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Simultaneous removal of organic carbon, nitrogen and sulfate, electricity generation and microbial community in anaerobic fluidized bed-microbial fuel cell

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ABSTRACT

The simultaneous desulfurization and denitrification technology has better economic and environmental values than conventional sulfate and nitrogen removal process. An anaerobic fluidized bed-microbial fuel cell (AFB-MFC) system was established to elucidate the effect of anodic different nitrate load on contaminant removal, electricity generation and anodic microbial community in a symbiotic ecosystem for sulfate reduction bacteria (SRB) and nitrate-reducing, sulphide-oxidizing bacteria (NR-SOB). Results showed that, when anodic load was 700 mg NO_3^-–N/(m^3 d), 2.1 kg SO_4^{2-}/(m^3 d) and 3.36 kg COD_{Cr}/(m^3 d), the removal rate of NO_3^-–N, SO_4^{2-} and COD_{Cr} was 96.43%, 52.68% and 98.02%, theoretical sulfur yield was 0.92 kg/m^3 d, and output voltage and power density were 7.23 mW m^{-2} and 385.46 mV, respectively. It proved that the AFB-MFC had a good performance in treating organic wastewater containing nitrates and sulfates. Sulfur had also been observed in effluent. As the anodic NO_3^- load increased, the diversity of microbial community increased, hydrogen-producing bacteria, SRB and strains related to nitrate removal also increased significantly. The main functional bacteria were *Sulfurovum*, *Desulfomicrobium*, *Thauera* and *Sulfurimonas*. AFB-MFC system provides a new approach to cost-effective treatment of organic wastewater containing nitrate and sulfate.

Keywords: Organic wastewater containing nitrate and sulfate; Microbial community; Electricity generation; Anaerobic fluidized bed; Microbial fuel cell

1. Introduction

Organic materials containing sulfur and nitrogen are used in many industrial processes (such as petrochemical, leather, food and paper processes), which results in the production of a large amount of rich organic wastewater containing high sulfate and nitrogen [1]. Rich organic wastewater is widely treated by anaerobic biological treatment for its cost-effective and environmentally friendly nature [2]. During anaerobic biological treatment, sulfate was biologically reduced to sulfide or H_2S. Therefore, sulfide is the main by-product of sulfate wastewater treatment [3]. Sulfide not only affects the removal of pollutants by inhibiting the growth of microorganisms, but is also corrosive and odorous, which can cause corrosion of sewage pipes. Sulfide removal is usually by oxidation, but oxygen solubility of wastewater is low, so additional energy is required for aeration. In addition, it easily escapes in a gaseous form under aerobic condition, causing pollution.

Therefore, autotrophic NR-SOB has attracted extensive attention. It uses nitrate as an electron acceptor and various types of sulfur-containing compounds (such as sulfides, polysulfides, sulfur, sulfate and sulfite) as electron donors to remove nitrates and sulfides simultaneously [4]. Besides, NR-SOB can oxidize sulfide to sulfur or sulfate, thereby eliminating the inhibition of end

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product of sulfate-reducing bacteria (SRB). And SRB can consume organic matter, which is beneficial to the process of autotrophic denitrification. So SRB and NR-SOB have a synergistic relationship. Thus, simultaneous removal of organic carbon, sulfate and nitrate by anaerobic process is theoretically feasible, and it has been confirmed in some anaerobic reactors such as anaerobic expanded granular sludge bed and anaerobic fixed bed [4–6]. However, relatively low conversion rate of sulfur limited its development, and it was strict to control operating condition to maintain the activity and quantity of functional microorganisms in the long-term continuous operation [7].

Microbial fuel cells (MFCs) are a new (bio) electrochemical system that can be used to recover energy and nutrients from various organic wastes. MFCs could produce electricity while removing organic carbon and nitrogen contaminants to improve the economics and efficiency of wastewater treatment. And in MFCs, sulfur was the main product of electrochemical oxidation of sulfide, which meant the conversion rate of sulfur was improved [8]. But study of MFCs on simultaneous removal of carbon, nitrogen and sulfate is still in early stage, and related applications in this aspect are still not enough. Based on this, this experiment constructed an AFB-MFC reactor to study how to improve both treatment efficiency of carbon, nitrogen and sulfate and power generation performance.

2. Materials and methods

2.1. Experimental set-up and operating conditions

The experimental setup is shown in Fig. 1. The system was made of plexiglass, the anode was an anaerobic fluidized bed (AFB) reactor (10.66 L effective volume) with three-phase separation, and the cathode was aerobic activated sludge zone (9.96 L aeration zone + 3.81 L settlement zone). The cathode and anode were separated by a proton exchange membrane (PEM). The anode chamber whose wall was excavated partly was attached to a porous (7 × 15 hole) plexiglass plate (180 mm × 450 mm). The cathode chamber whose wall was also excavated partly was attached to the other side of the porous plexiglass plate. PEM (Nafion 117, Dupond, USA) was sandwiched between the cathode wall and the plexiglass plate. Nafion membrane parameters were as follows: thickness, 183 um; gram weight, 360 g/m^2; electrical conductivity, 0.083 s/cm; exchange capacity, 0.89. Anode and cathode electrodes (effective area of 200 cm^2) were all made of porous carbon paper (HCP030). Parameters of HCP030 carbon paper were summarized as follows: thickness, 0.3 mm; density, 0.78 g/cm^3; porosity, 75%; resistivity, 3 mΩ cm; gas resistance, 12 mm H_2O; bending radius, 10 cm. In addition, in this experiment, a porous polymer material was added to anode chamber of AFB-MFC, and its parameters were as follows: wet particle size: 0.56 mm; dry particle size: 0.32 mm; skeleton density: 1,320 kg m^{-3}; wet bulk density: 1,010 kg m^{-3}; pore volume: 0.30 mL g^{-1}; wet specific surface area: 5,357 m^2 m^{-3}.

