Isolation of two iron-reducing facultative anaerobic electricigens and probing the application performance in eutrophication water

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Abstract

Purpose: Sediment microbial fuel cell (SMFC) is a promising bioremediation technology in which microbes play an important role. Electricigens as the bio-catalysts have effect on pollution control and electricity generation. It is of great significance to screen the microorganisms with the ability of generating electricity.

Methods: The SMFC anode biofilm was used as microbiological source to study the feasibility of electricigens with iron-reducing property for eutrophication water treatment. Preliminarily, we isolated 20 facultative anaerobic pure bacteria and evaluated their cyclic voltammogram (CV) through the three-electrode system and electrochemical workstation. The power generation performance of strains was verified by air-cathode microbial fuel cells (AC-MFCs) under different single carbon sources.

Result: According to its morphological, physiological, and biochemical characteristics, along with phylogenetic analysis, the two strains (SMFC-7 and SMFC-17) with electrical characteristics were identified as Bacillus cereus. Compared with SMFC-7, SMFC-17 exhibited efficient NH$_4^+$-N and NO$_3^-$-N removal and PO$_4^{3-}$-P accumulation from eutrophic solution with a removal rate of 79.91 ± 6.34% and 81.26 ± 1.11% and accumulation rate of 57.68 ± 4.36%, respectively.

Conclusion: The isolated bacteria SMFC-17 showed a good performance in eutrophic solution, and it might be a useful biocatalyst to enable the industrialized application of SMFC in eutrophic water treatment.

Keywords: Sediment microbial fuel cell (SMFC), Electricigens, Cyclic voltammograms (CVs), Biocatalyst, Eutrophic water, Bacillus

Introduction

Eutrophication of water body has an important impact on the living environment of human beings (Morgane et al., 2019). The input of nutrients, such as the diffusion loss of nitrogen and phosphorus, is the main drivers of eutrophication (Beusen, Bouwman, Van Beek, Mogollón, & Middelburg, 2016). Exogenous pollution has been kept down to some extent with the strengthening of water pollution management and treatment, whereas the treatment of endogenous pollution has become the important issue of eutrophication treatment (Wang et al., 2017; Wang et al., 2019). As an important component of the water environment, the sediment presents not only a natural reservoir but also a potential source of long-term releasing contaminants to the water. Thus, the remediation of sediment is the key link to improve the quality...
of the water environment (Karra et al., 2014; Li & Yu, 2015).

Sediment microbial fuel cells (SMFCs) have been used to utilize bioelectricity from water-based ecosystems, where electro-chemically active microbes metabolize biodegradable organic matter (OM) in sediments and produce electrons (Xu et al., 2017). Due to the capacities of power generation and pollution control, SMFCs have lots of potential for future application. For instance, SMFCs have been used for bioremediation of the overlying water in the sediment-water systems (Sajana, Ghangrekar, & Mitra, 2013; Sajana, Ghangrekar, & Mitra, 2014), sediment remediation by biodegradation of organic pollutants (Hong, Kim, & Chung, 2010), and phosphorus immobilization (Martins et al., 2014). As the bio-catalysts, electricigens play a role in pollution control and electricity generation. Electricigens are a kind of microorganisms that can directly or indirectly oxidize and decompose pollutants to obtain electrons and transfer them to anode to generate current (Lovley, 2006). They may be denitrifying bacteria (Virdis, Rabae, Rozendal, Yuan, & Keller, 2010; Xiao, Zheng, Wu, Yang, & Zhao, 2015), phosphorous accumulating bacteria (Tao et al., 2014), or bacteria that degrade other difficult pollutants (Pham et al., 2003; Xu et al., 2005; Biffinger et al., 2011). To date, many studies have focused on characterizing the microbiological community structure of the anode biofilm to analyze the microbial species (Ewing, Ha, & Beyenal, 2017; Kabutey et al., 2019; Xu et al., 2017). Referring to the reported literatures on electricigens (Kumar, Singh, & Zularisam, 2016), we can speculate which bacteria in the biofilm play a role in electricity production. However, the specific electricity-producing properties under specific conditions still need to be further verified, because the electricity-producing properties of each strain are different under different conditions. Moreover, the types of electricigens obtained by analysis were difficult to specific species. Therefore, it is of great significance to screen the microorganisms with the ability of generating electricity. Several studies have developed methods and devices that can be used for isolating electricigens, such as U-tube MFCs (Zuo, Xing, Regan, & Logan, 2008), WO₃ nanocluster probe (Yang et al., 2016), and electrodeplate-culture (EPC) method (Ueoka, Kouzuma, & Watanabe, 2018). At present, there is no unified screening method for electricigens, and most of the electricigens are obtained through anaerobic separation technology. Wu et al. (2014) used traditional aerobic separation technology to obtain 3 electricigens from micro-oxygen anode. The screened electricigens had similar direct extracellular electron transfer mechanism, indicating that micro-oxygen anode could be oriented to screen for electricigens with similar electrochemical properties. Successive experiments have proved that many iron-reducing bacteria have the ability to produce electricity (Liu et al., 2016; Liu & Wang, 2016; Park et al., 2001) and most of the electricigens are facultative anaerobic bacteria (Seo & Roh, 2018). Screening iron-reducing bacteria capable of rapidly transforming organic matter into electricity-producing bacteria has become an urgent need to promote the development of MFC. It is a trend to isolate and screen iron-reducing bacteria suitable for MFC directly from well-functioning MFC anodes, so that the physiological conditions (such as pH, temperature, and electron donors) of the strains are close to their original habitats. Thus, we speculate that screening the microorganism with iron-reducing property in the anode biofilm by micro-oxygen condition is beneficial to obtain the electrogenic microorganism with a similar property quickly.

