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Regeneration of Fe(II) from Fenton-derived ferric sludge using a novel biocathode

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Abstract

Fenton reactions are widely applied when degrading recalcitrant pollutants, but reusing the resulting ferric sludge remains a challenge. A novel concept for regenerating Fe(II) solution at pH 6 based on ferric sludge from neutral Fenton was herein proposed. The microbial fuel cell (MFC) with biocathode and citric acid was used for the first time to promote the regenerated rate of Fe(II) from ferric sludge. The concentration of dissolved Fe(II) reached 120 mg/L in biocathode, which was much higher than that obtained in abiotic cathode (<1 mg/L). The main chemical cost of regenerating Fe(II) was only 3.3% of the commercial Fe(II). Subsequently, the regenerated Fe(II) solution was used to activate H₂O₂, to remove pharmaceuticals from the municipal wastewater effluent. A wide range of pharmaceuticals was successfully removed at neutral pH in 60 min, and the efficiency of the treatment was similar to when the same dosage of commercial Fe(II) was applied.

**Keywords:** Fenton; Regeneration; Ferric sludge; Iron-reducing bacteria; Biocathode.
1. Introduction

Recalcitrant pollutants (e.g., pharmaceuticals) are frequently detected in municipal wastewater effluents, which are discharged into receiving water bodies and harm aquatic ecosystems (Giannakis et al., 2016). The classic Fenton process, using Fe(II) and H₂O₂ to produce OH• with strong oxidation potential (E°=2.8V), has already been proven to effectively remove recalcitrant pollutants (Neyens and Baeyens, 2003; Wang et al., 2018). However, two major issues limit its application. First, acids and bases are needed to adjust the wastewater to pH 3 or similar, before the treatment, and then adjust it back to a neutral pH after treatment for disposal (Miralles-Cuevas et al., 2014). Second, a large amount of iron sludge is inevitably formed, causing secondary pollution and substantial subsequent sludge treatment costs (Rodríguez et al., 2016). To overcome the first issue, a neutral Fenton has become more popular, as it can avoid having to adjust the pH level, thereby resulting in significantly reducing the cost consumption of acid and alkali (Amanollahi et al., 2019) at the cost of higher dosage and sludge production. Addressing the second issue, current methods, such as electrochemical reduction, for the regeneration of Fe(II) from iron sludge require high acid dosages (pH <3) and an additional power supply, which limit their application (Bolobajev et al., 2014; Zou et al., 2020). It was reported that iron-reducing bacteria (IRB) can effectively reduce Fe(III) at a neutral pH via conventional biological processes (Li et al., 2019; Nevin and Lovley, 2002). However, the disadvantages of conventional biological processes (like excess sludge production, continuous organic carbon consumption and high residual organic carbon in regenerated Fe(II) solutions) limit its combination with the Fenton approach (Li et al., 2019). The development of efficient and cost-effective dissolved Fe(II) regeneration technology may reduce the purchasing of Fe(II) and the production of iron sludge, and thereby contribute to the development of a neutral Fenton.
Compared to conventional biological processes, a microbial fuel cell (MFC), which combines electrochemical and biological processes to harvest electrical energy from organic matter in wastewater (Logan, 2009), may save the need to use organic carbon for iron reduction and separate the regenerated Fe(II) solution (catholyte) from wastewater (anolyte) via a cation exchange membrane. Metal reduction (such as Cr(VI) and Cu(II)) with MFC has been achieved in previous studies (M. Li et al., 2018; H. Wang et al., 2020). However, the current understanding of iron reduction in the MFC cathode is mostly based on using Fe(III) at pH 1.6, or potassium ferricyanide as an electron acceptor to generate electricity (Borsje et al., 2016; Ter Heijne et al., 2011). How to regenerate dissolved Fe(II) with MFC at a near-neutral pH for a neutral Fenton is still a technical conundrum.

