three groups of control tests were held at +0.2 V vs Ag/AgCl. The three different experiments were (1) abiotic

setup, (2) control with the same microbial community but without ethanol, and (3) control with the same microbial community but without sulfate. All of the tests were repeated three times.

2.3. Analyses

2.3.1. Chemical analyses

The concentrations of sulfate and thiosulfate ions were determined by the ICS-3000 ion chromatograph (Dionex Co., USA). Samples were passed through a syringe filter (0.45 μm) before the analysis. The concentrations of chemical oxygen demand (COD) were measured by 5B-1 Fast COD Detection Instrument (Lian-hua Tech. Co., Ltd., China). The pH value of solution was measured by UB-7 pH Meter (Denver Instrument, USA). pH measured at the end of test was defined as terminal pH.

The surface morphology of activated carbon cloth was observed by the S-4800 Field Emission Scanning Electron Microscopy (SEM) (Hitachi, Ltd., Japan) equipped with Energy Dispersive X-ray Analysis (EDAX) (Genesis XM2) for the detection of elements.

2.3.2. Electrochemical monitoring

The current generated by the BESs was monitored with 2 min intervals using the Series 4300 Battery Tested System in the condition of constant potential +0.2 V vs Ag/AgCl. The quantity of electric charge detected by Battery Tested System and calculated by COD were respectively marked as Q and Q_t (Eq. (1)) [22]. The coulombic efficiency (CE) is equal to the ratio of Q and Q_t.

$$Q_t = Fb\Delta CODV/m \tag{1}$$

where F = 96485 C mol^{-1} is Faraday constant, b = 4 means removal of 1 mol COD theoretically produce 4 mol electrons, $\triangle$COD is the total consumed COD during the test whose unit is g L^{-1}, V = 0.5 L is the volume of liquid in the BES, m = 32 g mol^{-1} is the molecular weight of oxygen.

2.3.3. Microbiological analyses

When the performance of acclimatized sludge was stable after three-month preparatory stage, acclimatized sludges at different pHs were collected for microbiological analyses. The total DNA of samples were obtained by phenol–chloroform extraction [23]. The extracted DNA was purified using DP 214 Universal DNA Purification Kit (Tiangen Biotech, Co., Ltd., China). The purified DNA was amplified as a template by touchdown polymerase chain reaction (PCR) using the Mastercycler gradient (Eppendorf, Co., Ltd., Germany). The universal primers 341f-GC containing a GC clamp (5′–CCTACGGGAGGCAGCAGCGCCCGCCGCGCGCGGCGGGCGGGGCG GGGGCACGGGGGG-3′) and 534r (5′-ATTACCGCGGCTGCTGG-3′) were employed for amplifying 16S rRNA gene [24]. The procedure of PCR reaction (50 μl) was set up as followed: denaturation at 95 °C for 4 min, followed by 10 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s with 0.5 °C decrement every cycle repeated, and extension at 72 °C for 45 s. After this step, 20 cycles of denaturation at 95 °C for 30 s, annealing at 51 °C for 30 s and extension at 72 °C for 45 s were performed. A final extension at 72 °C for 10 min was conducted before storing at 4 °C.

The PCR products were used for analysis of denaturing gradient gel electrophoresis (DGGE) by the Dcode™ Universal Mutation Detection System (Bio-Rad Laboratories, Inc., USA). The products were loaded onto 10% (W/V) polyacrylamide gels with the denaturant gradient ranging from 30% to 45%. After scanning the gel by the Ettan™ DIGE Imager System (GE Healthcare Ltd., USA), bands were excised from the gels and re-amplified with the primer set 341f/534r. The PCR products were purified with DP 1601 Gel Purification Kit (Spin-column) (BioTeke Co., China). The purified products were cloned using the Takara pMD®19-T Vector and then sent for sequencing (Majorbio, China). The sequences were

compared with the known sequences in the Ribosomal Database Project and National Center for Biotechnology Information Gen-Bank. The sequence data have been submitted to Genbank data-base and the accession numbers are JX548529-JX548555. The Quantity One® 1-D Analysis Software (Bio-Rad Laboratories, Inc., USA) was used for cluster analysis.