In pursuance of our quest for the desired microbes, this research work presents the isolation and identification of two bacterial strains from a lab-scale SMFC used for the treatment of eutrophic water. The objectives of the study include the following: (1) verifying the feasibility of the screening method through screening iron-reducing bacteria under micro-oxygen environment to obtain electricigens and (2) exploring the nitrogen removal performance and phosphorus accumulation efficiency of the isolated strains under experimental conditions to demonstrate the potential applications of the microbe in the treatment of eutrophic water bodies.

Materials and methods

Growth medium, inoculation, and cultivation

The sample was obtained from the anodic biofilm of a SMFC (Xu et al., 2017). The biofilm attached to the anode graphite felt was scraped off in aseptic condition and inoculated into an anaerobic bottle (100 mL) containing 50 mL of LB medium (peptone, 10 g/L; yeast extract, 5 g/L; NaCl 10 g/L, pH = 7.0 ± 0.2, sterilization at 121 °C for 20 min). The anaerobic bottle was incubated at 30 °C for 1 day, and then 1.00 mL of culture broth was added into the iron-reducing medium (IRM). The composition of the IRM was 2.5 g/L NaHCO₃, 0.6 g/L KH₂PO₄, 1.5 g/L NH₄Cl, 0.5 g/L yeast extract, 20 mM CH₃COONa, ferric citrate 20 mM (pH 7.0 ± 0.2), and sterilization at 121 °C for 20 min. The liquid medium was incubated at 30 °C for 4 days under a micro-oxygen condition (DO <1 mg/L) that was purged with 99% N₂ gas. The culture broth was serially diluted and then spread on the solid IRM, which contained 2.0% agar. The plates were cultivated at 30 for 4 days in an anaerobic incubator. According to the characteristics of colony morphology, color, and transparency formed on the plate, all the colony types that formed under solid IRM were picked and re-inoculated on the solid plates of
IRM and LB medium and then incubated at 30 °C for 3 days in an anaerobic incubator. This process was repeated at least 5 times to obtain a batch of purified strains.

**Electrochemical analysis**

Three-electrode system was prepared for testing cyclic voltammograms (CVs) of strains, which consist of a working electrode, counter electrode, and saturated calomel reference electrode (SCE, Hg/Hg2Cl2 saturated KCl, + 0.244 V vs. hydrogen standard electrode (SHE)). The purified strains were inoculated separately into IRM medium and cultured under anaerobic conditions for 3 days. Sixty milliliters of bacterial solution was taken under anaerobic conditions for CVs scanning. The CVs of the cell suspensions were obtained using a CS 2350 electrochemical workstation. The scan rate of 100 mV/s was employed over the range from −0.8 to 0.8 V.

To test the electricity production of strains that have obvious redox peaks, a series of membrane-free single chamber air-cathode microbial fuel cells (AC-MFCs, 28 mL working volume) were constructed to evaluate the current production of the purified strains under different single carbon sources. The reaction chamber is a cylinder with a column length of 4 cm and an inner diameter of 4 cm. Both electrodes were carbon cloth (the area is 3.14 cm²). The air cathode is a platinum-loaded carbon cloth with a platinum loading of 0.5 mg cm⁻² and four layers of polytetrafluoroethylene (PTFE) coated on the air to prevent leakage and regulate oxygen permeation. The anode was treated with acetone for 24 h to remove some non-compatible impurities from the surface of the carbon cloth.

