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Improved Fe(II) regeneration from actual ferric sludge using a biocathode with granular sludge

Guan Wang, Kai Tang, Yuechao Yao, Wenjing (Angela) Zhang, Henrik Rasmus Andersen, Yifeng Zhang *

Department of Environmental and Resource Engineering, Technical University of Denmark, DK-2800, Lyngby, Denmark

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A B S T R A C T

Ferric sludge pollution and unsustainable utilization of Fe(II) reagent were tough nuts to crack for homogeneous Fenton treatment. Previous Fe(II) regeneration methods are still limited by high reagent consumption or low regeneration rate. In this work, granular sludge was introduced into the biocathode of microbial electrolysis cell (MEC), to improve Fe(II)-regeneration rate from actual ferric sludge at near neutral pH. Wastewater constituents in the bioanode of dual-chamber MEC were used as the reducing power to regenerate Fe(II). In the flow-through biocathode, 175 ± 16 mg/L of dissolved Fe(II) was efficiently regenerated from actual ferric sludge. The regeneration rate of dissolved Fe(II) was 7.2 times higher than that of the previously reported biocathode. Besides, the actual and synthetic ferric sludge had a similar Fe(II) regeneration performance. Interestingly, recalcitrant organics and 20 mg/L NO_3–N in ferric sludge were removed by 96% and 100% during Fe(II) regeneration. The biomass in the granular sludge biocathode was 12 times that of the control biocathode, in which *Clostridium sensu stricto* dominated. These findings provide insights and theoretical support for developing a viable biotechnology platform, to close the gap for the efficient and sustainable iron cycle.

1. Introduction

Homogeneous Fenton and Fenton-like systems, based on dissolved Fe (II) and H_2O_2 to generate ·OH, are fundamentally practical for recalcitrant organics removal in wastewater. The process has the advantages of high efficiency, low interference of water matrix and no energy consumption (Neyens and Baeyens, 2003; Sun et al., 2020). However, accumulation of ferric sludge waste and consumption of Fe(II), caused by the slow transformation from ferric ion back to Fe(II), hinder their sustainability and practical application (Klein et al., 2017; Wiegand et al., 2017).

Many efforts have been made to alleviate the limitation of the unsustainable Fe(III)/Fe(II) cycle in homogeneous Fenton systems. Multiple reducing agents (e. g., hydroxylamine and natural polyphenols) have been introduced into homogeneous Fenton systems to assist the redox cycle of Fe(III)/Fe(II) (Chen et al., 2011; Ouyang et al., 2019). These agents reduced the accumulation of ferric sludge by accelerating the regeneration of Fe(II). However, adding sacrificial reagents can increase the operating costs and the risk of secondary pollution. Instead of adding reducing agents, the abiotic electrochemical systems provided electrons through electrical energy for Fe(II) regeneration from dissolved ferric ions at pH < 3 (Kishimoto et al., 2013; Li et al., 2007). In these systems, the pH of wastewater was adjusted to above 7.5 to separate ferric sludge from treated wastewater by sedimentation. The closed cycle of Fe (III)/Fe(II) was realized by reusing ferric sludge in the next Fenton treatment. However, the high consumption of pH-adjusting reagents and electrical energy in the systems limit their application. Therefore, there is still an urgent need to develop Fe(II)-regeneration technology with less reagent and energy consumption, for the broad application of homogeneous Fenton.

Microbial electrolysis cells (MECs) has become a prospective and sustainable technology for high-efficiency chemical production (e.g., H_2O_2 and H_2) from wastewaters (He et al., 2022; Li et al., 2016; Zhang and Angelidaki, 2014). Recently, our group found that dissolved Fe(II) can be regenerated from ferric sludge at near-neutral pH, in the biocathode of MEC (Guan Wang et al., 2022a). The regenerated Fe(II) solution from the biocathode effectively removed various recalcitrant organics, by activating H_2O_2 in Fenton treatment at near-neutral pH values (Wang et al., 2020a; Guan Wang et al., 2022). This approach significantly reduced the consumption of pH-adjusting reagents,

* Corresponding author. Department of Environmental and resource engineering, Technical University of Denmark, Denmark.
E-mail addresses: yifz@dtu.dk, yifzmfc@gmail.com (Y. Zhang).

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compared to abiotic electrochemical systems for Fe(II) regeneration at pH < 3. Besides, this technology avoided the addition of sacrificial reducing reagents for Fe(II) regeneration, using wastewater constituents in the bioanode of MEC as the reducing power. However, the regeneration rate of Fe(II) (0.5 mg/L/h) in the previously reported continuous-flow biocathode was still far from being applied, due to the thin cathode biofilm (Guan Wang et al., 2022). Also, it is unclear whether the Fe(II) regeneration rate from actual ferric sludge would decrease in the biocathode, due to the potential interference of recalcitrant organics and nitrate in the sludge.

Granular sludge is generally originated from conventional activated sludge, while the former possesses incomparable advantages over the latter, including dense and compact structure, excellent sludge settleability, high resilience to toxicants etc (Zhao et al., 2019; Zhou et al., 2021). Besides, adding plastic fillers to biological processes is also considered a good method to increase bioretention (Guochen Wang et al., 2022a; 2022b). However, this not only requires additional cost, but also makes it difficult for organisms attached to the filler to come into contact with the cathode electrode. Introducing granular sludge into the biocathode may increase the biomass in the continuous-flow operation without increasing operating cost. The "robust" characteristics of granular sludge may allow the stable operation of continuous-flow biocathode, using the actual ferric sludge as influent.

The aim of this study is to develop an approach for Fe(II) regeneration from actual ferric sludge with high and stable performance. This study specifically focuses on (i) the improved performance of Fe(II) regeneration in biocathode; (ii) a comparison of Fe(II) regeneration from synthetic and actual ferric sludge; (iii) removal efficiency of recalcitrant organics and nitrate during Fe(II) regeneration and (iv) improvement mechanism and community structure of the biocathode.