3. Results and discussion

3.1. Effect of pH value on the sulfate removal and power generation

After acclimating activated sludge at different pHs, an example of the current–time plots with the system of pH 6.5 was shown in Fig. 1a. Electricity can be generated after 50 h and a maximum current of 4.85 mA was obtained at 140 h. The test was stopped when the current gradually decreased and stabilized at 0.35 mA. At the ending, 86.6% of sulfate was removed from wastewater, 76.3% eth-anol was consumed and 23.2% CE was obtained.

The maximum currents at different pHs ranging from 2.5 to 10.5 were shown in Fig. 1b. A maximum current of 3.08, 4.93 and 5.66 mA were achieved at systems of pH 4.5, 6.5 and 8.5, respectively. At systems of pH 2.5 and 10.5, the maximum current decreased to about 10 μA. There was no current observed in the controls (Fig. S1). Compared with previous reports, the current density obtained here was relatively low. In the BES, the indirect electron transfer mechanism was the biologically reduced sulfate to sulfide, which was chemically oxidized to sulfur on anode sur-face. It should be noted that the electrical current from sulfide oxi-dation is dependent on sulfide concentration and electrode materials. Only 7 mM sulfate was added in this work, it means that a low bio-sulfide concentration can be produced for electrochemi-cal reactions. The current should be much higher if dosing a higher concentration of sulfate or using a high area electrode.

SEM images showed the surface morphology of working elec-trode (activated carbon cloth) before and after the test (Fig. 2). After the wastewater treatment, a layer of white material (Fig. 2b) on the surface of working electrode was scraped off to be observed on SEM. EDAX indicated the material covered in the carbon cloth was elemental sulfur. This indicated that the sulfide obtained from the sulfate reduction was oxidized to sulfur depos-ited in the surface of electrode and electrons transferred to the electrode relied on the role of electrochemistry and microbiology [25,26].

The sulfate removal, COD consumption, CE and terminal pH of system at different pHs were listed in Table 1. Systems of pH 2.5 and 10.5 removed sulfate of 0.8 and 0.6 mM, obtained CE of 0.1 and 1.3%, and consumed COD of 23.7 and 18.0 mM, respectively. The COD consumptions obtained at pH 2.5 and 10.5 were compa-rable with that obtained at pH 4.5, but the sulfate removal and CE were low in the two systems. These data may mean that some
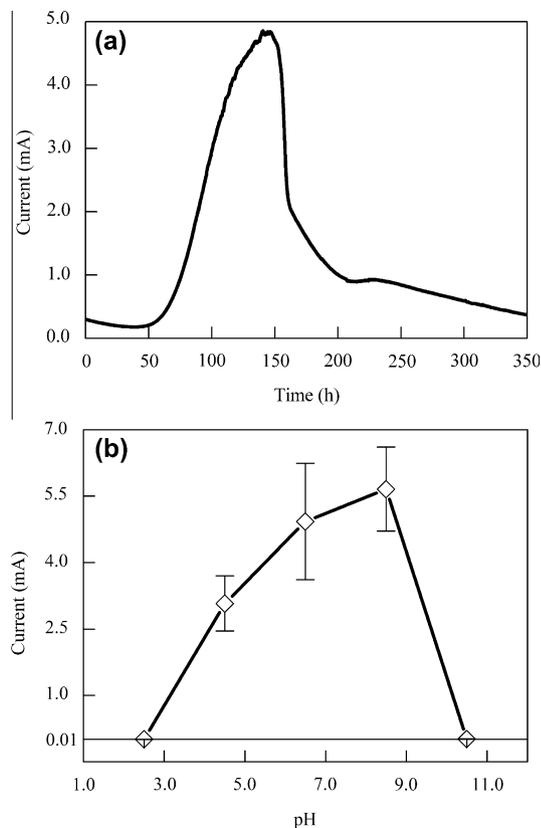