Bicathodes, which offer new and promising methods for metal reduction at a near-neutral pH, have the advantages of being cost-effective and sustainable (Huang et al., 2015; Jain and He, 2018). Mixed bacterial cultures, including IRB, have been used as biocathodes in MFC for the reduction of soluble metal electron acceptors such as vanadium(V) and Cr(VI) (Huang et al., 2015; Zhang et al., 2019, 2018). However, unlike soluble vanadium(V) and Cr(VI), if one reduces ferric sludge using IRB, the challenge of the very low Fe(III) solubility with a near-neutral pH has to be overcome. Increasing the solubility of Fe(III) is the key to successful reduction with a neutral pH (Zhao et al., 2018). EDTA was used in a previous study (Liu et al., 2019), but it was not suitable, as it complexates Fe(II) better and is itself a contaminant of concern, thus making inappropriate to add it to an effluent intended to be released. It is well known that the minor concentration of citric acid can increase the solubility of Fe(III) under neutral pH conditions (Villegas- Guzman et al., 2017). Therefore, combining IRB and low concentrations of citric acid in the cathode could be an efficient solution for Fe(III) reduction at a neutral pH. Subsequently, the regenerated Fe(II) may
effectively activate H$_2$O$_2$ to remove pharmaceuticals during a subsequent Fenton reaction, due to its higher solubility than Fe(III) at the neutral pH. To our knowledge, the regeneration of Fe(II) from iron sludge at a near-neutral pH in the MFC biocathode has not been previously reported. Furthermore, the integration of Fe(II) regeneration in an MFC biocathode-Fenton process has also not been studied.

In this work, a novel process to regenerate Fe(II) from iron sludge is proposed through the bioaugmentation of IRB in the MFC biocathode. The promotion effect of the MFC biocathode on the regeneration of Fe(II) was first investigated, following which the effects of the initial concentration of Fe(III) and the ratio of Fe(III) and citric acid were investigated. Thereafter, the contribution of an anode to the regeneration of Fe(II) was analysed, and lastly, the regenerated Fe(II) with a biocathode was initially reused in a neutral Fenton process, to remove pharmaceuticals spiked in deionised water and municipal wastewater effluent. The process can minimise the requirement for additional carbon sources and catalysts (e.g. iron), energy and acids for Fe(II) regeneration and the neutral Fenton reaction, which may offer insights into the development of an efficient and a cost-effective bioelectrochemical Fenton system.

2. Materials and methods

2.1. IRB source, medium and enrichment

The mixed culture IRB, used for biocathodes, was enriched from sediment collected from a metal-contaminated harbour (Esbjerg city, Denmark). Because many metal-reducing bacteria, including IRB, were found in metal-contaminated sites (Song Wang et al., 2020; Zhang et al.,
2020). The culture medium, containing NaCH$_3$COO (2.7 g/L), FeCl$_3$·6H$_2$O (1 g/L) and other elements was prepared as previously described to enrich IRB (Lovley and Phillips, 1988). The pH of the culture medium was 6.7. The mixed culture IRB was cultured for five generations in an N$_2$:CO$_2$ (80:20) atmosphere. The inoculum for the new generation came from the mixed culture IRB that was cultured for ten days. The concentration of Fe(II) regenerated by the mixed culture IRB was determined on a daily basis, and the temperature of the culture was 30 °C. All chemicals used in the culture medium were analytical-grade and purchased from Sigma (Darmstadt, German).