For the AC-MFCs inoculation, the pure bacteria under logarithmic stage were centrifuged and dissolved again in 50 mmol L⁻¹ PBS buffer (sterilization at 121 °C for 20 min), and this process was repeated 3 times. The bacterial suspension was mixed with a nutrient solution containing 20 mmol L⁻¹ single electron donor (sodium citrate, glucose, sodium acetate, sodium lactate, sodium oxalate, glycerol) in a ratio of 1:5 and then inoculated into AC-MFCs. All operations are carried out under aseptic conditions. External resistance is 1000 Ω, operating at 30 ± 1 °C constant temperature. The output voltage (V) of the AC-MFCs is measured using R6016/U (Shanghai Jisheng Electric) every 10 min.

**16S rDNA gene sequencing and analysis**

The single purified strains were used as template for PCR-mediated amplification of 16S rDNA with the universal primers 27f and 1492r. The PCR amplification was performed in a 30-μL reaction volume containing 1 μL of DNA, 1 μL of each primer (10 Mp), 15 μL of 2 × Power Taq PCR MasterMix, and 12 μL ddH₂O. PCR amplification was carried out with an initial pre-denaturation of DNA at 95 °C for 3 min, denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 50 s, 30 cycles, and finally at 72 °C for 5 min. Agarose gel electrophoresis analysis was amplified by product PCR for sequence determination. The 16S rDNA gene sequences were queried against the GenBank using the BioCloud (www.ezbiocloud.net/identify). Phylogenetic relationships were analyzed by the evolutionary distance matrix calculated using the neighbor-joining method with Kimura’s two-parameter method. A neighbor-joining tree was constructed with the program MEGA7.

Biochemical tests of the isolated strains were performed with Solarbio Gram stain kit and Bacillus cereus biochemical identification box.

**Performance analysis of electricigen**

**Effects of temperature and pH**

The pure bacteria under logarithmic stage were streaked on LB solid medium and placed in a constant temperature incubator at 4, 10, 20, 30, 35, 37, and 45 °C. To determine the optimum growth pH of the strain, the strain was inoculated in 50% LB liquid medium at a 1% inoculum, and the pH was adjusted to 4, 5, 6, 7, 8, and 9 with 1 M HCl and 1 M NaOH, respectively.

**Iron reduction performance**

The pure bacteria under logarithmic stage were centrifuged 3 times and resuspended in sterile water with an OD₆₀₀ of 0.5. The bacterial liquid was inoculated into 50 mL iron-reducing medium (IRM) with different concentrations of ferric citrate (5 mM and 20 mM) at a 1% inoculum, respectively. The anaerobic bottle is filled with a sterile N₂ (5 min) to maintain a micro-oxygen environment and then placed at 30 °C. The sample was centrifuged at 3500 r/min for 5 min every 24 h, and the contents of Fe(II) and Fe(III) in the supernatant were determined for 7 days. The medium without the addition of bacteria was blank control. All experiments were conducted in triplicate.

**Nitrogen removal efficiency and phosphorus accumulation rate**

To compare the performance difference of pure bacteria and the mixed bacteria, they were cultured to the logarithmic phase, centrifuged three times, and dissolved again in sterile water with an OD₆₀₀ of 0.5, respectively. The bacterial liquid was inoculated into the formulated eutrophic liquid medium (MgSO₄·7H₂O, 91.26 mg/L; CH₃COONa·3H₂O, 3.32 g/L; NH₄Cl, 305.52 mg/L; KNO₃, 300 mg/L; K₂HPO₄, 25 mg/L; PIPES buffer solution, 8.5 g/L; CaCl₂·2H₂O, 25.68 mg/L; NaCl, 20 mg/L; trace element solution 2 mL; pH 7.0 ± 0.2, sterilization at 121 °C for 20 min) at a 1%
inoculation amount and incubated at 30 °C for 7 days. The sample was centrifuged at 8000 r/min for 5 min every 24 h, and the contents of NO₃⁻, NO₂⁻, NH₄⁺, and PO₄³⁻ in the supernatant were determined for 7 days. The medium without the addition of bacteria was blank. All experiments were conducted in triplicate.