Fig. 1. Tests held at a constant potential of +0.2 V vs Ag/AgCl: (a) current–time plots of acclimatized sludge at the pH value of 6.5, (b) the maximum current for acclimatized sludge at different pHs.
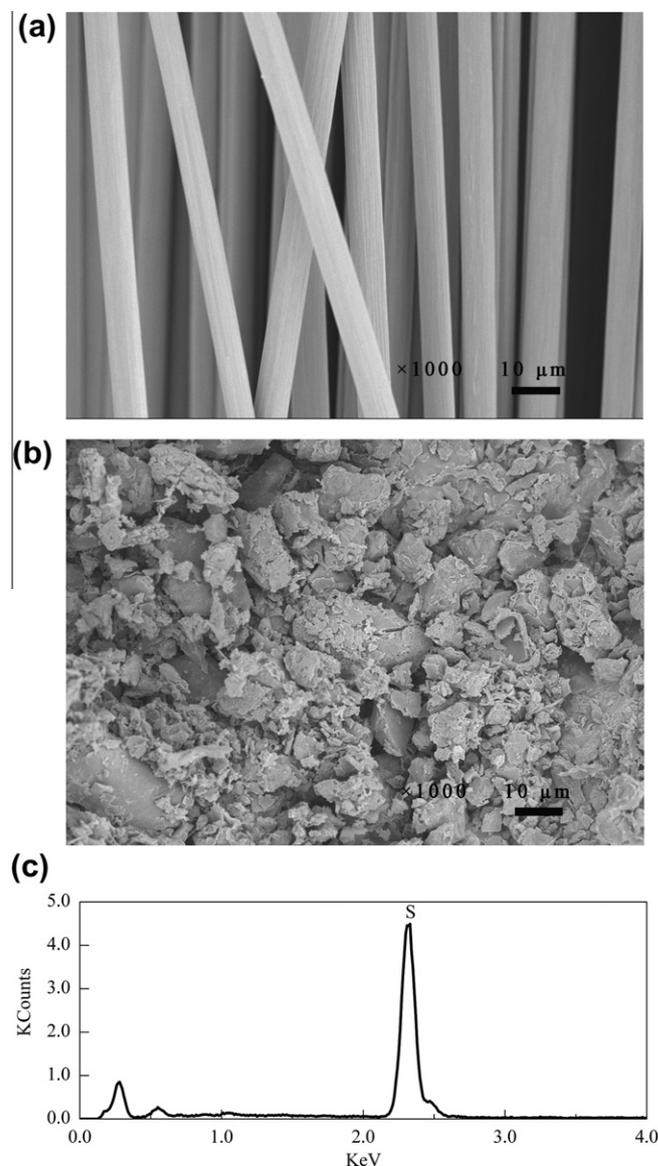

Fig. 2. SEM pictures of working electrode in the BES: (a) before using, (b) material (white color) scraped off from the activated carbon cloth after the test, and (c) EDAX of the material scraped off from the activated carbon cloth in Fig. 2b.

Table 1
The sulfate removal, COD consumption, CE and terminal pH of BES at different pHs.

pH	Sulfate removal (mM)	COD consumption (mM)	CE (%)	Terminal pH
2.5	0.8 ± 0.2	23.7 ± 10.7	0.1 ± 0.0	2.8 ± 0.4
4.5	6.5 ± 0.1	27.0 ± 11.1	27.1 ± 0.1	6.2 ± 0.3
6.5	5.8 ± 1.2	37.0 ± 7.9	27.7 ± 9.5	6.5 ± 0.2
8.5	4.6 ± 1.7	43.0 ± 1.4	35.8 ± 11.1	6.7 ± 0.2
10.5	0.6 ± 0.0	18.0 ± 3.9	1.3 ± 0.3	9.4 ± 0.4

bacteria at pH 2.5 and 10.5 can consume COD but not reduce sulfate nor produce electricity. The BESs of pH 2.5 and 10.5 would not be discussed in this study because they were not fit for the treatment of sulfate-rich wastewater.

The sulfate removal at systems of pH 4.5, 6.5 and 8.5 were higher than that of 2.5 and 10.5. The concentrations of thiosulfate were below the detection limit (data not shown). The maximum sulfate removal of 6.5 mM was achieved at system of pH 4.5. Meanwhile, 5.8 and 4.6 mM sulfate were reduced at systems of pH 6.5 and 8.5, respectively. Systems of pH 6.5 and 8.5 utilized 37.0 and 43.0 mM COD, respectively. At the same time, system of pH 4.5 consumed COD of 27.0 mM, which was the least among the three systems. Reducing the carbon dosage could reduce the cost correspondingly.