Wastewater was uniformly fed into AFB-MFC from the bottom, and was in full contact with anaerobic biological granular sludge. Organic matter was utilized by anaerobic microorganisms, and the water that flowed out entered

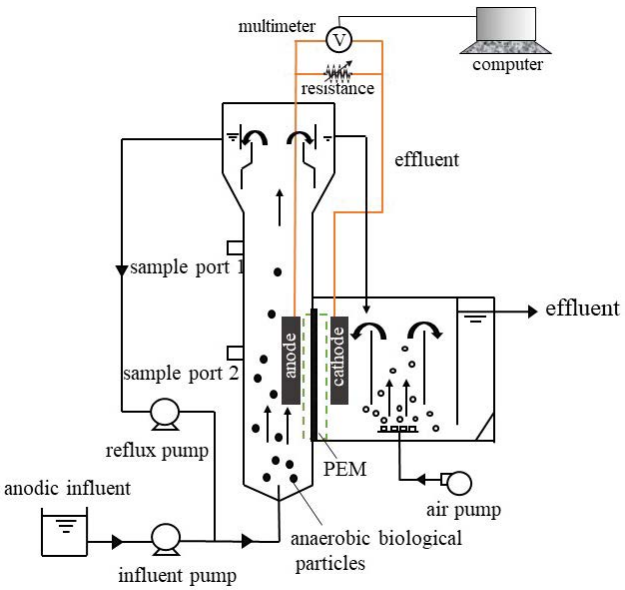

Fig. 1. Schematic representation of AFB-MFC.

aerobic tank (cathode chamber) through the upper hose. Gas (such as H_2S, N_2, CH_4 and CO_2) generated during anaerobic degradation was collected, and was discharged after passing through an alkaline solution. An electromagnetic constant temperature heater maintained temperature in AFB at 37°C ± 2°C to ensure anaerobic microbial activity. The cathode chamber was an aerobic biocathode, and was aerated by an air compressor and a microporous aeration head, and aeration amount was 2–4 L/min (controlled by a rotameter). The cathode and the anode were connected by an external circuit system, and the external resistance was fixed to 1,000 Ω. The output voltage was recorded by a computer connected to UT70B multimeter at intervals of 120 s.

2.2. Experimental wastewater and reactor operating parameters

This study used synthetic simulated wastewater to provide a continuous stable source of biodegradable organic pollutants. Added Na_2SO_4 and $NaNO_3$; glucose as organic carbon source; NH_4Cl and KH_2PO_4 in a ratio of 5:1(N:P); 32,500 mg/L $NaHCO_3$; 300 mg/L $CaCl_2$; and 400 mg/L $MgCl_2{\cdot}6H_2O$ to tap water. 1 mL of nutrient solution 1 and nutrient solution 2 was added to each liter of influent water. The composition of nutrient solution 1 was (g/L): EDTA, 15.00; $ZnSO_4{\cdot}7H_2O$, 0.42; $CuCl_2{\cdot}2H_2O$, 0.17; $NiSO_4{\cdot}6H_2O$, 0.21; H_3BO_3, 0.014; $CoSO_4{\cdot}7H_2O$, 0.28; $MnSO_4{\cdot}H_2O$, 0.85; $(NH_4)_2MoO_4$, 0.20. The composition of nutrient solution 2 was (g/L): EDTA, 15.00; $FeSO_4{\cdot}7H_2O$, 5.00. The experimental cycle was divided into six stages. Effect of $NaNO_3$ concentration on the performance of AFB-MFC was investigated in stage 1–5. Fixed influent COD_{Cr}/SO_4^{2-} to 1.6/1, that was, COD_{Cr} was 3,360 mg/L (load was 3.36 kg COD_{Cr}/(m^3 d)), SO_4^{2-} was 2,100 mg/L (load was 2.1 kg SO_4^{2-}/(m^3 d)), NH_4 was 84 mg/L (load was 0.084 kg N/(m^3 d)), and KH_2PO_4 was 16.8 mg /L. Influent NO_3^- (in terms of N) was 100 mg/L (stage 1), 200 mg/L (stage 2), 350 mg/L (stage 3), 500 mg/L (stage 4) and 700 mg/L (stage 5). When influent NO_3^-–N was

700 mg/L, the efficiency of AFB-MFC was deteriorated, so influent organic matter was adjusted to 5,000 mg/L in stage 6 to examine the recovery of the reactor performance.

The relevant reactor operating parameters were as follows: in anaerobic anode, the hydraulic retention time was 23.66–24.38 h, and the reflux was 4.8 L/h; in aerobic cathode, the reflux was 200% (20 L/d), the dissolved oxygen (DO) was controlled at 4.5–5.3 mg/L.

2.3. Analytical methods

NH_4^+–N, NO_2^-–N, NO_3^-–N, biomass concentration (MLSS) and volatile suspended solids (VSS) were analyzed according to Standard Methods [9]. SO_4^{2-} was measured by ion chromatography using a Dionex ICS-1100 (Thermo Fisher Scientific Inc., MA, USA). S^{2-} was measured by an iodometric titration method. Voltage was measured by a digital multimeter at definite time intervals and the volumetric power density was calculated as $P = U^2/RA$, where U is the recorded voltage (V), R is the external resistance (Ω) and A is the surface area of the anode electrode (m^2). After the reactor operated stably, COD_{Cr}, NH_4^+–N, NO_3^-–N, NO_2^-–N, S^{2-} and SO_4^{2-} of anaerobic and aerobic wastewater were measured every 3 d in each stage.

Microbial communities of the anodes in stage 2 (sample 1) and stage 5 (sample 2) were studied by 16S rRNA amplicon sequencing. Biomass samples in stage 2 and 5 were collected and immediately stored at –80°C. Subsequent work included DNA extraction, PCR amplification, high throughput sequencing and data analysis by Sangon Biotech Co., Ltd., (Shanghai, China). DNA extraction was performed by an EZNA™ Mag-Bind Soil DNA kit (Omega Bio-tek, Inc., Norcross, GA, USA).

After total genomic DNA was extracted from each sample, M-340F and GU1ST-1000R as the archaeal primer were used to amplify the V3-V4 region of the 16S rRNA gene, and 341F and 805R as the bacterial primers were used to amplify the V3-V4 region of the 16S rRNA gene. Prinseq, FLASH, Mothur, Uclust, Cytoscape, Oiime, Muscle, MEGAN, RDP and Fasttree were used for sequencing. Databases used for sequencing were the RDP classifier database, the Silva database and the Unite database.

3. Results and discussion

3.1. Organic carbon, nitrogen and sulfate removal performance of AFB-MFC

3.1.1. COD_{Cr} removal

The effluent COD_{Cr} concentration and removal efficiency at different stages were shown in Fig. 2. First, the average COD_{Cr} of anaerobic effluent decreased from 825.12 to 189.47 mg/L, and corresponding removal efficiency increased from 75.89% to 93.56% with the increase of the influent NO_3^- concentration in stage 1–5. It indicated that the amount of organic matter consumed by heterotrophic denitrification increased as the influent NO_3^- increased, and anaerobic organic matter removal ability was positively correlated with influent NO_3^-. In stage 6, influent COD_{Cr} increased from 3,360 to 5,000 mg/L, and the anaerobic effluent COD_{Cr} also increased from 189.47 to 492.75 mg/L, but the removal efficiency decreased slightly from 93.56% to 90.12%. This phenomenon could be explained by NO_3^- removal path. As shown in Fig. 3b, when influent NO_3^- was 700 mg/L, the anaerobic effluent sulfide was 96.45 mg/L in stage 5 and rose to 198.56 mg/L in stage 6, while the NO_3^-–N removal efficiency was always above 99%. This indicated that NO_3^-–N was removed mainly by both heterotrophic denitrification and autotrophic denitrification in stage 5 but by heterotrophic denitrification in stage 6. It is because the increase of influent organic matter had an inhibitory effect on NR-SOB [10]. Therefore, the consumption of the anode organic matter increased in stage 6, but COD_{Cr} in the anaerobic effluent also rose due to an excessive organic matter increase in the influent water.