The trace element solution was Na₂EDTA, 63.7 mg/L; MnCl₂·4H₂O, 5.06 mg/L; ZnSO₄, 2.2 mg/L; FeSO₄·7H₂O, 5.0 mg/L; CaCl₂, 5.5 mg/L; CuSO₄·5H₂O, 1.57 mg/L; Na₂MoO₄·4H₂O, 1.1 mg/L; CoCl₂·6H₂O, 1.61 mg/L; and pH 7.0 ± 0.2.

Analytical methods

Cell density in the form of OD was measured against the blank using a spectrophotometer at an absorbance of 600 nm. The reduction of Fe(III), concentration of nitrate-nitrogen (NO₃⁻), nitrite-nitrogen (NO₂⁻), ammonium-nitrogen (NH₄⁺), and phosphate (PO₄³⁻) were measured by O-phenanthroline spectrophotometry, sulfamic acid method, sulfanilic acid method, Nessler’s reagent method, and persulfate digestion method, respectively (State EPA of China, 2002).

In this study, the amounts of biological assimilation, organic nitrogen, gaseous nitrogen, and absorbance were not measured. Accordingly, the nitrogen removal efficiency was calculated only based on measured values of nitrate, nitrite, and ammonium in aqueous solutions.

The calculation formula is as follows (Islam, Ethiraj, Cheng, Youssuf, & Khan, 2017):

\[ R_\text{v} (\%) = \frac{[N\text{O}_3^-]+[N\text{O}_2^-]+[N\text{H}_4^+]-[N\text{O}_3^-]-[N\text{O}_2^-]-[N\text{H}_4^+]}{[N\text{O}_3^-]+[N\text{O}_2^-]+[N\text{H}_4^+]} \times 100 \]

Statistical analysis

The experimental data, which were statistically calculated and drawn by office 2010 and origin 2019, were analyzed with analysis of variance (ANOVA) and t test. All data were presented as the mean value and standard deviation (SD). The SPSS 18.0 was used to evaluate the significant differences among treatments by a one-way ANOVA test at the 0.05 probability level.

Results and discussion

Strain selection

According to the difference of colonies on LB and ferric citrate solid medium, a total of 20 strains were obtained from the anode biofilm, which were numbered SMFC-1 to SMFC-20. The purified 20 strains and the blank culture solution were tested for electrochemical activity. The peak value of pure bacterial suspension measured by cyclic voltammetry can represent the electrochemical activity of the strain (Wu et al., 2014). It was found that the system without adding microorganisms was no redox peak in the cyclic voltammetry curve, indicating that the system itself had substantially no electrochemical activity. The cyclic voltammetry curves of the strains SMFC-1, SMFC-7, SMFC-15, SMFC-17, and SMFC-20 (Fig. 1) show certain oxidation peaks or reduction peaks, indicating the selected microorganisms have different degrees of electrochemical activity. We found that the strains SMFC7 and SMFC17 had significant redox peaks, which indicated that they had strong electrogenetic properties (Kim et al., 2002).

Voltage output

Strains SMFC-7 and SMFC-17 were inoculated into AC-MFC, respectively. The results (Fig. 2) showed that under the condition of a single carbon source, both strains SMFC-7 and SMFC-17 could produce electricity using sodium citrate and sodium lactate and could not use glucose, sodium acetate, sodium oxalate, or glycerol as a single carbon source to produce electricity. When strain SMFC-7 was used to produce electricity by sodium lactate (SL), the voltage reached the maximum value of 63 mV in the first cycle after 24 h. When the electricity was produced by sodium citrate (SC), the voltage reached the maximum value of 18 mV in the first cycle after about 120 h. When strain SMFC-17 was used to produce electricity by sodium lactate, the voltage reached the maximum value of 65 mV in the first cycle after 75 h. When the electricity was produced by sodium citrate, the voltage reached the maximum value of 15 mV in the first cycle after about 80 h of starting. It can be seen that strains SMFC-7 and SMFC-17 have certain similarities in electrical performance. Compared with the control experiment (the highest voltage value is 2 mV when glucose, sodium acetate, sodium oxalate, and glycerol are the single carbon sources), we demonstrated that strains SMFC-7 and SMFC-17 possess a significant ability to produce electricity.