CE of the system was calculated by Eq. (1). The highest CE of 35.8% was obtained at the system of pH 8.5. For pH 4.5 and 6.5, 27.1% and 27.7% were achieved, respectively. In an aqueous solution with pH less than 7.0, sulfide exists mainly in the form of H_2S; sulfide presents in the form of HS^- when pH is between 7.0 and 13.9 [25]. Based on different forms of sulfide present in solutions with different pHs, conversion efficiency of sulfide oxidizes to elemental sulfur is different. According to the results, conversion efficiency in acidic solution was lower than that in basic solution, which caused CE in system of pH 4.5 decreased compared with that in pH 8.5. At the end of test, the pH value was measured. As shown in Table 1, all the pH in the systems moved towards the neutral direction. For the system of pH 4.5, pH rose from the initial 4.5 to terminal 6.2, which was beneficial to the discharge of effluent wastewater. Bacteria have a high activity at pH 8.5, and protons liberated from ethanol oxidation decrease pH of the system. At pH 4.5, some bacterial activity was inhibited due to acidification; however, the reduction of sulfate by SRB using ethanol liberates bicarbonate and increases pH of the system.

The BES of pH 6.5 and 8.5 achieved a bit higher current than that of pH 4.5. However, sulfate pollutants largely derive from acidic wastewater and the pHs are around 3–4. Sodium hydroxide is added into wastewater to adjust pH for bio-treatment. In practical application, a mass of sulfate-rich wastewaters are produced daily. The cost of sodium hydroxide is increasing exponentially with the amount of wastewater, but the power is not generated that much. Compared with the other two systems, the BES of pH 4.5 was the most economical way to treat sulfate-rich wastewater (Table 2). The BES of pH 4.5 had a higher sulfate removal, a less COD consumption, a reasonable CE and a suitable effluent pH. It can draw a conclusion that pH 4.5 was optimum for acclimatized
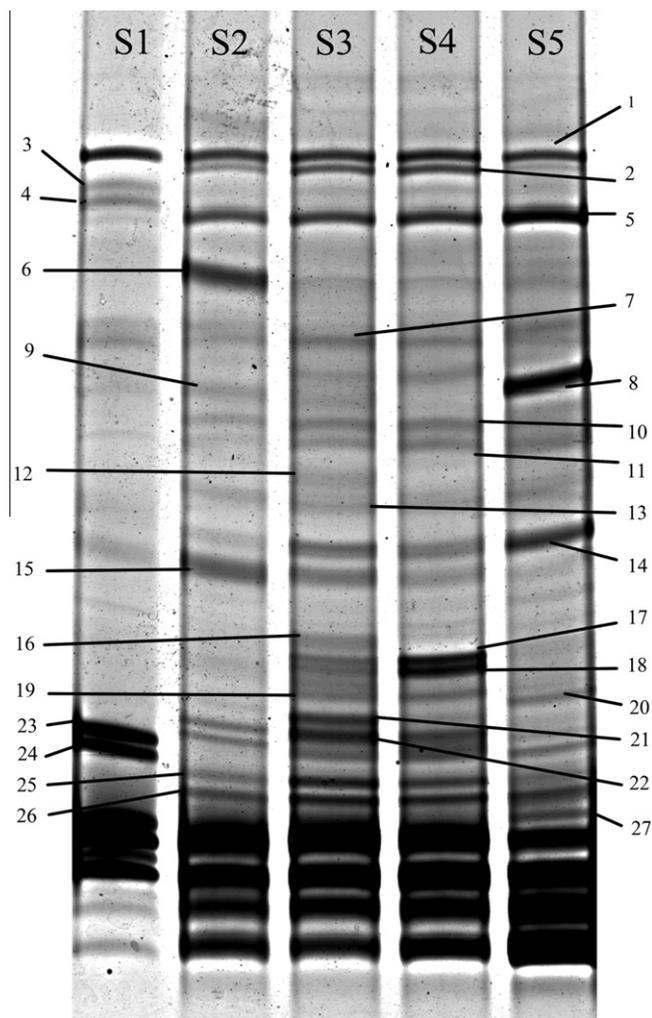

Fig. 3. DGGE profiles of acclimatized sludge at different pHs with an 10% polyacrylamide 30–45% denaturing gradient gel.

sludge treating sulfate. The results, to some extent, could provide a basis for the acidic wastewater treatment with the BES.

Sulfate-reducing bacteria theoretically should be the predominant bacteria in acclimatized sludge with sulfate. However, this

Table 2
Differences of cost and benefit between the systems of (1) pH 4.5 and 6.5, (2) pH 4.5 and 8.5.