2. Materials and methods

2.1. Chemicals and Fenton-derived ferric sludge

100 L of municipal wastewater effluent (35 mg/L chemical oxygen demand (COD), pH 7.2, 5 mg/L NO_3–N, 6.5 mg/L total solid (TS), Lundtofte wastewater treatment plant, Lyngby, Denmark), including 15 recalcitrant organic contaminants (detailed in Text S1), was treated by Fenton oxidation (9 mg/L of H_2O_2 and 15 mg/L of dissolved Fe(II)). The actual Fenton-derived ferric sludge was collected after the treatment (see Fig. S1), which mainly contained 300 mg Fe(III)/L and unremoved recalcitrant organics. The regeneration process from ferric sludge in this work was similar to the practical application. Besides, the synthetic ferric sludge contains 0.57 g/L $Fe(OH)_3$ (300 mg Fe(III)/L), 0.1 g/L $NaHCO_3$, 0.05 g/L NH_4Cl, 0.01 g/L $NaH_2PO_4{\cdot}H_2O$, 0.1 g/L KCl, trace elements (detailed in Text S2) and vitamins (detailed in a previous study (Survey, 1993)). The synthetic wastewater in the anode contains 1.0 g/L $C_2H_3NaO_2$, 0.3 g/L NH_4Cl, 11.5 g/L $Na_2HPO_4{\cdot}12H_2O$, 2.3 g/L $NaH_2PO_4{\cdot}2H_2O$, 0.1 g/L KCl, trace elements and vitamins (detailed in our previous study (Wang et al., 2020b)). All chemicals used in this work were purchased from Sigma, Denmark (analytical reagents).

2.2. Establishment of the MEC with granular-sludge biocathode

As shown in Fig. 1a, a dual-chamber MEC with a biocathode was separated by a cation exchange membrane (CMI 7001, Membrane international, NJ), which allows the H^+ produced at the anode to be transported to the cathode. Commercial carbon fiber brushes (diameter 5.9 cm, length 6.9 cm, Mill-Rose, USA) were used both as anode and cathode electrodes. The large surface area of the carbon brushes can provide many attachment points for granular sludge in biocathode (Baek et al., 2021). They were connected with a 1 mm diameter through an external resistance of 10 Ω for recording voltage by a data logger (USB DAQ 280G, China). The practical volumes of bioanode and biocathode chambers were 180 mL and 145 mL, respectively.

Granular sludge (anaerobic sludge blanket reactor, Colsen, Netherlands) was first cultured by the synthetic ferric sludge in a serum bottle under H_2 atmosphere, to enrich iron-reducing bacteria. Subsequently, 40 mL enriched granular sludge was inoculated into the biocathode of the MEC. The mature bioanode biofilm from a previous study (Wang et al., 2020b) was used in the dual-chamber MEC of this work.

A 0.3 V power supply was connected in series between the bioanode and biocathode to start the MEC operation. During the enrichment stage, anolyte and catholyte were replaced by the synthetic wastewater and actual ferric sludge, respectively, every five days. The concentrations of different iron forms were measured continuously. The enrichment of granular-sludge biocathode was completed until the regeneration rate of dissolved Fe(II) was stable after 15 days' operation.

Duplicate MECs were used in this work to reduce experimental error. Their biocathodes were inoculated by granular sludge (granular-sludge biocathode). Another MEC was set up as a control (see Fig. 1b). Sea sediment and *Geobacter sulfurreducens* were used as inoculum for the biocathode in previous studies for Fe(II) regeneration. They was used as a control to better compare the granular sludge biocathode with previous studies. Various bacteria associated with Fe(III) reduction have been found in sea sediments (Boyd and Ellwood, 2010; Kim et al., 2012; Tagliabue et al., 2017). *Geobacter sulfurreducens* not only can reduce Fe (III), but also a bacterium that has received extensive attention in bioelectrochemical systems. Therefore, the control biocathode was inoculated by sea sediment and *Geobacter sulfurreducens*.

2.3. Recycling actual ferric sludge and removal of pollutants in biocathode

The actual ferric sludge was used as the influent of the granular-sludge biocathode of MEC, to verify the feasibility of simultaneously producing Fe(II) and removing recalcitrant organics. Meanwhile, the synthetic wastewater was used as the influent of the bioanode of MEC, to provide electron donor and reduction potential for Fe(II) regeneration. The MEC was operated for 30 days at continuous flow mode with two days' HRT. The pH values of bioanode and biocathode were seven and

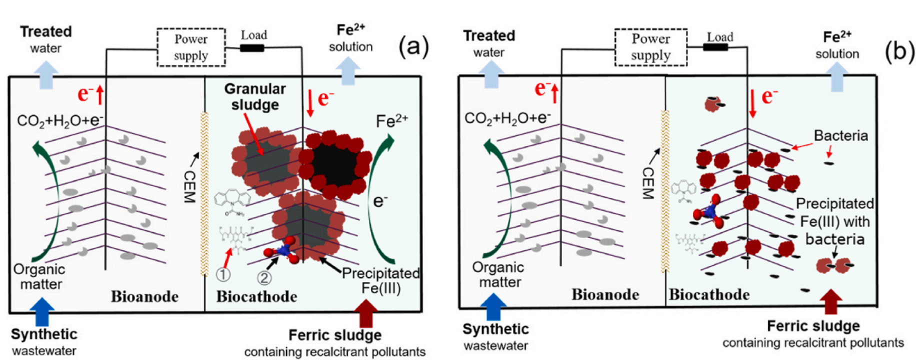

Fig. 1. Schematic diagram of the dual-chamber MEC with biocathode: (a) granular sludge was used as the inoculum of biocathode; (b) sea sediment and Geobacter sulfurreducens were used as the inoculum of biocathode (control biocathode). CEM: cation exchange membrane. ①: recalcitrant micropollutants; ②: nitrate. Compared with using sea sediment and Geobacter sulfurreducens as inoculum, granular sludge was hard to wash out by Fenton sludge due to its large weight and volume.

6.3 ± 0.2, respectively. Fe(III) is the only electron acceptor in the synthetic ferric sludge. However, interfering substances (e.g., nitrate and recalcitrant organics) in the actual ferric sludge may have a negative impact on Fe(II) regeneration. Synthetic ferric sludge was used as a control influent to compare Fe(II) regeneration performance with actual ferric sludge used as influent.

Nitrate and bicarbonate in actual ferric sludge were potential competing electron acceptors for Fe(II) regeneration. The granular-sludge biocathode was operated for another 24 days, using actual ferric sludge with different nitrate concentrations (5, 10, 20 mg N/L) as influent. The effect of nitrate on Fe(II) regeneration and potential bicarbonate reduction products (e.g., CH_4, acetate and formate) were also investigated during the operation.

The concentrations of Fe(II), Fe(III), recalcitrant organics, nitrate, CH_4 and volatile fatty acids (VFAs) were measured during the operation. After the experiments, the bacteria in the biocathodes fed with actual ferric sludge and synthetic ferric sludge were collected for 16S rRNA measurement, respectively.