While numerous electricigens have been observed, the majority of research focused on a group of highly elec troactive species, such as Geobacter and Shewanella. Geobacter, however, was a strict anaerobe with a relatively high level of electroactivity, but it was usually accompanied by slower growth rates, which could pose challenges in real-world applications. On the contrary, Shewanella was a facultative anaerobic microorganism with diverse metabolic modes (Ong et al., 2014), which was an example of a less picky electricigen. Doyle and Marsili (2018) have suggested that some strains may quickly be ruled out because they produce a low current. However, the weakly electroactive populations and communities may have certain application prospects in bioprocesses, biosensors, and bioremediation. Some researchers have classified microbes that produce low currents or low coulomb efficiencies as weak
Fig. 1 Cyclic voltammetry curves of strains: a SMFC-7, b SMFC-17, c SMFC-1, d SMFC-15, e SMFC-20

Fig. 2 The output voltage of strain under different carbon source
electricigens (Doyle & Marsili, 2018). Weak electricigens could be broadly distributed across nature (Chabert, Amin, & Achouak, 2015; Courret, Délia, Bergel, Roques, & Bergé, 2010). Compared with strong electricigens, weak electricigens could compensate for metabolic shortcomings and realize more stable bioprocess in a mixed community (Doyle & Marsili, 2018). Bacillus is a type of weak electricigen. Bacillus thuringiensis DRR-1 from cow rumen produced a small potential and current when cultivated in an MFC with an unspecified medium (Jothinathan & Wilson, 2017). Nonetheless, Bacillus spp. may also play a synergistic role in co-culture bioelectrochemical devices. When co-cultured Bacillus subtilis RH33 with Shewanella oneidensis MR-1, Liu, Yu, Chen, and Chen (2017) found that the power output increased significantly and confirmed that MR-1 could efficiently utilize the high concentration of riboflavin produced by RH33 to improved MFC performance. Wu, Xiabo, et al. (2014) and You et al. (2018) also found that Bacillus could secrete flavins which were able to act as electron shuttles, strengthening the electron transfer from microorganism to the electrode. According to the above studies, Bacillus had the ability to secrete riboflavin. Previous researches have been reported that some species of Geobacter, Shewanella, Bacillus, and other microorganisms could rely on flavin molecules for mediated electron transfer (MET) (Kumar et al., 2016; Li, Tiedje, Chiu, & Worden, 2012; Wu et al., 2013; You et al., 2018). Therefore, as an electron shuttles, riboflavin played an important role in extracellular electron transfer.

Identification of strains SMFC-7 and SMFC-17
Morphological character
The colony morphology of SMFC-7 on nutrient agar under aerobic conditions was faint yellow, domed, and was approximately 3 mm in diameter. The edge condition was regular and the varnish was glossy. Compared with SMFC-7, the diameter of SMFC-17 is bigger, approximately 5 mm under the same culture time, and its color is milk white. They were facultative anaerobe, Gram-positive bacteria. A subset of the study reported that electricigens are facultative anaerobic bacteria, e.g., Shewanella putrefaciens (Rabaey, Boon, Siciliano, Verhaege, & Verstraete, 2004), Pseudomonas aeruginosa (Nimje et al., 2009), Bacillus subtilis (Zhou et al., 2017), and Citrobacter freundii (Seo & Roh, 2018). Since facultative strains possess many desirable properties compared to anaerobic strains, such as their ability to survive in anaerobic and aerobic conditions, these strains represent a promising exoelectrogenic species in engineering of biological catalysts for microbial electrochemistry.

Phylogenetic analysis
One thousand four hundred two base pairs and 1425 bp target fragments were amplified by PCR using strain SMFC-7 and strain SMFC-17 genome DNA as template. The sequence similarity of the 16S rDNA gene was compared with those of reference microorganisms obtained from GenBank data libraries. The result showed that the isolated strains SMFC-7 and SMFC-17 belonged to the genus Bacillus (Fig. 3). Bacillus cereus was the nearest neighbor of both strains, with a 16S rDNA sequence similarity of 100% (SMFC-7) and 99% (SMFC-17). In the systematic classification and identification, when the homology of 16S rDNA gene sequence is greater than 97%, it can be considered as one species (Embley & Stackebrandt, 1994). According to its morphological, physiological, and biochemical characteristics, along with phylogenetic analysis, these two strains were identified as Bacillus cereus (Table 1). The highest proportion of Bacillus was found by testing the microbial community structure of the anode biofilm, reaching 22.5% of microbial community (Xu et al., 2017). Through our experiment, it can be proved that the type of electricigens present in the anode of SMFC belongs to Bacillus.

Some performances of SMFC-7 and SMFC-17
Optimum growth conditions and growth curve
It was found that two strains both can grow in the range of 20–45 °C and grows fastest between 30–37 °C. From the growth curves of the two strains at different pH, we know that the optimum pH condition of SMFC-7 is 6–8, and the optimum pH condition of SMFC-17 is 6–9 (Fig. 4). Compared with SMFC7, SMFC17 is more tolerant to acid and base conditions.