In aerobic cathode, aerobic sludge maintained high activity and had good resistance to load fluctuation after adapting to new substrate. As shown in Fig. 2, the aerobic effluent COD_{Cr} gradually decreased from 187.73 to 75.95 mg/L and maintained below 80 mg/L in stage 2–6.

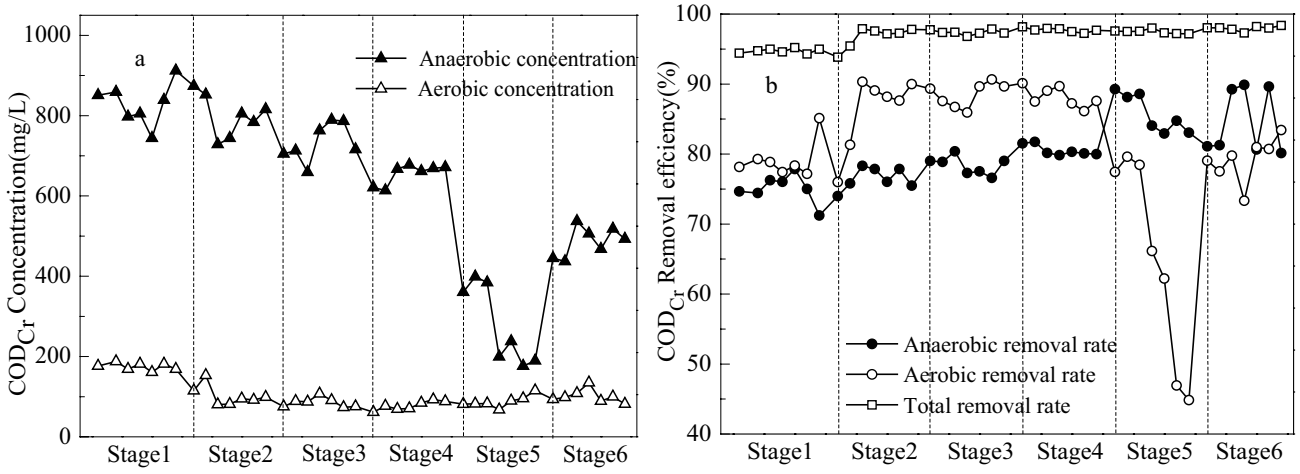

Fig. 2. Effluent COD_{Cr} concentration (a) and removal efficiency (b) at stage 1–6.

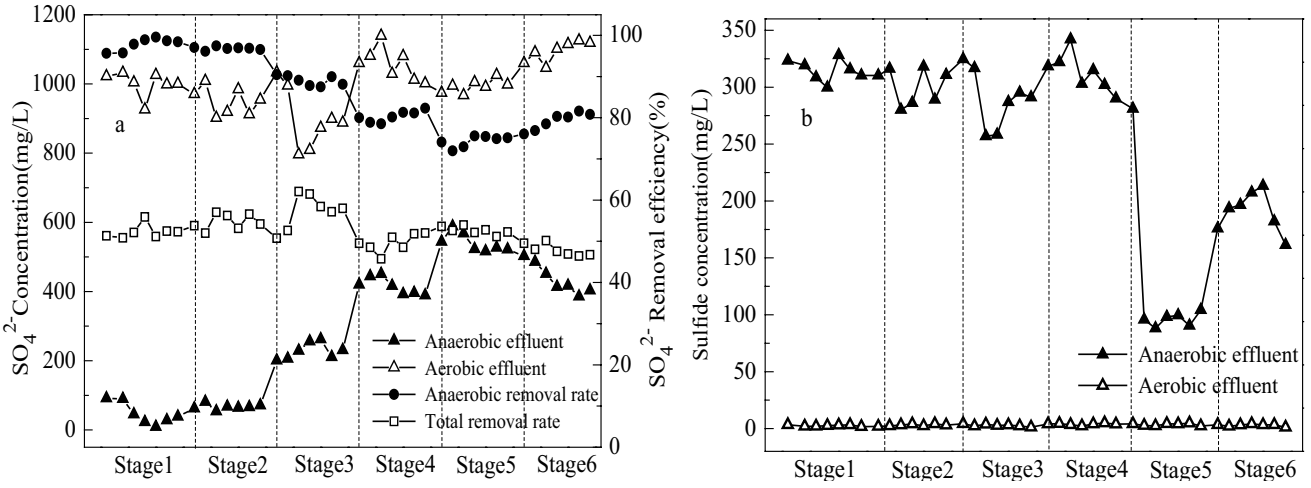

Fig. 3. Effluent SO_4^{2-} (a) concentration and removal efficiency and S^{2-} (b) concentration in stage 1–6.

From the perspective of entire AFB-MFC system, total removal efficiency of COD_{Cr} could be maintained above 95% throughout the experiment, that is, the AFB-MFC system can effectively remove organic carbon.

3.1.2. SO_4^{2-} removal

As shown in Fig. 3a, SO_4^{2-} in the anaerobic effluent gradually increased from 65.48 to 512.67 mg/L, and the removal efficiency decreased from 96.78% to 75.48% with the increase of NO_3^- in stage 1–5. That is to say, the removal efficiency of SO_4^{2-} was negatively correlated with the influent NO_3^-. It could be explained in two ways. (1) In anaerobic biological treatment, the heterotrophic denitrification takes precedence over the sulfate reduction. When a large amount of nitrate was present, the utilization of the electron donor by SRB was inhibited, which was not conducive to the progress of sulfate reduction [11]. Therefore, the growth of denitrifying bacteria was promoted as the increase of NO_3^-, denitrifying bacteria competed with the SRB for substrate, and the organic matter that SRB used for sulfate reduction was reduced, which made the removal efficiency of SO_4^{2-} decreased. (2) Denitrifying bacteria reduced nitrate to nitrite, and nitrite inhibited the reduction of sulfate by inhibiting its metabolic pathway [12]. Therefore, the nitrite produced by denitrification increased as the nitrate increased, which was not conducive to SO_4^{2-} removal. When COD_{Cr} increased to 5,000 mg/L in stage 6, SO_4^{2-} in anaerobic effluent gradually decreased from 512.67 to 407.85 mg/L, and the removal efficiency recovered from 75.48% to 81.62%.