2.2. Biocathode MFC setup and operation

A schematic of the MFC-biocathode setup is shown in Fig. 1. The dual-chambered MFC was separated by a cation exchange membrane (CMI 7001, Membrane international, NJ), which can transport the H$^+$ produced in an anode to the cathode. The working volume of each chamber was 180 mL. The commercial carbon fiber brushes (diameter 5.9 cm, length 6.9 cm, Mill-Rose, USA) were used in the anode and the cathode as electrodes, the latter of which were connected with a 1 mm-diameter titanium wire through an external resistance of 1,000 Ω during anode bacteria enrichment. The anode was first inoculated with municipal wastewater collected from a primary clarifier at Lundtofte wastewater treatment plant (Lyngby City, Denmark) and mixed with sodium acetate (1 g/L). Potassium ferricyanide (12 g/L) containing a phosphate buffer solution was used as the cathode solution. After four weeks of operation, the maximum voltage of MFC was stable at approximately 0.65 V, which indicated the anodic biofilm was successfully enriched. The anolyte was replaced with synthetic wastewater after the matured anode biofilm was produced. It contained: 1.0 g/L sodium acetate, 11.5 g/L Na$_2$HPO$_4$·12H$_2$O, 2.3 g/L NaH$_2$PO$_4$·2H$_2$O, 0.3 g/L NH$_4$Cl, 0.1 g/L KCl, trace elements and vitamins. The pH of the anolyte was 7.0. Details of these trace elements and vitamins can be found in a previous study (Zou et al., 2020).
Subsequently, the potassium ferricyanide in the cathode was replaced by the ferric sludge solution for biocathode enrichment. The cathode was inoculated with the mixed culture IRB from Section 2.1. Meanwhile, external resistance was changed from 1000 to 10 Ω. The ferric sludge mixture used for the catholyte contained: 0.15 g/L NaHCO₃, 0.05 g/L NH₄Cl, 0.01 g/L NaH₂PO₄·H₂O, 0.025 g/L yeast extract, 1 g/L FeCl₃·6H₂O and trace elements. The pH of the catholyte (conductivity: 2.0 mS/cm) was adjusted to 7 by NaOH (2 M) to form ferric sludge (Kishimoto et al., 2013). Half of the cathode solution was taken out every week and replaced with a new ferric sludge solution and mixed culture IRB (1 mL). The pH in the cathode was controlled at 6.0 by a pH controller with 0.1 M HCl. The concentration of Fe(II) was regularly determined during biocathode enrichment, and the anode was given enough sodium acetate (1.0 g/L) to ensure a sufficient electron donor. Both the anode and the cathode were fluxed with nitrogen before the operation. The enrichment of the biocathode lasted for about 45 days, and all setups were duplicated.

2.3. Batch experiments

2.3.1 The potential of Fe(II) regeneration in different setups

To examine the ability to generate Fe(II) from insoluble Fe(III) in ferric sludge by IRB, batch experiments conducted in 230 mL of anaerobic serum bottles (Sigma, Denmark), including IRB (1 mL), sodium acetate (1 g/L) and ferric sludge (100 mL, 200 mg/L Fe(III)), were studied. The experiments were carried out on an inoculator (KS 4000, IKA Shakers, Germany) at 200 rpm and 30 °C for 27 days. Besides, citric acid (3.6 mM) was included in the serum bottles thereafter, to investigate its effect on Fe(II) regeneration by increasing the solubility of Fe(III).
Different to the operational conditions in the above batch experiments, Fe(II) was regenerated from the ferric sludge mixture (including 200 mg/L Fe(III), details in section 2.2) at two parallel MFCs with biocathodes, and then it was separated with the synthetic wastewater (including 1 g/L sodium acetate, details see section 2.2) in an anode via the cation exchange membrane. The synthetic wastewater in the anode was used to provide the electron donor for Fe(II) regeneration, and the IRB attached to the cathode electrode was used for transferring electrons to Fe(III) in the ferric sludge mixture. Moreover, experiments with or without citric acid (3.6 mM) in the biocathode were used to examine its influence on the regeneration of Fe(II). Besides, to verify the importance of cathode bacteria, the other two parallel MFCs with an abiotic cathode were used for comparison with the biocathode in Fe(II) regeneration. The experiments conducted in the abiotic cathode were set as blank control 1 (without citric acid) and control 2 (with 3.6 mM citric acid), respectively. The pH levels of the anaerobic serum bottles and the MFC cathodes were all controlled at pH 6 (because the performance of Fe(II) regeneration in the MFC biocathode at pH 6 was better than at pH 7 in the preliminary experiment). The descriptions of operational conditions in different reactors can be found in Tab. 1 (E-supplementary data for schematic diagrams of different setups can be found in e-version of this paper online). All batch experiments were conducted in duplicate.