Iron reduction rate
Most of the electricigens have iron reducing properties (Liu et al., 2016; Liu & Wang, 2016; Park et al., 2001). We enriched iron-reducing bacteria through SMFC anode biofilm and found that strains SMFC-7 and SMFC-17 had strong redox properties and could use organic carbon sources to produce electricity. Electrochemical experiment verified the feasibility of obtaining electricigens by screening iron-reducing bacteria. Test results of the iron reduction ability of two strains of electrogenic microorganisms were shown in Fig. 5, and we found that both strains SMFC-7 and SMFC-17 have significant iron reducibility. The maximum iron reduction rate under the condition of 20 mM ferric citrate is 15.92 ± 0.13% and 15.16 ± 0.80% (p = 0.450), respectively. It increased under 5 mM ferric citrate conditions, reaching 29.59 ± 1.99% and 21.43 ± 1.67% (p = 0.011), respectively.

Strains SMFC-7 and SMFC-17 had the largest accumulation of Fe(II) on the first day, which may be due to
Fig. 3 Phylogenetic tree of strain SMFC-7, SMFC-17: a SMFC-7, b SMFC-17
the stronger reduction of Fe(III) during the growth of strains SMFC-7 and SMFC-17 (García-Balboa et al., 2010; Liu & Wang, 2016). At the ferric citrate concentration of 20 mM, we found the decrease on Fe(III) reduction compared to 5 mM ferric citrate. Compared with the 5 mM ferric citrate solution, the 20 mM ferric citrate solution had more Fe(III), and these excess Fe(III) may negatively impact the enrichment of Fe(III)-reducing bacteria (Liu & Wang, 2016). The reason may be that the excess amount of Fe (III) caused Fe(III) reducing bacteria to form an iron mineral coatings on cell surface, which was detrimental to cell growth and microbial activity (Liu, Zachara, Gorby, Szecsody, & Brown, 2001).

### Concentration variation of nitrogen and phosphorus

As shown in Fig. 6a–c, the concentration of NO$_3^-$-N decreased sharply on the second day compared with the initial concentration ($p = 0.000$) and fluctuated within a small range in the following days in the system of inoculation of strain SMFC-7. The lowest concentration reached 3.72 ± 0.26 mg/L and the removal rate was 83.48 ± 1.16%. The concentration of NH$_4^+$-N declined slowly compared with SMFC-17 and the mixed bacteria system, and the removal rate reaches 18.97 ± 3.17% on the 7th day. The concentration of NO$_2^-$-N rose sharply on the second day, reaching 37.6 ± 0.04 mg/L, and then basically stayed the same. The concentration changes of NO$_3^-$-N and NO$_2^-$-N in the system when inoculating strain SMFC-17 is similar to the one observed after the inoculation of SMFC-7. The lowest concentration of NO$_3^-$-N reached 4.22 ± 0.25 mg/L and the removal rate was 81.26 ± 1.11%, while the concentration of NH$_4^+$-N dropped significantly on the third day and dropped to 29.10 ± 9.19 mg/L on the 7th day; the removal rate reaches about 79.91 ± 6.34% on the 7th day. For the pure bacteria system, the concentration of NO$_3^-$-N decreased while the concentration of NO$_2^-$-N increased. We confirmed the capability of the high capability of Bacillus strain to convert nitrate in nitrite, as already observed by Cho and Rhee (2019). The concentration of NO$_2^-$-N increased was higher than that of NO$_3^-$-N, which may be due to the conversion of ammonium to nitrite. Yang, Lin, and Huang (2017) isolated an aerobic denitrifying bacterium from a bio-trickling filter treating NOx, Bacillus sp. K5. It was found that hydroxylamine oxidase (HAO) played a key role. Higher nitrite accumulation was observed during ammonium oxidization by a pure strain of ammonia-oxidizing bacteria (AOB) or a population of AOB in an activated sludge system (Fumassoli et al., 2017; Miao et al., 2017; Zou, Yao, & Ni, 2014). We can suspect the possibility that isolated bacteria SMFC-7 and SMFC-17 also oxidize ammonia by HAO.