As shown in Fig. 3b, the sulfide in the anaerobic effluent was maintained at a high level (300 ± 15 mg/L) in stage 1–4. But in stage 5, the sulfide decreased significantly to 96.45 mg/L. After the influent organic load increased, the anaerobic effluent sulfide again rose to 198.56 mg/L in stage 6. The nitrate removal pathway can explain the phenomenon: the nitrate was mainly removed by the heterotrophic denitrification, the sulfide obtained by the sulfate reduction was mainly removed by the biological oxidation and electro-oxidation in stage 1–4, and the excess sulfide flew out through anaerobic effluent. But when NO_3^-–N raised to 700 mg/L, a part of the nitrate was removed by autotrophic denitrification, that is, part sulfide was oxidized to sulfur by NR-SOB in stage 5 [13]. So the sulfide in anaerobic effluent decreased. After organic matter was added, the heterotrophic denitrification again became the main role of nitrate removal in stage 6, so the effluent sulfide rose again.

After anaerobic effluent entered the cathode, various types of sulfur-containing compounds (such as sulfide, thiosulfate, and sulfite) in the anaerobic effluent were oxidized to sulfur and sulfate by sulfide-oxidizing bacteria (SOB). Therefore, the sulfide in the aerobic effluent was extremely low, and was kept below 3 mg/L, which avoided the secondary pollution caused by the sulfide produced in the traditional sulfate wastewater treatment.

During this experiment, the anaerobic effluent was milk-white turbid liquid, and pale yellow particles were found on the top of the AFB and in the anaerobic effluent, confirming the production of sulfur. The pH in the anode chamber was 6.99–7.68 during the experiment, so the sulfide in the anode chamber was in the form of S^{2-} or HS^-. It was because that only pH is 6, the sulfide is present in the form of H_2S [14]. Additionally, there were not H_2S and SO_3^{2-} detected. Based on above, theoretical sulfur yield of the entire AFB-MFC system can be calculated by the discrepancy of dissolved inorganic sulfur between anaerobic influent and aerobic effluent. The results are shown in Fig. 4, showing a trend of increase first and then decrease. The theoretical sulfur yield gradually increased from 0.75 to 0.82 kg/m^3 d in stage 1–4, and rose rapidly to the maximum value of 0.92 g/m^3 d in stage 5, but dropped to 0.80 kg/m^3 d in stage 6. Results confirmed that when organic substrate became insufficient due to the increase of influent nitrate, NR-SOB reduced nitrate and oxidized sulfide to obtain sulfur. In addition, the sulfide in stage 5 was significantly lower than other stages, which alleviated the inhibition of sulfide to sulfate reduction and sulfur electrochemical oxidation. Both were conducive to the accumulation of sulfur.

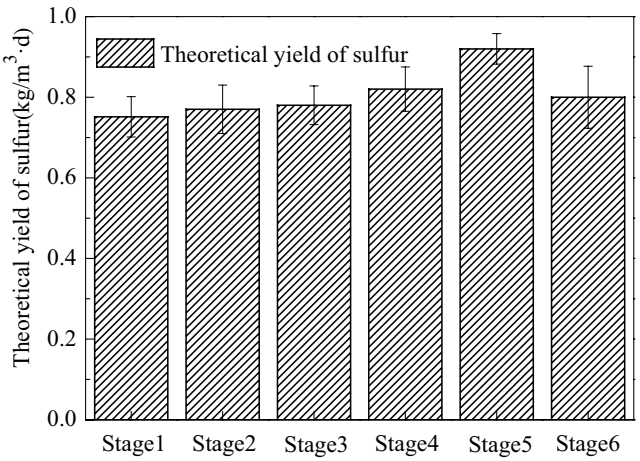

Fig. 4. Theoretical yield of sulfur of AFB-MFC at stage 1–6.

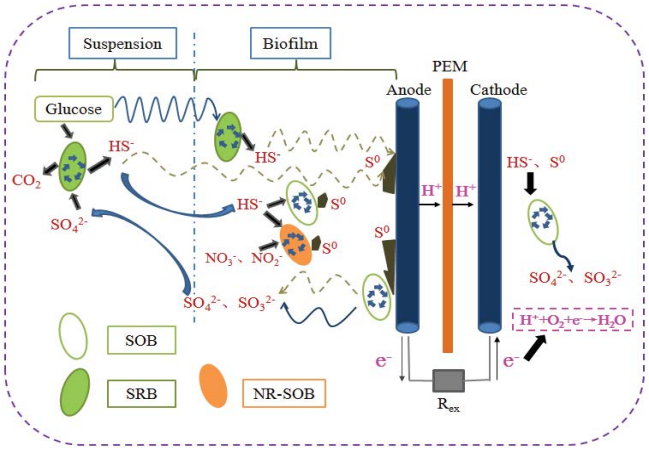

Fig. 5. Removal and transformation mechanism of SO_4^{2-} in AFB-MFC system.

Based on the above analysis, the removal and conversion pathway of sulfate in the AFB-MFC system was shown in Fig. 5. In anaerobic anode, when sulfate and nitrate existed simultaneously in influent, there were two transformation ways of SO_4^{2-}. (1) SO_4^{2-} was assimilated and absorbed by microorganisms into organic components of themselves [15]. (2) SO_4^{2-} was reduced to HS^-, S^{2-} or H_2S by SRB. H_2S escaped from the reactor and was absorbed by alkaline solution. Sulfide (HS^-, S^{2-}) was removed in three ways, namely biological oxidation, electrochemical oxidation and autotrophic denitrification, and eventually became polysulfide, thiosulfate, sulfate or sulfur [8]. Sulfur was deposited on the surface of PEM and anode electrode and the top of the reactor, or discharged through the effluent. And sulfate and other oxidation products of sulfide re-entered the system or flowed out with anaerobic effluent.