2.4. Analytical methods

Samples from the biocathode were passed through a 0.2 μm polytetrafluoroethylene membrane to obtain dissolved Fe(II). The concentration of dissolved Fe(II) was measured using ferrozine to form a purple complex with Fe(II) in acetate-buffered saline solution (Stookey, 1970). The absorbance of the complex solution was measured by a spectrophotometer (DR 3900, Hach, USA) at 562 nm. 5 M HCl was used to dissolve precipitated Fe(II) for measuring total Fe(II). The total Fe(II) concentration was calculated from the sum of the dissolved and precipitated Fe(II) concentrations. The total iron includes total Fe(II) and total Fe(III). Of the two, Fe(III) cannot be measured directly. A reducing reagent, 10 M hydroxylamine hydrochloride, was used for reducing all the Fe(III) to Fe(II) at pH 2 when measuring total iron. The concentration of total Fe(III) was obtained by calculating the difference between total iron and total Fe(II).

Moreover, concentrations of CH_4 in the biocathode were determined by gas chromatography. Test kits were used to measure concentrations of nitrate (Hach, LCK339), ammonia (Hach, LCK304) and COD (Hach, LCI500).

Recalcitrant-organic concentration was analyzed using a high-performance liquid chromatography system (Agilent 1290 Infinity, USA, HPLC) with a tandem mass spectrometer (Agilent 6470 series, USA, MS/MS). The detail of measurement parameters can be found in a previous study (Wang et al., 2021). Four scan rates were used to analyze the CV of the biocathode. Total DNA extraction was performed using a Power Soil DNA Isolation Kit (MoBio Power Soil, Carlsbad, CA). Total genomic DNA amplification, using universal primers 515F/806R, was conducted on the v4 hypervariable region of the 16S rRNA gene, and amplicons were sequenced by an Illumina MiSeq desktop sequencer (Ramaciotti Centre for Genomics, Kensington, Australia). The morphologies of material were investigated by scanning electron microscopy (SEM) (Zeiss SUPRA 55) with an accelerating voltage of 10 kV, combined with energy-dispersive X-ray spectroscopy (EDX) for elemental analyses.XRD patterns were measured with a Rigaku XRD-6000 diffractometer with Cu Kα radiation (λ = 0.1542 nm) at 40 kV and 30 mA with a scan step of 0.2° and a scan range from 10 to 65°. Measurement method of biomass in the biocathode can be found in Text S3. Calculation of current efficiency can be found in Text S4.

3. Results and discussion

3.1. Improvement of Fe(II) regeneration performance during enrichment stage

Fig. 2a shows the Fe(II) regeneration performance in the granular-sludge biocathode during the enrichment stage. Its performance was significantly improved than that of the control biocathode. On the one hand, the highest dissolved Fe(II) concentrations increase from 94 mg/L (control biocathode) to 174 mg/L (granular sludge biocathode). On the other hand, the dissolved Fe(II) regeneration rate was stable after 460 h in the granular sludge biocathode. However, the regeneration rate of dissolved Fe(II) was still unstable after 624 h in the control biocathode. The results show that the enrichment time can be shortened in the granular sludge biocathode for recycling actual ferric sludge.

Apart from dissolved Fe(II), changes in total Fe(II) and total Fe(III) at the granular-sludge biocathode were investigated (Fig. 2b). The results showed that total Fe(III) concentration decreased from 300 mg/L to below the detection limit with increased Fe(II) concentration. After two cycles, the total Fe(III) concentration decreased to below the detection limit after 24 h of reactor operation. Total Fe(II) concentration rapidly increased to 300 ± 20 mg/L, which was the opposite of the trend of total Fe(III). Both total Fe(II) and total Fe(III) were not detected in the bioanode of MEC. The above results showed that all the Fe(III) in actual ferric sludge was reduced to Fe(II) at the biocathode, in the last two cycles. It is worth mentioning that the total Fe(II) concentration was higher than dissolved Fe(II). The reason may be that some Fe(II) precipitated with CO_3^{2-} and S^{2-} (Microbial reduction product from sulfate), due to the low K_{sp} values of $FeCO_3$ and FeS (Detailed in Text S5) (Heron et al., 1994; Rickard, 2006). Since the concentrations of carbonate and sulfate in ferric sludge were limited, they could only form a limited amount of precipitation with Fe(II). The ratio of dissolved Fe(II) and total Fe(III) should increase when increasing the concentration of Fe (III) in the ferric sludge solution. Back to the enrichment perspective, the reduction rate of Fe(III) and the regeneration rate of dissolved Fe(II)

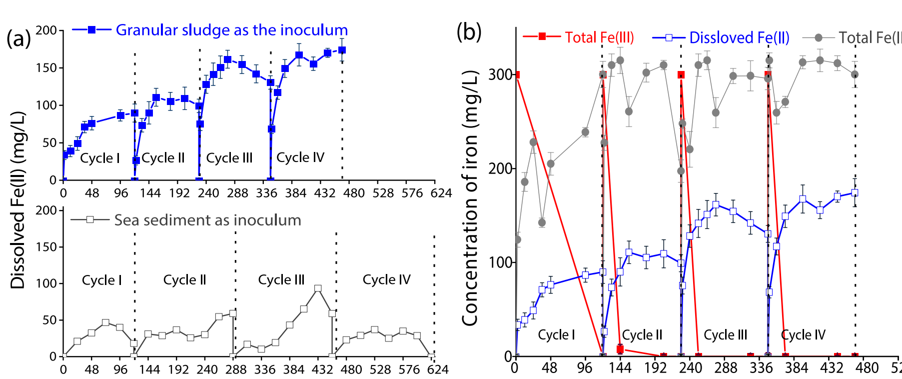

Fig. 2. Comparison of Fe(II) regeneration performance during the enrichment stage: (a) changes in the concentration of dissolved Fe(II); (b) changes in the concentration of various forms of iron in granular-sludge biocathode. Total Fe(II) includes dissolved Fe(II) and precipitated Fe(II). Granular sludge biocathode performed significantly better than the control biocathode.

were stable after four cycles. Therefore, the MECs with granular-sludge biocathode were used in subsequent experiments and switched from batch to continuous flow.