### Table 1

| Characteristics          | SMFC-7 | SMFC-17 | Bacillus cereus |
|--------------------------|--------|---------|-----------------|
| Gram reaction            | +      | +       | +               |
| Dynamic test             | +      | +       | ±               |
| Nitrate test             | +      | +       | ±               |
| VP test                  | –      | –       | ±               |
| Catalase                 | +      | +       | +               |
| Xylose-gelatin           | –      | –       | +               |
| Mannitol                 | –      | –       | –               |
| Lysozyme tolerance       | +      | +       | ±               |
| Protein toxin crystal    | –      | –       | –               |

![Fig. 4 Growth curve under different pH: a SMFC-7, b SMFC-17](image-url)
Fig. 5 Iron reduction rate of SMFC-7 and SMFC-17: a 20 mM ferric citrate, b 5 mM ferric citrate

Fig. 6 Concentration changes in the three groups in the eutrophic medium: a $\text{NO}_3^-$, b $\text{NO}_2^-$, c $\text{NH}_4^+$, d $\text{PO}_4^{3-}$
concentration of NO$_3^-$-N and NH$_4^+$-N decreased by 17.72 mg/L and 114.20 mg/L, respectively, and NO$_2^-$-N increased by 40.94 mg/L. After the second day, the concentration of NO$_3^-$-N and NO$_2^-$-N showed no significant change, while NH$_4^+$-N decreased significantly ($p = 0.000$). All these results indicate that strain SMFC-17 preferred to remove ammonium in the mixed N-source, which might be attributed to the higher enzyme activity of ammonium oxidation than that of nitrate reduction (Yang, Liu, & Wang, 2019).

The variation trends of nitrogen and phosphorus concentration of mixed bacteria system and pure bacteria system were different. In the mixed bacteria system, the concentration of NO$_3^-$-N changes slowly, and the lowest concentration reaches 18.74 ± 0.45 mg/L on the 6th day. The NH$_4^+$-N concentration displayed obvious fluctuations, presenting a first decline and then slowly increasing during the first 3 days. It had a perfect performance in the removal of NH$_4^+$-N, the highest removal rate reached to 90.49 ± 1.86%, and almost no nitrite nitrogen accumulation in the first 4 days. Nakano, Shimizu, Okumura, Sugahara, and Maeda (2008) constructed a consortium comprising AOB, and denitrifying bacteria removed roughly 90% of NH$_4^+$-N in 37 days when they were incubated in an ANA3 medium containing equal concentrations of NH$_4^+$-N and NO$_3^-$-N (56 mg L$^{-1}$). However, the NO$_3^-$-N in the medium increased to 74.2 mg L$^{-1}$. Yang, Liu, and Wang (2019) isolated a consortium of AOB from landfill leachate through persistent domestication, and the consortium showed approximately 90.85 ± 0.80% and 77.88 ± 1.86% removal of NH$_4^+$-N in a bioaugmentation treatment of eutrophic wastewater with an initial concentration of 1.80 ± 0.04 mg L$^{-1}$ and 40.31 ± 0.57 mg L$^{-1}$, respectively. A high efficiency of ammonification and relatively low accumulation of nitrite were obtained by these mixed bacteria systems in the present study compared with those consortiums. In the 5th day, the concentration of NH$_4^+$-N was accumulated, it probably owing to the strains entered decline phase and the release of ammonium from the dead bacteria cell caused by insufficient organic compound (Yang, Wang, Chen, & Lyu, 2019). The removal efficiency of NO$_2^-$-N in mixed bacteria is much lower than that in pure bacteria, indicating that denitrification exists to convert NO$_2^-$-N or NH$_4^+$-N into NO$_3^-$-N. The maximum nitrogen removal efficiency of inoculation with SMFC-7, SMFC-17, and mixed bacteria reached about 8%, 55%, and 76%, respectively. The mechanism of nitrogen removal needs to be explained by further experiments.

The concentration of PO$_4^{3-}$ varied under three systems (as shown in Fig. 6d). The inoculation of strain SMFC-7 produced minimal variation in phosphate concentration and the lowest concentration was around 8.39 ± 0.00 mg/L, while significant variations were observed after inoculating strain SMFC-17 and the mixed-species inoculum. When inoculating strain SMFC-17, phosphate content stabilized to 4.59 ± 0.08 mg/L. With the mixed-species inoculum, the concentration dropped to the lowest amount of 1.29 ± 0.10 mg/L.