3.1.3. NO_3^-–N and NH_4^+–N removal

Keep the influent organic matter and sulfate unchanged in stage 1–5, the removal efficiency of NO_3^-–N decreased first and then increased, as shown in Fig. 6a. Specifically, after the anode sludge adapted to the new matrix in stage 1–2, NO_3^-–N of the anaerobic effluent could be kept below 3 mg/L and the removal efficiency was high. The reason was organic matter as the electron donor in the denitrification was sufficient due to the lower NO_3^-. But NO_3^-–N increased to 350 mg/L in stage 3, the anaerobic effluent NO_3^-–N rose to 14.59 mg/L, the removal efficiency decreased from 98.12% to 96.12%. Since the organic matter in this stage as an electron donor became insufficient, and the NR-SOB began to appear but was in a small amount, the NO_3^- removal efficiency in the anode was lowest. In stage 4 and 5, the NR-SOB was enriched, excess NO_3^- was removed as an electron donor by autotrophic desulfurization and denitrification, so that anaerobic effluent NO_3^-–N again decreased to 9.48 and 2.56 mg/L, respectively. The above process was represented by Fig. 7, that is, NO_3^- was removed by both heterotrophic denitrification and autotrophic denitrification. The organic matter was consumed by the process of sulfate reduction, thereby, the inhibition of organic matter on autotrophic denitrification was alleviated [7]. In stage 6, the COD_{Cr} was increased to 5,000 mg/L, and the effluent NO_3^-–N was below 1.5 mg/L, and the removal rate was above 99.80%. Although the NO_3^- removal efficiency was the highest, the increase of organic matter had an inhibitory effect on the autotrophic desulfurization and denitrification process [10]. That is to say, NO_3^-–N was mainly depended on the heterotrophic denitrification, which was not conducive to the removal of S^{2-}. Throughout the stage 1–6, the anaerobic effluent NO_2^-–N was 0.12 mg/L with no significant change as shown in Fig. 6b. In summary, the nitrogen removal and desulfurization efficiency of the anode was optimal in stage 5 for this AFB-MFC.

The influent NH_4^+–N kept at 84 mg/L, and the removal of NH_4^+–N in the anode gradually increased after stabilization during the experiment, as shown in Fig. 8. From stage 1 to 4, the anaerobic effluent NH_4^+–N decreased from 72.56 to 67.56 mg/L, and the anaerobic removal rate increased from 13.61% to 19.57%. Subsequently, NH_4^+–N rapidly decreased to 42.56 mg/L in stage 5, and the anaerobic removal rate also increased to 49.33%. But there was no significant change in stage 6. The increase in removal efficiency of NH_4^+–N may be due to the increase of the influent NO_3^- and NO_2^- produced by denitrification, resulting in an increase of NH_4^+–N removed by the anaerobic ammonium oxidation (ANAMMOX). Due to the slow growth of ANAMMOX bacteria and the inhibition of autotrophic ANAMMOX bacteria by the high organic matter, the NH_4^+–N removal rate increased significantly in stage 4–5 [16].

Although the NH_4^+–N in the anaerobic effluent varied from 37.59 to 73.49 mg/L, the aerobic NH_4^+–N removal rate stabilized above 93.59%, and the total removal rate was stable above 98.26%, as shown in Fig. 8. It can be concluded that the aerobic biocathode had good resistance to impact load, and AFB-MFC had good removal efficiency for NH_4^+–N.

It was worth noting that there was different amount of aerobic effluent NO_3^-–N. In stage 1–5, the NO_3^-–N in the aerobic effluent gradually increased from 36.78 to 56.76 mg/L, because of the incomplete denitrification caused by the high DO (4.5–5.3 mg/L), the inflow of NO_3^-–N in the anaerobic effluent. Besides, the most important reason was that the anaerobic effluent COD_{Cr} dropped from 825.12 to

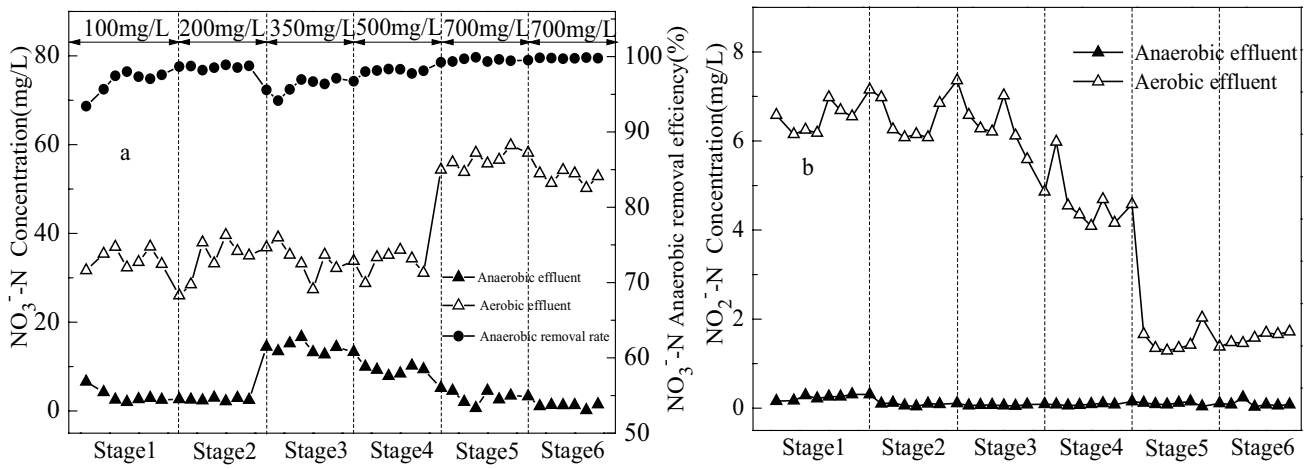

Fig. 6. NO_3^-–N (a) concentration and removal efficiency and effluent NO_2^-–N (b) in stage 1–6.

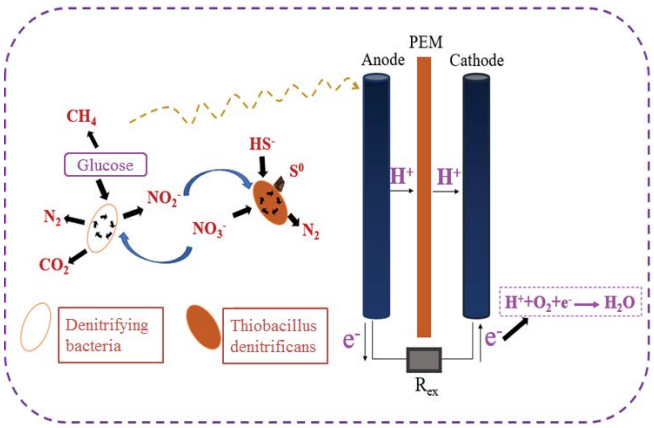

Fig. 7. Removal and transformation mechanisms of NO_3^-–N^- in AFB-MFC.

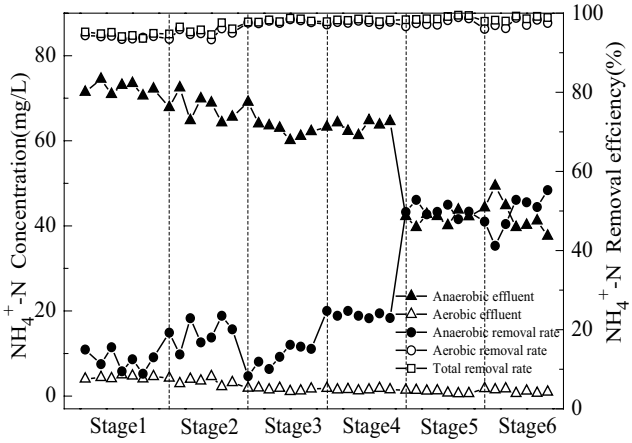

Fig. 8. NH_4^+–N concentration and treatment efficiencies in stage 1–6.