BES	Cost differences				Benefit difference		Total value ($)
	pH adjustment		Ethanol dosage		Electricity		
	M_{NaOH} (g)	Value ($)[a]	V (ml)	Value ($)[b]	W (Wh)	Value ($)[c]	
6.5 vs 4.5	$2 \times 10^{-3.5}$	$-10^{-6.5}$	0.10	-10^{-4}	0.05	0.5×10^{-5}	-0.95×10^{-4}
8.5 vs 4.5	$2 \times 10^{-3.5}$	$-10^{-6.5}$	0.15	-1.5×10^{-4}	0.11	1.1×10^{-5}	-1.39×10^{-4}

[a] Assuming industrial sodium hydroxide values 5×10^{-4} \$ g^{-1}.
[b] Assuming industrial absolute ethyl alcohol values 10^{-3} \$ ml^{-1}.
[c] Assuming an electricity values 0.1 \$ kw h^{-1}.

result was different from the previous study of pH 6–8 suitable for growth and metabolism of sulfate-reducing bacteria. Therefore, the microbial community was studied to see whether other microorganisms involved in this process besides sulfate-reducing bacteria.

3.2. Effect of pH value on the microbial community

Microbial communities of five acclimatized sludge at different pHs were analyzed by DGGE. Sludge acclimatized at pH 2.5, 4.5, 6.5, 8.5 and 10.5 were marked as S1, S2, S3, S4 and S5, respectively. The DGGE profiles of these five samples were summarized in Fig. 3. The differences in numbers and brightness of bands for each sample showed the differences in microbial community. The microbial diversities of S2, S3 and S4 were much more abundant than that of S1 and S5. Cluster analysis of the DGGE pattern according to the
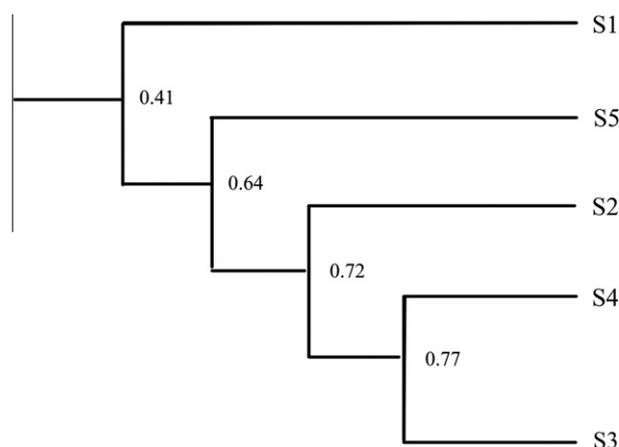

Fig. 4. Cluster analysis of the DGGE pattern.

single linkage clustering in Quantity One® 1-D Analysis Software was shown in Fig. 4. The similarities between S3 and S4 reached up to 77% corresponding to the similar maximum current systems of pH 6.5 and 8.5 achieved (Fig. 1b). These were due to the pH 6.5 and 8.5 are closed to neutral. The similarities between S1 and any other sample were lower than 50%, whereas the similarities between any other two samples except S1 were higher than 50%. The results indicated that some specific microorganisms existed in extreme acidic condition.

Table 3 visually showed the microbial diversity of samples. The acclimatized sludge community was mainly composed of *Deltaproteobacteria* (33.3%), *Bacteroidetes* (14.8%), *Epsilonproteobacteria* (11.1%), *Clostridia* (7.4%), *Chloroflexi* (7.4%), *Chlorobia* (3.7%), *Betaproteobacteria* (3.7%), *Gammaproteobacteria* (3.7%) and *Bacilli* (3.7%). According to Genbank or references about bacteria in the same genus, possible function of each species was displayed in Table 3. The descriptions showed that sulfate reducers, sulfur reducers and sulfur oxidizers were dominant bacteria in the microbial community. In the 17 bands which can be initially determined functionality, eight bands had high homology with sulfate-reducing bacteria, five bands with sulfur-reducing bacteria and four bands with sulfur-oxidizing bacteria. The five BESs showed different desulfurization performance since different bacteria had different adaptabilities to pH.