3.2. Enhanced regeneration rate of Fe(II) in continuous-flow mode

The actual ferric sludge containing recalcitrant organics was used as the influent of the flow-through biocathode to study the feasibility of simultaneously producing Fe(II) and removing recalcitrant organics. As shown in Fig. 3a, 175 ± 16 mg/L of dissolved Fe(II) was stably obtained in the flow-through biocathode, using actual ferric sludge as influent after 20 days of operation. 134 ± 16 mg/L of dissolved Fe(II) was stably produced in a control biocathode using synthetic ferric sludge without recalcitrant organics as cathode influent (Fig. 3b). The concentration of HCl-extractable Fe(III) in actual ferric sludge decreased from 300 mg/L (influent) to less than 5 mg/L (effluent) (Fig. S2). This result shows that dissolved Fe(II) can be efficiently obtained not only from synthetic ferric sludge, but also from actual ferric sludge with high efficiency.

Furthermore, as shown in Fig. 3c, the regeneration rate of dissolved Fe(II) in the flow-through biocathode (3.6 ± 0.2 mg/L/h) was 7.2 times higher than the previously reported biocathode (0.5 ± 0.2 mg/L/h) (Guan Wang et al., 2022). HRT of the flow-through biocathode was reduced from 7 days (previous work) to 2 days (this work). This can significantly reduce the volume of biocathode for recycling actual ferric sludge. Moreover, as shown in Fig. 3d, the current efficiency for Fe(II) regeneration was only 9% in the previously reported biocathode (Guan Wang et al., 2022). However, the current efficiency for Fe(II) regeneration increased to 23% in granular-sludge biocathode (this work), which was 2.5 times that of the previous work. The degradation of organic pollutants in the anode wastewater of MEC could provide the current for the biocathode (Yi et al., 2022). The enhancement of current efficiency significantly improved the conversion of organic pollutants and ferric sludge waste into a Fe(II) solution resource.

Overall, this work demonstrated for the first time the high Fe(II)-regeneration concentration and regeneration rate of the granular-sludge biocathode for regenerating Fe(II) from actual ferric sludge, providing more solid information and parameters for its application.

3.3. Recalcitrant organics removal during Fe(II) regeneration

The combination of Fenton treatment and ferric sludge rececying consists of three steps (detailed in S5 of Supplementary Material). Firstly, a municipal wastewater effluent containing 15 recalcitrant organics (9 groups) was treated by a Fenton oxidation (Fe(II)/H_2O_2) at initial pH 7.2. As shown in Fig. 4, nine recalcitrant organics (5-cholobenzotriazole, mefenamic acid, diclofenac, bezafibrate, ketoprofen, sulfamethoxazole, atenolol, ciprofloxacin and mycophenolic acid) were removed by 60%–100%. However, the removal efficiencies of 6 recalcitrant organics (iohexol, iomeprol, metoprolol, venlafaxine, clarithromycin and carbamazepine) were only 13%–39%. Then, ferric sludge from Fenton oxidation was separated from the treated effluent by settlement, which still contained some of the unremoved parts of these recalcitrant organics with a total concentration of 95 μg/L. The distribution proportion of recalcitrant organics in the ferric sludge can be found in Fig. S3. Among the 15 recalcitrant organics, only three recalcitrant organics were completely removed in the supernatant of ferric sludge.

Thereafter, the ferric sludge containing recalcitrant organics was recycled by the granular-sludge biocathode. The concentration change of recalcitrant organics was investigated, during Fe(II) regeneration from actual ferric sludge. Interestingly, the concentration of recalcitrant organics in ferric sludge decreased from 95 to 3.8 μg/L at the biocathode (Fig. 4). Among 15 recalcitrant organics, the concertations of 11 recalcitrant organics were decreased into below the detection limit. The

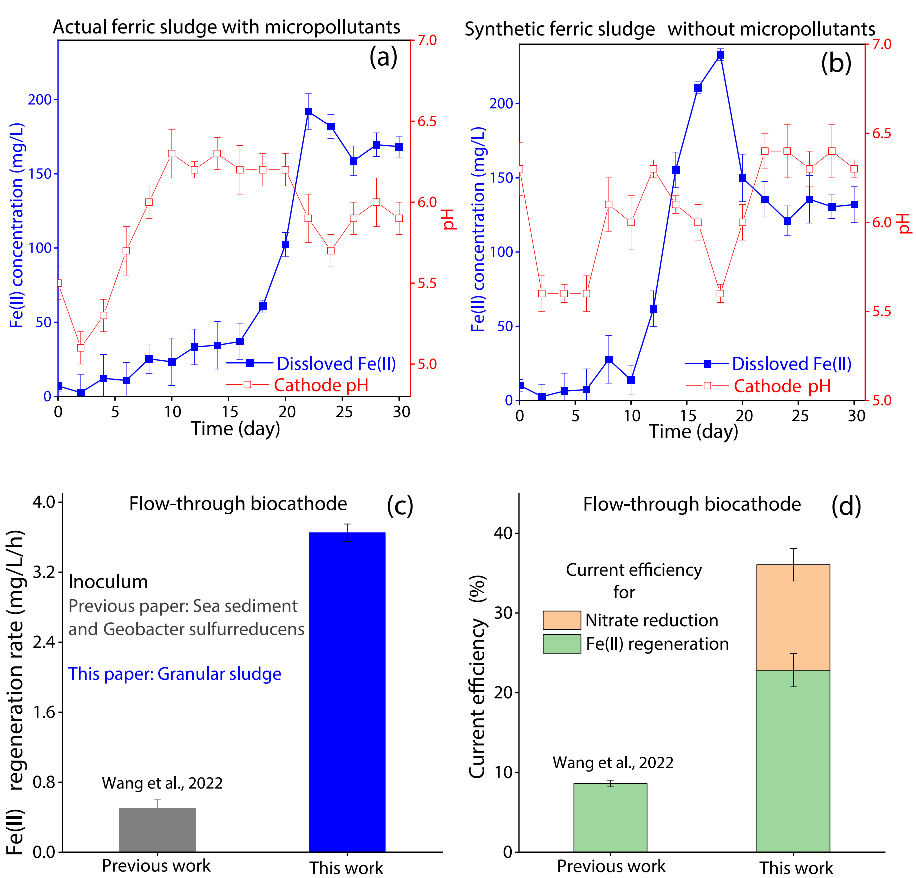

Fig. 3. Performance of regenerating Fe(II) from ferric sludge in the granular-sludge biocathode under continuous-flow mode: (a) actual ferric sludge as cathodic influent; (b) synthetic ferric sludge as cathodic influent. (c) Comparison of dissolved Fe(II) regeneration rate in the flow-through biocathode with previous work. (d) Comparison of current efficiency in the granular-sludge biocathode with previous work. The regeneration rate and current efficiency for Fe(II) regeneration in this work were significantly higher than those of the previous biocathode.