189.47 mg/L from stage 1 to 4. Reduction of COD_{Cr} lead to insufficient carbon source in aerobic pool, which was conducive to the growth of autotrophic nitrifying bacteria but not to the growth of denitrifying bacteria. So aerobic influent COD_{Cr} rose to 492.75 mg/L in stage 6, the effluent NO_3^-–N also decreased to 51.42 mg/L.

3.2. Sludge concentration of AFB-MFC

MLSS and MLVSS are commonly used indicators for evaluating sludge biomass, and MLVSS/MLSS indirectly reflect the biological activity of sludge [17]. As shown in Fig. 9a, MLSS and MLVSS in the anode showed an upward trend, while MLVSS/MLSS showed a trend of increasing first and then decreasing from stage 1 to 6. As the influent NO_3^-–N increased from 100 to 350 mg/L, MLSS, MLVSS, MLVSS/MLSS rose from 23.4 g/L, 18.52 g/L and 0.79 to 28.58 g/L, 23.70g/L and 0.83, respectively. Above results could be explained by the fact that low concentration of nitrate provided microorganisms with required nitrogen source, which helped to improve electron transport efficiency to facilitate the growth of anaerobic granular sludge [18]. After influent NO_3^-–N increased to 700 mg/L in stage 5, MLSS increased to 32.65 g/L and MLVSS increased to 24.32 g/L, but MLVSS/MLSS decreased to 0.77. It could be explained from two aspects: (1) high concentration of nitrate and nitrite obtained by incomplete denitrification inhibited the growth of microorganisms such as SRB, acid-producing bacteria and electrolyzed bacteria [12,18]; (2) Sulfur was generated by bio-oxidation and electro-oxidation of sulfides and accumulated. Part of it was stored in anaerobic sludge, which increased the proportion of inorganic substances, thereby lowering the MLVSS/MLSS. In stage 6, COD_{Cr} rose to 5,000 mg/L, electron donors of microorganisms such as SRB, denitrifying bacteria and electrogenic bacteria were increased, and the growth of anaerobic microorganisms was promoted, so MLSS and MLVSS increased to 33.12 and 24.52 g/L in order. But MLVSS/MLSS dropped slightly to 0.74. It was speculated that the continued accumulation of sulfur lead to a decrease in MLVSS/MLSS.

As shown in Fig. 9b, sludge of the aerobic cathode first reduced and then increased, and MLVSS/MLSS first stabilized and then fell, from stage 1 to 6. Specifically, in stage 1–3 MLSS decreased from 8.52 to 7.59 g/L, MLVSS

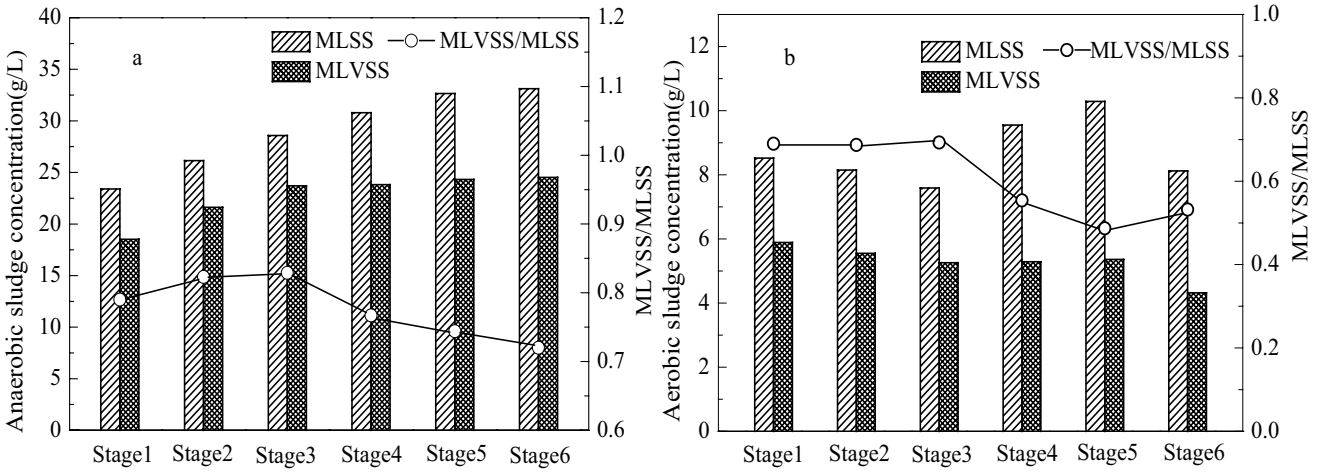

Fig. 9. Sludge concentration at the anode (a) and cathode (b) of AFB at stage 1–6.

decreased from 5.89 to 5.26 g/L, and MLVSS/MLSS showed no significant change (with an average of 0.69). The reduction of sludge mainly because microorganisms that did not adapt to the new environment were continuously eliminated as the influent water changed. The surviving functional bacteria were enriched, and the aerobic sludge gradually increased so that MLSS and MLVSS increased to 10.29 and 5.36 g/L in stage 5, respectively. But the sulfur produced by the anode entered the aerobic tank and the inorganic content in the aerobic sludge was continuously increased, resulting in a decrease in the MLVSS/MLSS value.

In addition, organic matter in the anaerobic effluent from stage 1 to 5 gradually decreased from 825.12 to 189.74 mg/L, sulfide in anaerobic effluent was 300 ± 15 mg/L in stage 1–4, and the NO_3^-–N in aerobic effluent increased to 56.76 mg/L. It meant that NR-SOB and nitrifying bacteria in the aerobic sludge continuously enriched and became the dominant species. Based on this, both MLSS and MLVSS decreased in stage 6, for the increase of organic matter was not conducive to the growth of autotrophic bacteria.