Bands 1, 4 and 24 had high homology with *Desulfurella* sp., which had the ability to reduce sulfur. Different from bands 4 and 24 only existed in pH 2.5, band 1 had a strong growth activity at a wide pH range. Band 2, the dominant bacteria at pH 4.5–10.5, was phylogenetically related (99% similarity) to *Desulfuromonadaceae* bacterium enrichment culture clone B31212. Previous study demonstrated that *D. acetoxidans*, belongs to *Desulfuromonadaceae* bacteria, produced power in a two-chambered sediment fuel cell [27]. This strain might play a certain role in producing electricity in the systems of pH 4.5, 6.5 and 8.5. At pH 8.5, band brightness

Table 3
The identifications of DGGE bands.

Band	Closest GenBank match	Class	Identity (%)	Accession no.	Description
1	*Desulfurella multipotens* strain RH-8	*Deltaproteobacteria*	96	NR_026461	Sulfur reducer [32]
2	*Desulfuromonadaceae* bacterium enrichment culture clone B31212	*Deltaproteobacteria*	99	HQ133036	Sulfur reducer [33]
3	*Acidithiobacillus thiooxidans*	*Gammaproteobacteria*	99	AF359940	Sulfur oxidizer (Chemolithotrophic) [26]
4	Uncultured *Desulfurella* sp. clone SK665	*Deltaproteobacteria*	96	DQ834026	Sulfur reducer
5	Uncultured *Chloroflexi* bacterium clone QEDN8AA01	*Chloroflexi*	99	CU926200	–
6	Uncultured *Paludibacter* sp. clone 17–13	*Bacteroidetes*	98	FJ888420	Sulfate reducer
7	Uncultured *Desulfobulbaceae* bacterium clone DBB4	*Deltaproteobacteria*	97	EF187874	Sulfate reducer
8	Uncultured *Clostridium* sp. clone MBfR_NS-181	*Clostridia*	96	JN125737	–
9	Uncultured *Chlorobi* bacterium clone QEDN2DF11	*Chlorobia*	100	CU927353	Sulfur oxidizer (phototrophic green sulfur bacteria)
10	*Desulfomicrobium hypogeium*	*Deltaproteobacteria*	98	AB237494	Sulfate reducer
11	*Desulfomicrobium* sp. SA2	*Deltaproteobacteria*	99	AY548759	Sulfate reducer
12	*Sulfurospirillum* sp. NO3A	*Epsilonproteobacteria*	100	AY135396	Sulfur reducer [34]
13	Uncultured *Bacteroidetes* bacterium clone KWK23F.45	*Bacteroidetes*	93	JN656899	–
14	Uncultured *Chloroflexi* bacterium clone D25_30	*Chloroflexi*	99	EU266904	–
15	Uncultured *Bacteroidetes* bacterium clone QEDQ3DC06	*Bacteroidetes*	99	CU922962	–
16	Uncultured *epsilon proteobacterium* clone MC1_bact_cl30	*Epsilonproteobacteria*	99	DQ295689	–
17	Uncultured bacterium clone W6-38-SC1-100	–	100	AF486679	Sulfate reducer [35]
18	*Desulfomicrobium* sp. PR6_H04	*Deltaproteobacteria*	99	HE600843	Sulfate reducer
19	Uncultured *Bacteroidetes* bacterium clone 3A10	*Bacteroidetes*	98	HQ003601	–
20	Uncultured *Firmicutes* bacterium clone QEDR1DD02	–	98	CU922223	–
21	Uncultured *Bacteroidetes* bacterium clone 2H27	*Bacteroidetes*	97	GU074133	–
22	*Sulfuricurvum* sp. enrichment culture clone D2CL_Bac_16S_Clone8	*Epsilonproteobacteria*	100	EU498374	Sulfur oxidizer (Chemolithotrophic) [36]
23	Uncultured *Thiobacillus* sp. clone SK673	*Betaproteobacteria*	96	DQ834033	Sulfur oxidizer (Chemolithotrophic)
24	Uncultured *Desulfurella* sp. clone SK688	*Deltaproteobacteria*	97	DQ834048	Sulfur reducer
25	*Bacillus funiculus* strain NK20.3	*Bacilli*	100	AY169820	–
26	Uncultured *Desulfovibrio* sp. clone ZZ-S12F11	*Deltaproteobacteria*	99	EF613482	Sulfate reducer
27	*Desulfotomaculum carboxydivorans* strain CO-1- sulfate-reducing bacteria; DSM 14880; VKM B-2319	*Clostridia*	95	NR_043297	Sulfate reducer