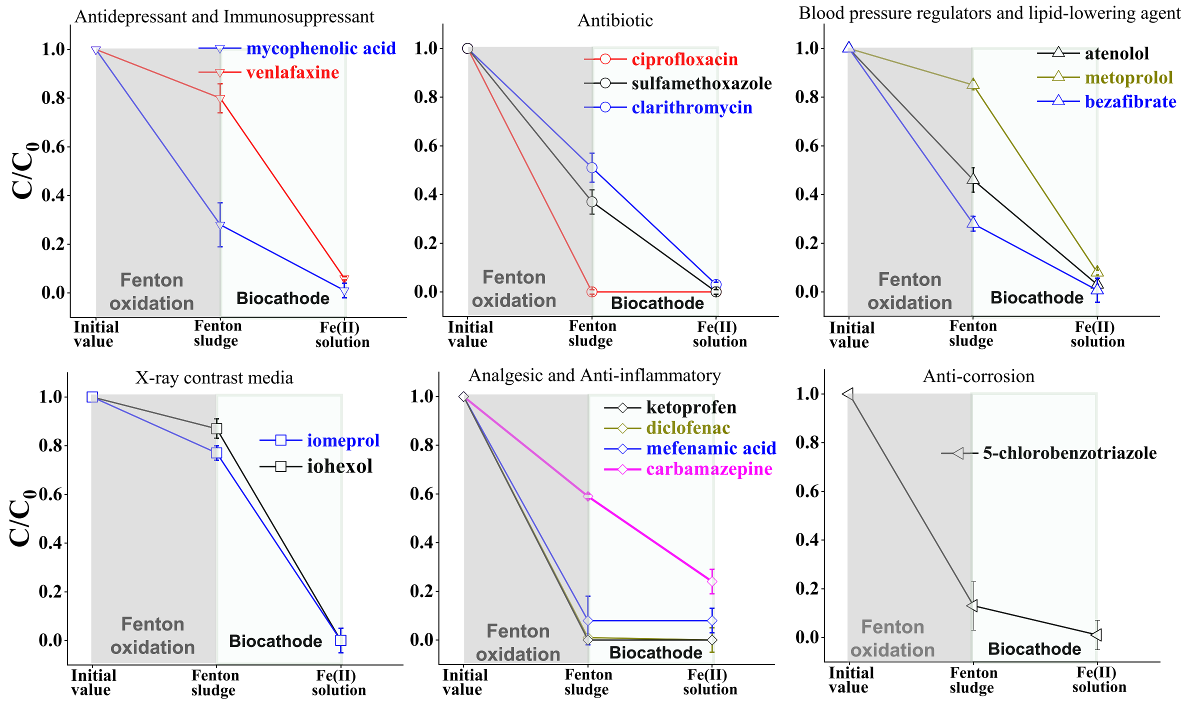

Fig. 4. Concentration change of recalcitrant organics in Fenton oxidation and biocathode. Thanks to the granular-sludge biocathode, a Fe(II) solution with less recalcitrant organics could be obtained from the Fenton sludge containing recalcitrant organic pollutants.

concentration of the other four recalcitrant organics was also significantly decreased. The total removal of recalcitrant organics (96%) was well above the removal efficiency (80%) required by the Switzerland standard for recalcitrant organics removal (The Swis Federal Council, 2018). Regarding the excellent removal performance of recalcitrant organics, in previous biocathode MEC without ferric sludge, recalcitrant organics could be removed by several mechanisms (oxidation and adsorption) (Yan et al., 2019). Microorganisms, the electrode and the ion exchange membrane can adsorb some recalcitrant organics to a limited extent (Werner et al., 2015). However, these materials and granular sludge were less effective at adsorbing some recalcitrant organics (e.g., atenolol and sulfamethoxazole) (Cai et al., 2021; Tang et al., 2020; Zou et al., 2020). Recalcitrant organics were found can be oxidized during bacteria-mediated Feammox reaction (Huang and Jaffé, 2019; Wan et al., 2022). In this work, both Fe(III) and ammonium (10–20 mg/L of NH4–N) are present at the biocathode, which may allow the oxidation of the recalcitrant organic during the Feammox reaction (anaerobic ammonium oxidation coupled to Fe(III) reduction).

Finally, COD change in anode wastewater was also measured, during Fe(II) regeneration from ferric sludge. It was found that 75% of COD in anode wastewater (initial and final COD: 780 and 195 mg/L) was removed during Fe(II) regeneration. Overall, the above results indicated that ferric sludge waste could be converted into valuable Fe(II) solution with less recalcitrant organics, while COD degradation in anode wastewater.

Recalcitrant organic pollutants in ferric sludge were a significant issue for treating and recycling ferric sludge. This work verified for the first time the feasibility of fighting ferric sludge with wastewater to obtain Fe(II) solution with less recalcitrant organics via biocathode. These findings provide a sustainable and profitable platform for ferric sludge recycling.

3.4. Effect of nitrate on the Fe(II) regeneration and bicarbonate reduction

NO_3–N and bicarbonate in ferric sludge are two of the potential competing electron acceptor for Fe(III) reduction. The effect of NO_3–N in ferric sludge on the Fe(II) regeneration in the biocathode was also investigated. Actual ferric sludge with three NO_3–N concentrations (5, 10, 20 mg/L) were adopted for the influent of granular-sludge biocathode, respectively. As shown in Fig. S4, the results show that NO_3–N were all decreased to below detection in the effluent of granular-sludge biocathode. Notably, 173 ± 13 mg/L of dissolved Fe(II) can still be obtained at the flow-through biocathode, even (though) the concentration of NO_3–N in ferric sludge was 20 mg/L. The difference in the average concentration of dissolved Fe(II) was less than 2 mg/L at three different NO_3–N concentrations. The current in the MEC were 0.95, 1.04, and 1.19 mA with 5, 10, and 20 mg/L NO_3–N in ferric sludge, respectively. This result indicated that NO_3–N up to a concentration of 20 mg/L did not adversely affect the regeneration of Fe(II) from ferric sludge. Conversely, NO_3–N can be simultaneously removed during Fe(II) regeneration. These stable performances were significantly improved compared with our previously reported biocathode (Guan Wang et al., 2022), which was susceptible to disturbances by the change in ferric-sludge composition. Besides, ferric sludge with high precipitated Fe(III) concentration can be obtained by sedimentation. Mixing ferric sludge with nitrate-contaminated low-carbon wastewater may simultaneously recycle ferric sludge waste and sustainably treat NO_3–N-contaminated wastewater at the biocathode.