3.3. Power generation performance of AFB-MFC

The power generation performance of AFB-MFC is shown in Fig. 10. In stage 1, the voltage increased from 529 to 586 mV and the power density increased from 15.96 to 17.17 mW m^{-2} as the microbes adapted to the new environment. In stage 2–5, the voltage and power density of each stage decreased rapidly and then rose slowly, indicating that it all took some time for microorganisms to adapt to different concentrations of nitrate. From the overall situation of stage 1–6, power production of AFB-MFC decreased, output voltage dropped from 582 to 384 mV, and power density decreased from 15.74 to 7.43 mW m^{-2}. The above phenomena showed that the power generation performance of MFC was related to influent NO_3^-. On one hand, the increase of NO_3^- increased the ion and the conductivity of the anolyte, which was beneficial to power generation. It was proved that adding a certain amount of nitrate in MFC helped to reduce the internal transfer resistance of the anode and improve the electron transfer efficiency [18]. On the other hand, nitrate for heterotrophic denitrification competed with the anode electrode for organic substrate, and it replaced anode as electron acceptor, which inhibited power generation [19]. At the same time, sulfide reduced the anode potential and acted as a soluble redox mediator, which contributed to electron transfer and thus increased the output of power generation [20]. However, part of the nitrate consumed sulfide through the autotrophic denitrification in stage 5, resulting in poor power generation performance of the MFC. In addition, the accumulation of sulfur on the electrode surface and PEM lead to electrode surface loss [21] and increase the internal resistance of AFB-MFC (from stage 1 to 5, internal resistance increased from 276.82 to 525.12 Ω), which was not conducive to electronic transfer. In general, the increase of nitrate lead to a decrease in the power generation of AFB-MFC, which was also confirmed in other studies [8,22].

In stage 6, the influent COD_{Cr} increased to 5,000 mg/L, which meant the electron donor of the anode increased. However, due to the accumulation of sulfur, the internal resistance rose from 525.12 to 539.02 Ω, and the output voltage and the power density rose from 384 mV and 7.43 mW m^{-2} to 418 mV and 8.86 mW m^{-2}, respectively. In summary, power generation capacity of sulfate, nitrate and organic acted as a co-substrate in AFB-MFC was weaker than that of sulfate and organic as a co-substrate.

3.4. Microbial community analysis of anodes

In this experiment, genomic high-throughput sequencing of microbial communities in stage 2 (sample 1) and stage 5 (sample 2) was performed. The distribution of major bacteria was shown in Table 1 and Fig. 11. The most abundant bacteria in both sample 1 and sample 2 was *Sulfurovum*, accounting for 57.4% and 30.92%, respectively. *Sulfurovum* belong to the *Helicobacteraceae* family and was a kind of chemoautotrophic sulfur-oxidizing bacteria isolated from the deep sea hydrothermal sediments of Okinawa, Japan. It used sulfur or thiosulfate as an electron donor and oxygen or nitrate as an electron acceptor to achieve simultaneous removal of nitrogen and sulfur [23]. A variety of acid-producing bacteria had also appeared in sample 1 (21.53%)

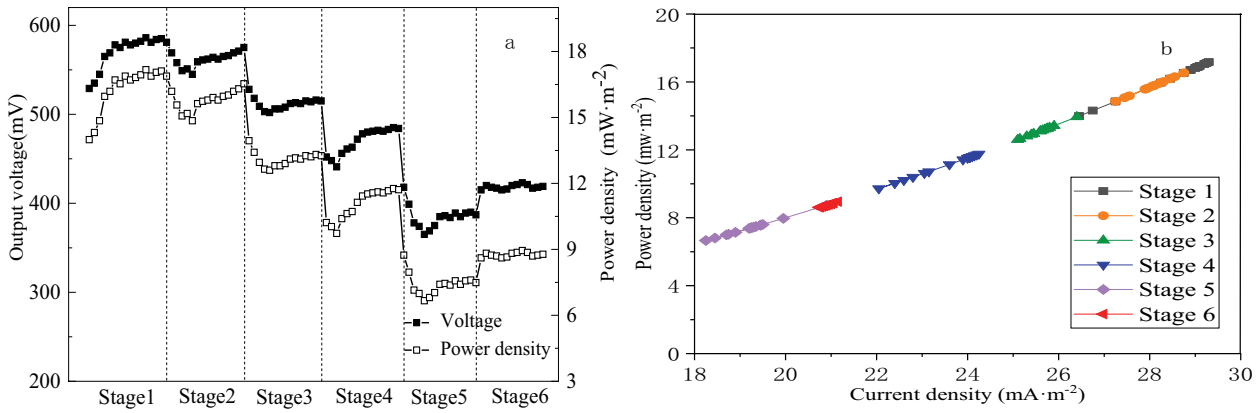

Fig. 10. Voltage output and power density (a) and the polarization curves (b) in stage 1–6.

and sample 2 (13.07%). Among them, *Levilinea saccharolytica* was a kind of mesophilic anaerobic acid-producing bacteria, which hydrolyzed macromolecular organic matter and produced small molecular fatty acids such as acetic acid and lactic acid, as well as ethanol, CO_2 and H_2 [24]. *Aminivibrio pyruvatiphilus* (2.71%/1.41%) hydrolyzed fermented macromolecular organics and produced propionate [25]. *Mesotoga infera* (1.43%/0.81%) was a kind of medium-temperature anaerobic rod-shaped bacteria that could use glucose and lactic acid as energy sources to produce acetate, CH_4 and CO_2 [26]. The above three acid-producing bacteria provide abundant electron donors and carbon sources for SRB and heterotrophic denitrifying bacteria. The proportion of *Enterobacter* increased to 15.03% in sample 2. *Enterobacter* belonged to *Enterobacter aerogenes* and was facultative anaerobic. It produced hydrogen efficiently by using glucose and sucrose. *Enterobacter* sp. Z-16 strain had a hydrogen conversion rate of 2.35 mol H_2/mol glucose under the condition of glucose as substrate, pH = 7.0 and reaction temperature of 35°C [27]. *Enterobacter aerogenes* sp. had also been shown to be a kind of facultative anaerobic bacteria, which was easy to operate and has a high hydrogen production rate, making it a highly promising fermentative hydrogen generator [28]. Therefore, *Enterobacter* provided electron donors H_2 for autotrophic denitrification, which facilitated the removal of nitrate by autotrophic denitrification [29]. In addition, *Actinomyces* (4.06%) in sample 2 was also a member of hydrolyzed acid-producing bacteria, such as *Actinomyces naeslundii* strain, which was a homotypic lactic acid fermenting bacterium that used CO_2 as a growth factor [30].