seemed to be the biggest, which indicated that sulfur reduction and power generation of this strain might be better in neutral partial alkali condition. The gene sequence of bands 3 and 23 both showed similarities of more than 95% to *Thiobacillus* sp. They had the biggest brightness at pH 2.5 showed that they were acidophilic bacteria. Band 5 could be alive at pH 2.5–10.5, but at pH 2.5 its activity should be lower than at other pHs. According to the brightness of band in DGGE profile, pH 10.5 may be more close to its optimum pH.

Band 6 showed the distinct difference between S2 and other samples. It had a similarity of 98% with uncultured *Paludibacter* sp. clone 17-13, which was found among the sulfate-reducing microbial community in sediments of an acidic mine pit lake. Moreover, *Paludibacter propionicigenes* strain WB4 has been confirmed to reduce sulfate during growth [28]. Taken together the previous results that system of pH 4.5 achieved the maximum sulfate removal, *Paludibacter* sp. should have a good removal capacity of sulfate at pH 4.5. Band 6 had the highest activity at pH 4.5. The desulfurization performance could be further improved if its proportion in the system could increase.

Bands 7–13 were relatively weak, and band 7, 10 and 11 had high homology with sulfate-reducing bacteria. Band 7, existed in all the samples with almost the same abundance, had a similarity of 97% with uncultured *Desulfobulbaceae* bacterium clone DBB4. Previous article reported that *D. propionicus* could transfer electrons to Fe(III) and graphite electrodes along with its own growth [29]. The electricity production capacity of *Desulfobulbaceae* bacteria were likely to be affected by the pH value combined with the electricity production data. Band 8, existed mainly in the sample of pH 10.5, had a similarity of 96% with uncultured *Clostridium* sp. clone MBfR_NS-181. It not belonged to sulfur bacteria and may be the bacteria consumed COD but not reduced sulfate nor produced electricity mentioned above. The growth pH range for band 14 and 15 was wide, but the two strains had different optimum pH. Band 14 seemed more likely alkaline condition, and band 15 was more suitable for acidic condition. Band 15 might have the highest activity at pH 4.5 according to DGGE profile, which meant that the strain was essential to the system performance. However, we knew little about the function of the strain as the homology sequence belonged to an uncultured organism clone.

Bands 16 and 21 were dominant bacteria in neutral condition. Band 17 and 18 had high homology with sulfate-reducing bacteria, which indicated that the two strains may be important for sulfate reduction in partial alkaline condition. Bands 20 and 27 both only existed at pH 10.5, showed that they maybe alkalophilic bacteria. Bands 22, 25 and 26 had higher activity at pH 4.5–10.5. Band 26 had high homology with sulfate-reducing bacteria and was bright in each lane, implied that the strain might play an important role in sulfate reduction. The gene sequence of band 26 showed a 99% similarity to uncultured *Desulfovibrio* sp. clone ZZ-S12F11, which was obtained from *in situ* benzene degradation under sulfate-reducing conditions [30]. It has been observed that *D. desulfuricans* could produce current with sulfite and thiosulfate removal using microbial fuel cells [31]. Hence, the *Desulfovibrio* sp. contributed to the electricity production at pH 4.5–10.5 in all possibility.

According to the DGGE analysis, *Desulfuromonadaceae* sp., *Desulfobulbaceae* sp. and *Desulfovibrio* sp., which were reported as sulfate reducers or sulfur reducers, were inferred to play key roles in power generation. Based on the speculation, *Paludibacter* sp. should have a good removal capacity of sulfate at pH 4.5.

4. Conclusions

In this study, sulfate removal, power generation and microbial community were analyzed on the basis of acclimatizing sludge with sulfate at different pHs. According to the performance of acclimatized sludge, pH 4.5 was optimum for sulfate pollutant treatment by the BES. *Paludibacter* sp. might play the most important role in sulfate removal in this system; in addition, several bacteria were inferred to make contribution to power generation by DGGE profiles and sequence analysis. These results could help to reduce capital cost and assist in optimization of sulfate-rich wastewater treatment.

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2012.12.021.

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