Additionally, the possible reduction products of bicarbonate (e.g., CH_4, acetate, formate, etc) was not detected in biocathode. On the one hand, bicarbonate reduction may be inhibited in the competition of the cathodic reactions. On the other hand, the produced methane can also be oxidized by methanotrophs using Fe(III) as an electron acceptor (eq. (1)) (Cai et al., 2018; Zheng et al., 2020). Cathode bacteria can also consume the organic reduction products (VFAs) from bicarbonate as a carbon source (Zhang et al., 2022).

3.5. Mechanism studies

3.5.1. Cathode electrode and cathode bacteria

The electrode and biomass on the electrode from granular-sludge biocathode were studied, to investigate the reasons for the improved Fe(II) regeneration performance. Surprisingly, the carbon brush attached a large amount of granular sludge (see Fig. 5a–c) instead of

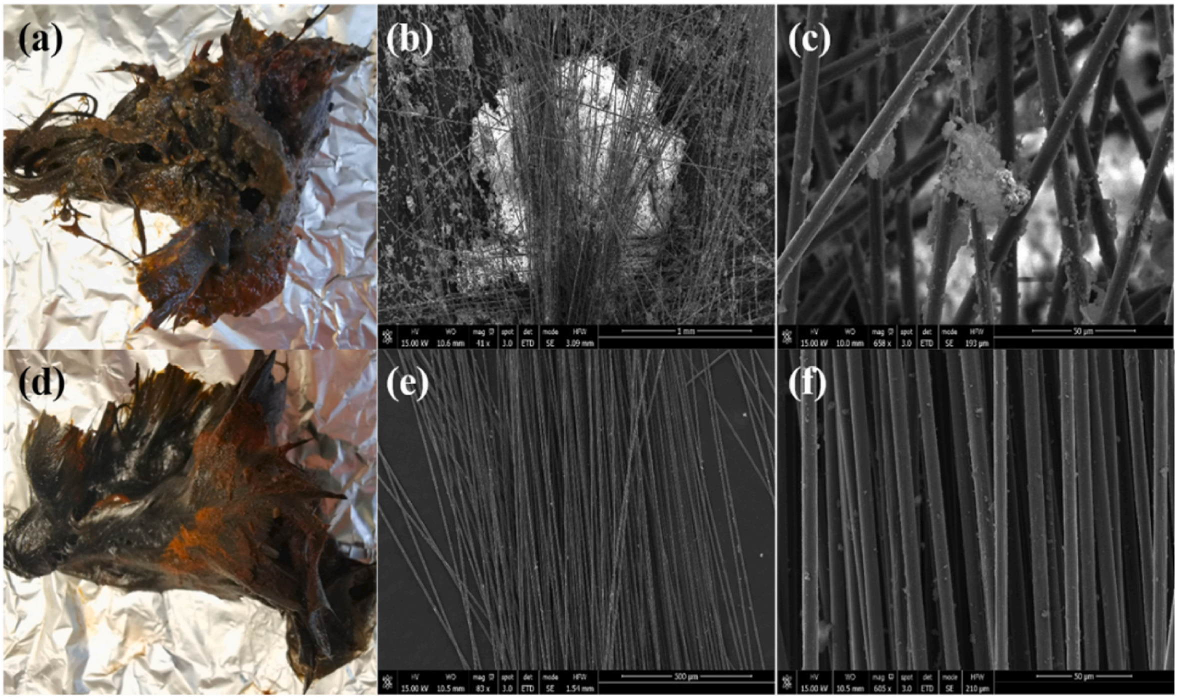

Fig. 5. Characterization of cathode electrode and cathode bacteria: (a–c) images of the electrode including bacteria from granular-sludge biocathode; (d–f) images of the electrode including bacteria from the control biocathode.

settling to the bottom of the cathode. However, only a small amount of precipitate was found in the electrode of the control biocathode (Fig. 5d–e). The total weight of the dry biomass attached to the electrode was 0.95 g/electrode, 12 times the control biocathode (0.08 g/electrode). The large volume and weight of granular sludge may make it less susceptible to being washed out by ferric sludge in the flow-through biocathode.

Fe(II) regeneration rate was limited by thin biofilm in the control biocathode and destabilized by low-concentration organic (Guan Wang et al., 2022). The cathodic bacteria can transfer from the cathode electrode into the catholyte to obtain the electron donor (acetate), easily adsorbed by ferric sludge and washed out in flow-through mode. This further reduced the biomass of the control biocathode. In this work, only 9% of the biomass of the granular-sludge biocathode was in the catholyte. Besides, even if little granular sludge was in the catholyte, it was hard to wash out by ferric sludge due to its large volume and heavy weight advantages. Conversely, the hard COD remaining in the actual ferric sludge not only hardly affected the stability of Fe(II) regeneration, but also might provide the electron donor for increasing Fe(II) regeneration.