SRB played an important role in the anode microbial community. *Desulfomicrobium*, a kind of SRB, was 4.78% in sample 1 and rose to 15.17% in sample 2. *Desulfomicrobium* was Gram-negative, strictly anaerobic sulfate-reducing bacterium that incompletely oxidized pyruvate and lactate to acetate and CO_2 in the presence of sulfate [31]. The strain associated with nitrate removal was also significantly increased in sample 2. *Thauera* appeared in sample 2, accounting for 4.17% of the community. *Thauera* belonged to *Rhodobacter*, which was mostly rod-shaped. And acetate used benzoate and ethanol as electron donors for heterotrophic denitrification to remove nitrogen under anaerobic conditions [32]. Moreover, studies have shown that there

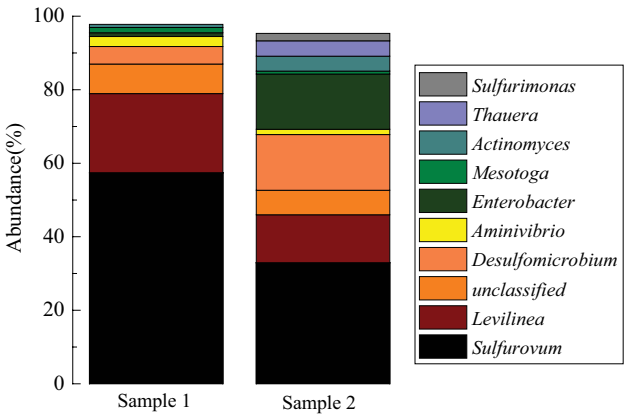

Fig. 11. Abundance of genus of bacteria in anode chamber (sample 1 and sample 2).

were strains of *Thaueus* that directly use H_2 for autotrophic denitrification to remove nitrogen [33]. Additionally, there was *Sulfurimonas* (2.04%) in sample 2, which belonged to *Helicobacteraceae*. *Sulfurimonas denitrificans* use sulfide and thiosulfate as electron donors to reduce nitrates while autotrophic growth, that is, it was a kind of NR-SOB [34].

Compared with bacteria, the diversity of archaeal communities was relatively low, and archaea were almost obligate anaerobic methanogens (Table 2 and Fig. 12). Specifically, the abundance of *Methanothrix* (31.03%/30.71%), *Methanolinea* (33.07%/21.17%), *Methanobacterium* (9.2%/10.22%), *Methanomassiliicoccus* (4.11%/11.87%) and *Methanospirillum* (0.08%/13.21%) was high. *Methanothrix soehngenii* sp. used acetate as energy source, sulfide as sulfur source and ammonia as nitrogen source to produce methane and CO_2 [35]. *Methanolinea tarda* NOBI-1T used formate, H_2 and CO_2 to grow and produce methane [36]. It is worth noting that due to the emergence of *Enterobacter* (15.03%) in sample 2, the proportion of *Methanomassiliicoccus luminyensis* strain B10, which reduced methane by hydrogen to produce methane, increased from 4.11% to 11.87%, and the proportion of hydrogen-nutrient *Methanobacterium aggregans* sp. also increased from 9.3% to 10.22% [37,38]. In addition, *Methanospirillum lacunae* sp. in sample 2 increased

Table 1
Phylum distribution of bacteria in anode chamber (sample 1 and sample 2)

Bacteria (sample 1)	Number	Abundance/%	Bacteria (sample 2)	Number	Abundance/%
Sulfurovum	25,328	57.40	*Sulfurovum*	11,736	32.92
Levilinea	9,202	21.53	*Desulfomicrobium*	6,028	15.17
Unclassified	3,440	8.05	*Enterobacter*	6,070	15.03
Desulfomicrobium	2,043	4.78	*Levilinea*	5,059	13.07
Aminivibrio	1,160	2.71	Unclassified	2,574	6.65
Enterobacter	456	1.02	*Thauera*	1,614	4.17
Mesotoga	540	1.43	*Actinomyces*	1,571	4.06
Actinomyces	371	0.86	*Sulfurimonas*	810	2.04
			Aminivibrio	540	1.41
			Mesotoga	306	0.81

Table 2
Phylum distribution of archaea in anode chamber (sample 1 and sample 2)

Archaea (sample 1)	Number	Abundance/%	Archaea (sample 2)	Number	Abundance/%
Methanolinea	11,594	33.07	*Methanothrix*	10,498	30.71
Methanothrix	11,337	31.03	*Methanolinea*	6,942	21.17
Unclassified	7,510	21.92	Unclassified	2,327	12.03
Methanobacterium	3,148	9.3	*Methanospirillum*	4,678	13.21
Methanomassiliicoccus	1,140	4.11	*Methanomassiliicoccus*	3,721	11.87
Methanospirillum	33	0.08	*Methanobacterium*	2,999	10.22

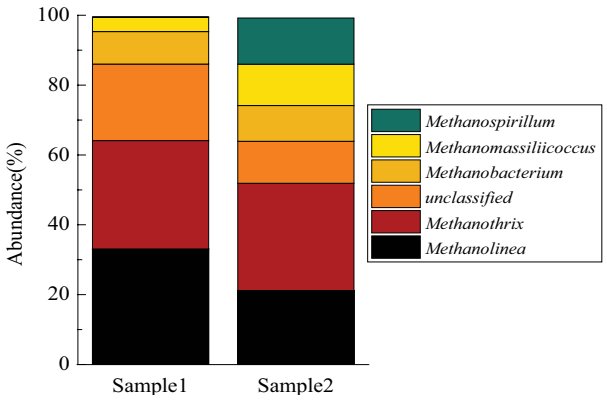

Fig. 12. Categories and abundance in genus of archaea in anode chamber.

significantly, used hydrogen and formic acid to produce methane, and built a harmonious symbiotic relationship with a variety of bacteria [39]. Therefore, it can be seen that the diversity of anodic microorganisms increased, the abundance of hydrogen-producing bacteria and SRB increased significantly, and strains related to nitrate removal also appeared, as the increase of influent NO_3^-–N.

4. Conclusion

The pollutant treatment performance and power generation capacity as the increase of influent nitrate and COD in the AFB-MFC system were explored. When anaerobic NO_3^-–N, SO_4^{2-} and COD_{Cr} loads were 700 mg NO_3^-–N/(m^3 d), 2.1 kg SO_4^{2-}/(m^3 d) and 3.36 kg COD_{Cr}/(m^3 d), the removal rate of NO_3^-–N, SO_4^{2-} and COD_{Cr} was 96.43%, 52.68% and 98.02%, the theoretical sulfur yield was 0.92 kg/m^3 d, the output voltage was 7.23 mW m^{-2} and power density was 385.46 mV, respectively. This proved that the AFB-MFC had a good performance on treating organic wastewater containing nitrates and sulfates. NO_3^-–N was removed by heterotrophic denitrification and autotrophic denitrification. The anaerobic effluent was milky white turbid and pale yellow particles were found on the top of the AFB and in the anaerobic effluent, which confirmed the production of sulfur. High-throughput sequencing of two samples showed that the diversity of microbial community increased, the abundance of hydrogen-producing bacteria, SRB and the strains related to nitrate removal increased significantly in sample 2 with a higher influent nitrate concentration. The main functional bacteria were *Sulfurovum*, *Desulfomicrobium*, *Thauera* and *Sulfurimonas*.

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