PO$_4^{3-}$-accumulating organisms (PAOs) can accumulate abundant PO$_4^{3-}$ under aerobic conditions and transfer poly-PO$_4^{3-}$ to ATP under anaerobic conditions to assist in the synthesis of polyhydroxalkanoates (Han et al., 2018). The strain SMFC-7 had weak absorption capacity of PO$_4^{3-}$, the accumulation rate only reaches 15.25 ± 0.00%, and the change is small within 7 days; we can speculate that the strain is not a PAO. Strain SMFC-17 has a strong absorption capacity for PO$_4^{3-}$, and its absorption efficiency is as high as 57.68 ± 4.36%; the loss might be due to adsorptive PO$_4^{3-}$ removal by extra polymeric substances (EPS) of the microbial cell (Rout, Bhunia, & Dash, 2017). Due to the decrease of electron acceptors or DO, there were changes after the 4th day. Strain SMFC-17 was metabolically capable of utilizing nitrate or nitrite without any inhibitory effect on phosphate uptake, so it might be a denitrifying phosphate-accumulating organism (DPAO). Under the mixed bacteria condition, the phosphorus absorption capacity reached to 86.97 ± 1.01% on the third day, followed by a small amount of release, which might be owing to the strains entering the decline phase and the release of PO$_4^{3-}$ from the dead bacteria cell caused by insufficient organic compound; the production of NO$_2^-$-N in the 4th day has a certain toxic effect on some strains or the system DO drops (Rout et al., 2017; Weon, Lee, Lee, & Koopman, 2002; Zhou et al., 2010; Zhou, Pijuan, & Yuan, 2007). During the system of inoculating strain SMFC-17 and mixed bacteria, the nitrogen removal performance and phosphorus accumulation efficiency proved that P removal could occur during with heterotrophic nitrification aerobic denitrification process.

The reason for the little change in pollutant content after dropping to a certain level may be that high microbial growth resulted in depletion of oxygen in the medium which might be responsible for inhibiting enzyme system, consequently repressing the nutrient removal activity. Taking cost-effectiveness into account, a simultaneous heterotrophic-nitrifying, aerobic-denitrifying, and polyphosphate-accumulating microorganism is highly essential for efficient treatment of nutrient-rich wastewater. Owing to the significant nutrient removal efficiency, it was suggested that strain SMFC-17 had a broad application prospect in terms of wastewater or sediment treatment emphasizing on simultaneous organic, nitrogen and phosphorus removal in the same reactor. Previous studies have showed that the microbial composition of the anode, especially the
et al. (2019) co-cultivated the active carbon fibers with strain SMFC-17 to the anode of SMFC might also be an effective strategy to induce biofortification performance (Liu, Li, Shi, Zhu, & Gao, 2013; Wang et al., 2013), fixing strain SMFC-17 to the anode of SMFC might also be an effective means to improve SMFC performance. Shi et al. (2019) co-cultivated the active carbon fibers with S. oneidensis MR-1 at 150 rpm and 30 °C for 6 days to ensure the strain MR-1 got immobilized on the anode surface sufficiently. As a facultative anaerobe, SMFC-17 could not only be fixed in the anaerobic anode but also in the aerobic cathode, which is expected to improve the treatment effect of SMFC on sediments and overlying water pollutants. In order to improve the performance of strain SMFC-17 in the treatment of reagent contaminants, it can also be considered to be combined with other technologies. Seo and Roh (2018) used the combination of biologically synthesized Pd-FeS and Bacillus cereus to enhance the efficiency of aerobic nitrogen removal at a circumneutral condition.

Conclusion
In this study, two strains of iron-reducing electricigens were obtained by screening from the anode biofilm under micro-oxygen conditions. According to its morphological, physiological, and biochemical characteristics, along with phylogenetic analysis, these two strains were identified as Bacillus cereus. Compared with SFMC-7, the strain SMFC-17 exhibited outstanding ability to remove nitrogen and accumulate phosphorous simultaneously from eutrophic solution under experimental condition and the efficiency of NH$_4^+$-N, NO$_3^-$-N removal, and PO$_4^{3-}$–P accumulation with the removal rate of 79.91 ± 6.34% and 81.26 ± 1.11% and accumulation rate of 57.68 ± 4.36%, respectively. Strain SMFC-17 might be a useful biocatalyst to enable the industrialized application of SMFC in eutrophic water treatment.

Additional files

Additional file 1 Genne sequence.
Additional file 2 Original data.

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Competing interests
The authors declare that they have no conflict of interest.

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N/A.

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N/A.

Authors’ contributions
XZ performed the experiments and drafted the manuscript. XZ, Hpq, and QZC analyzed the data. CW and YqZ contributed reagents/materials/analysis tools. QHZ and ZBW conceived of the study and participated in its design and coordination. The authors read and approved the final manuscript.

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