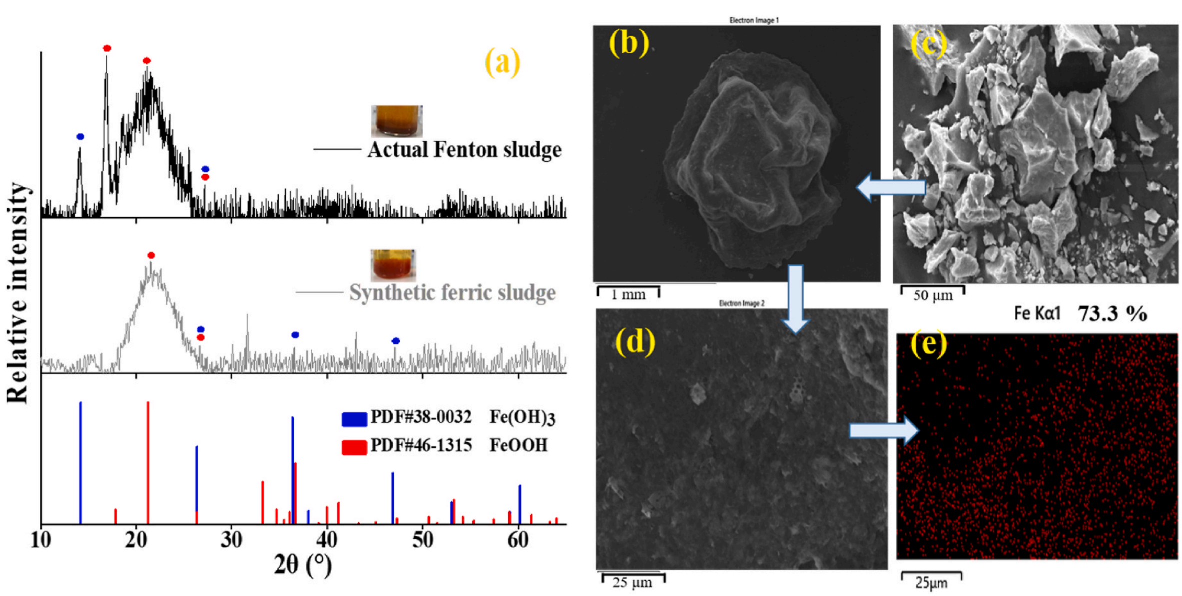

Fig. 6. Characterization of granular sludge and ferric sludge: (a) XRD spectra of actual ferric sludge and synthetic ferric sludge; SEM images of (b) granular sludge from the biocathode, (c) actual ferric sludge and (d) the surface of granular sludge from the biocathode; (e) distribution of Fe on the surface of granular sludge from the biocathode. Poorly crystalline ferrihydrite was observed. Precipitated Fe(III) in ferric sludge was adsorbed on the surface of granular sludge.

3.5.2. Granular sludge and ferric sludge

The physicochemical characteristics of the Fe(III) (hydr)-oxide are essential factors influencing Fe(III) (hydr)oxide transformation and microbial Fe(II) regeneration (Wang et al., 2019). Even though the color of the actual ferric sludge was different from that of the synthetic ferric sludge (Fig. S1), broad characteristic peaks are both found in actual and synthetic ferric sludge (Fig. 6a), indicating their weakly crystalline structure. In addition, a few characteristic peaks appeared in both actual and synthetic ferric sludge, and these characteristic peaks may correspond to $Fe(OH)_3$ (PDF# 38-0032) and FeOOH (PDF# 46–1315). Compared with the synthetic ferric sludge, more characteristic peaks appeared in the actual ferric sludge. In previous studies (Wang et al., 2018, 2019), it was shown that $Fe(OH)_3$ with a weakly crystalline structure is more bioavailable, which can result in a higher reduction rate of Fe(III) reduction in this work. Furthermore, SEM was used to analyze the morphology of the ferric sludge. As shown in Fig. 6c and S5, both the actual ferric sludge and the synthetic ferric sludge have irregular shapes and non-uniform particle sizes.

Besides, the contact behavior of granular and ferric sludge was also investigated. As shown in Fig. 6b–e, granular sludge with an intact ball-like structure was found in the biocathode. It had a rough surface and contained 73% of the iron element. However, the iron element on the surface of raw granular sludge was below the detection limit. These results indicated that a large amount of precipitated iron in the ferric sludge was adsorbed to the surface of the granular sludge.

3.5.3. Community structure of the granular-sludge biocathode

16S rRNA was measured to study the community structure of biomass in the granular-sludge biocathode. Q20 of three samples (Initial inoculum, Biocathode_synthetic ferric sludge and Biocathode_actual ferric sludge) were all higher than 99.7%, implying that the results can reflect the actual situation of bacteria. As shown in Fig. 7a, the results show that the Chao index and Simpson index both decreased in the biocathodes, compared with the initial inoculum. The results indicated a decrease in microbial community richness and diversity, demonstrating the higher selectivity in the biocathodes (Ma et al., 2022). In two biocathodes with different ferric sludges as influents, the Chao index and Simpson index of the "Biocathode_actual ferric sludge" were slightly higher. This may be since the chemical components of the actual ferric sludge was more complex (Wang et al., 2019), compared with synthetic ferric sludge.

Furthermore, PCA figure shows that the distance between the two biocathodes was closer (Fig. 7b). The result indicated that the community structure of biocathodes shifted significantly, compared with the initial inoculum. Thereafter, the community structures of the three samples were investigated at the Family level. The relative abundance of *Methanobacteriaceae* and *Methanosaetaceae* in granular-sludge biocathodes decreased obviously, compared with the initial inoculum. However, *Clostridiaceae* and *Betaproteobacteriales* increased obviously in the biocathodes. The dominant Family in the "Biocathode_actual ferric sludge" shifted from *Methanobacteriaceae* into *Clostridiaceae*.

Finally, the community structures were further studied at the Genus level. *Clostridium sensu stricto* was dominant in the "Biocathode_actual ferric sludge", which was shown to be electrochemically active (Guo et al., 2020). Besides, *Clostridium sensu stricto* and *Methanobacterium*, high relative abundance of Genus, were both found can use Fe(III) oxides and nitrate as electron acceptors (Bond and Lovley, 2002; Fan et al., 2018; Yi et al., 2020). Interestingly, Genus related to the degradation of recalcitrant organics also had a high relative abundance in the "Biocathode_actual ferric sludge". For example, *Clostridium sensu stricto* also dominated the anaerobic reactor's bacterial community for treating textile wastewater (Köchling et al., 2017). *Methanobacterium* were previously found can improve dechlorination performance (Holliger et al., 1992; Wen et al., 2015). Besides, the relative abundance of *Geobacter* in the "Biocathode_actual ferric sludge" increased from 0.02% (initial inoculum) to 0.4%.