**Tab. 1 is here**

2.3.2 The effect of key parameters on Fe(II) regeneration and anode contribution in an MFC biocathode

To investigate the effect of initial Fe(III) concentration on Fe(II) regeneration, the initial concentrations of Fe(III) in the MFC biocathode were set to 200 mg/L, 400 mg/L, 800 mg/L and 1600 mg/L, with a fixed amount of 3.6 mM of citric acid. The operation times were all set to 120
h. Additionally, to investigate the effects of the molar ratios of citric acid/Fe(III) on Fe(II) regeneration, two molar ratios, namely 1 and 0.01, were applied with a fixed initial 200 mg/L of Fe(III). The concentration of Fe(II) in the biocathode was measured at several set times. Furthermore, the performance of Fe(II) regeneration in the MFC biocathode in the closed circuit and the open circuit, under two low citric acid to Fe(III) molar ratios (0.1 and 0.01), was studied to evaluate the contribution of the anode.

2.3.3 Comparison of pharmaceutical removal by regenerated and commercial Fe(II) in the Fenton process

To study pharmaceutical removal success, in water-based regenerated Fe(II) or commercial Fe(II), in a Fenton process, a stock solution of mixed pharmaceuticals (including antibiotics (azithromycin, clarithromycin and erythromycin); beta-blockers (atenolol); analgesics (diclofenac); antidepressants (citalopram and sertraline); contrast media (iomeprol) and antiandrogens (bicalutamide), all purchased from Sigma, Denmark), dissolved in methanol was first spiked in the 250 mL bottles (Simax, Czech Republic), before the solution was allowed to evaporate for two hours, to get rid of the effect of methanol as additional Chemical oxygen demand (COD). Second, 100 mL of municipal wastewater effluent (Lundtofte wastewater treatment plant, Lyngby City, Denmark), as well as deionised water, was transferred to the separated bottles and then mixed by magnetic stirring (ARE, Fisher Scientific, USA). Immediately, the regenerated Fe(II) from the MFC biocathode (operation condition: initial Fe(III), 200 mg/L; molar ratio of citric acid/initial Fe(III), 0.01) and the commercial FeCl₂ (Analytical grade, Sigma, German) with a fixed dosage of 5 mg/L of H₂O₂, was added to the respective bottles. The nominal dosages of Fe(II) were set to 5, 10 and 15 mg/L. After 60 min, a 1.5 mL sample was taken and quickly filtered with a 0.22 μm PTEE filter (Agilent, USA) into a sample bottle containing tert-butanol (radical scavenger), following which it was preserved at -20 °C in a freezer before analysing by HPLC-MS/MS. The
dissolved organic carbon (DOC) in the wastewater effluent was $15.7 \pm 3.3$ mg/L. The initial reaction pH levels in the deionised water and municipal wastewater effluent were 6.5 and 7.5, respectively. The initial concentrations of the pharmaceuticals in the experiments can be found in Tab. 2.

**Tab. 2 is here**

2.4. Analytical methods

For the dissolved Fe(II) measurement, which was based on the ferrozine method (Stookey, 1970), 0.5 mL of the sample was collected and immediately filtered with a 0.22 µm PTEE filter in an N₂ atmosphere, after which the pH levels of the samples were immediately adjusted to below 1 with 5M HCl, in order to slow down the Fe(II) oxidation rate. A total of 200 µL of the sample was grabbed and reacted with the ferrozine to form the complex (a 500 µL sample was used if the concentration of Fe(II) was below 1 mg/L). The absorbance of the complex was measured with a spectrophotometer (Hach, DR3900) at 562 nm. When determining the total concentration of Fe(II) (including HCl-extractable and dissolved Fe(II)), the steps were the same as seen for the dissolved Fe(II), except the sample was not filtered. Moreover, the total iron concentration was measured by ICP-MS (inductively coupled plasma mass spectrometry, Thermo Scientific, USA).