It would be interesting to analyze the relationship between microbial community and higher regeneration rate of Fe(II). The regeneration performance of Fe(II) from actual ferric sludge was slightly better than that from synthetic ferric sludge. When the actual ferric sludge is the

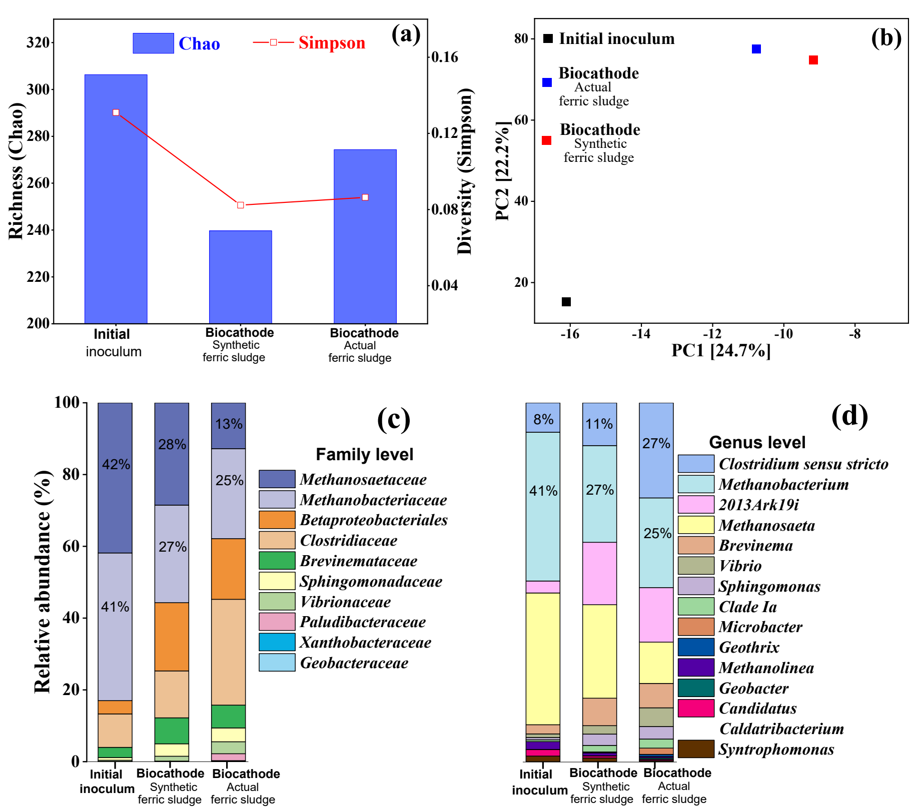

Fig. 7. Comparison of community structure between initial inoculum (granular sludge), "Biocathode_synthetic ferric sludge" and "Biocathode_actual ferric sludge": (a) Alpha diversity analysis; (b) Principal component analysis; (c) Community barplot analysis at Family level; (d) Community barplot analysis at Genus level. Blank: granular sludge had been activated before adding into the biocathode. Biocathode_synthetic ferric sludge: granular-sludge biocathode using synthetic ferric sludge as influent. Biocathode_actual ferric sludge: granular-sludge biocathode using actual ferric sludge as influent.

influent, the relative abundance of most electroactive bacteria (27% of *Clostridium sensu stricto*, 0.6% of *Geothrix and* 0.4% of *Geobacter*) was significantly higher than that of the synthetic ferric sludge as the influent (11% of *Clostridium sensu stricto*, 0.2% of *Geothrix and* 0.01% of *Geobacter*). These bacteria were found to reduce insoluble Fe(III) (Bond and Lovley, 2002; Fan et al., 2018). However, the relative abundance of *Methanosaeta* (11%) was obviously lower than that of the synthetic ferric sludge as the influent (26%). The higher Fe(II) regeneration rate from actual ferric sludge may be related to the higher relative abundance of electroactive bacteria, compared with that synthetic ferric sludge. In our previous study, we also found that the increase in the relative abundance of electroactive bacteria (such as *Geobacter sulfurreducens*) can increase the regeneration rate of Fe(II) (Guan Wang et al., 2022).

3.6. Comparison of Fe(II) regeneration and H_2O_2 production using MEC

Besides Fe(II) reagent, H_2O_2 is also a kind of Fenton reagent, which can be generated by O_2 reduction at the cathode of MEC. The economical value of Fe(II) regeneration via MEC with biocathode is compared with H_2O_2 generation via MEC with abiotic cathode. Compared with previously reported biocathode, the energy consumption of Fe(II) regeneration of this work (0.5 kW h per kg Fe(II)) was reduced by 83% (detailed in Text S6). 2 kg Fe(II) (1.5 €/kg) can be produced using per kW h of electricity (0.2 €/kWh) in the MEC. More than 2 kg ferric sludge (−3 €/kg) can be recycled. The output of the biocathode MEC for Fe(II) regeneration was more than 45 times the input, which was much higher than that of using MEC for H_2O_2 production (Li et al., 2017; Zhou et al., 2020). Fe(II) regeneration from Fe(III) requires only one electron (1-electron reduction) (eq. (1)). However, 2 e^- transfer was needed during the reduction of O_2 to H_2O_2 (eq. (2)) (Wang et al., 2021).

$$Fe(OH)_3 + 3H^+ + e^- \rightarrow Fe^{2+} + 3H_2O \tag{1}$$

$$2H^+ + O_2 + 2e^- \rightarrow H_2O_2 \tag{2}$$

This improvement of Fe(II) regeneration may be related to 1-electron reduction, and higher current efficiency, compared with H_2O_2 production. The ability of the biocathode MEC to convert waste into products also increases its value to the environment.

4. Conclusions

This work introduced a wastewater-driven granular-sludge biocathode that efficiently regenerated dissolved Fe(II) from actual ferric sludge. The key findings of this study are recapped as follows.

(1) Compared with the control biocathode, the granular-sludge biocathode had a shorter enrichment time, a higher Fe(II) regeneration rate, stable regeneration performance and higher current efficiency.
(2) The regeneration performance of Fe(II) from actual ferric sludge was slightly better than that from synthetic ferric sludge. 175 ± 16 and 134 ± 16 mg/L of dissolved Fe(II) were regenerated from actual and synthetic ferric sludge, respectively.
(3) The removal efficiencies of recalcitrant organics and NO_3–N were 96% and 100%, during Fe(II) regeneration from ferric sludge at the biocathode.
(4) The biomass in the granular sludge biocathode was 12 times that of the control biocathode. 91% of the biomass was on the electrode of the granular-sludge biocathode.

CRediT authorship contribution statement

Guan Wang: Investigation, Methodology, Validation, Experiment, Formal analysis, Writing – original draft. **Kai Tang:** Formal analysis, Writing – review & editing. **Yuechao Yao:** SEM characterization, Writing – review & editing. **Wenjing (Angela) Zhang:** Writing – review & editing. **Henrik Rasmus Andersen:** Conceptualization, Supervision, Resources, Funding acquisition, Writing – review & editing. **Yifeng Zhang:** Conceptualization, Supervision, Resources, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2023.136118.

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