Pharmaceutical concentration was analysed using a high-performance liquid chromatography (Agilent 1290 Infinity, USA, HPLC) system with a tandem mass spectrometer (Agilent 6470 series, USA, MS/MS). For analysis, 900 µL of the freezer sample, after reaching room temperature, was transferred into an HPLC vial (Agilent, Germany), and 100 µL of internal standards was then added into the same vial. 10 µL of the sample from the HPLC vial was injected
and analysed by HPLC-MS/MS. Details of the operating parameters of HPLC-MS/MS can be found in a previous study by our group (Tang et al., 2020).

The method used to analyse the H₂O₂, based on a previous study (Nadas et al., 2018), was the colorimetric method, which uses potassium titanium oxide oxalate to form a yellow pertitanic acid complex in the presence of hydrogen peroxide. The mixed solution was measured at 400 nm using a spectrophotometer. Total organic carbon (TOC) and COD were determined by the TOC analyser (Shimadzu TOC 5000 A) and a COD cuvette test (Hach, LCK114 and LCK314), respectively. The voltage (V) of the MFCs was monitored at 30-min intervals, using a multimeter connected to a computer.

2.5. Economic analysis

The volume of HCl consumed by the cathode to control pH was obtained by weighing. The unit price of chemical reagents in the cost calculation was based on Alibaba's online supply prices, whilst the price of the regenerated Fe(II) solution was calculated as FeCl₂; the Fe(II) mass of the two was the same.

3. Results and discussion

3.1 Comparison of Fe(II) regeneration in different experimental conditions

The Fe(II) regeneration ability of the IRB was first studied in anaerobic serum bottles. As shown in Fig. 2a, concentrations of dissolved Fe(II) with (indicated by in blue line) or without citric acid (indicated by the black line) increased over time and reached the same maximum concentration of Fe(II) at 172 mg/L. The results indicate that the IRB was able to generate Fe(II)
from ferric sludge at pH 6. The regenerated concentration of dissolved Fe(II) with citric acid (72 mg/L) was 2.3 times higher than without citric acid (31 mg/L) on day 8. It was also found in previous studies that the bioavailability of insoluble Fe(III) improved after it formed soluble complexes (Harrington and Crumbliss, 2009; Shu Wang et al., 2020). Moreover, it has been demonstrated that citrates bind tightly to Fe(III) in the form of Fe(OH)$_3$ and stabilise it, thus lowering the reduction potential of Fe(III)/Fe(II) that is favourable for the enrichment of IRBs (Li et al., 2019). In this paper, citric acid might have the same effect during Fe(II) regeneration in anaerobic serum bottles.

**Fig. 2 is here**

The potential ability to regenerate Fe(II) at a biocathode enriched with IRB was further investigated. As shown in Fig. 2b, all of the Fe(III) in the ferric sludge was reduced to dissolved Fe(II) (200 mg/L) within 32 h at pH 6, following the addition of citric acid. It was found that the dissolved Fe(II) concentration was almost equal to that of the total Fe(II) (E-supplementary data for the concentrations of dissolved Fe(II) and total Fe(II) can be found in e-version of this paper online), thereby indicating that nearly all of the regenerated Fe(II) at the biocathode was dissolved. The maximum current was 1.3 mA (E-supplementary data for the current change can be found in e-version of this paper online). Furthermore, the concentration of dissolved Fe(II) at the biocathode without citric acid addition was only 19 mg/L at 32 h (Fig. 2b), which was 9.5 times lower compared to the biocathode with added citric acid. In the abiotic cathode with added citric acid, the concentration of dissolved Fe(II) was only 0.9 mg/L at 32 h (Fig. 2b), while it was below the detection limit (0.2 mg/L) in the abiotic cathode without citric acid (Fig. 2b). The results show that the biocathode with citric acid significantly enhanced the regeneration of dissolved Fe(II) from ferric sludge at pH 6.
In previous studies on metal reduction by biocathodes, the soluble electron acceptors (e.g. Cr(VI) and V(V)) were reduced to a precipitated state (e.g. Cr(OH)$_3$ and insoluble V(IV)) (Huang et al., 2011; Zhang et al., 2019). However, Fe(III) has a lower solubility than that of Cr(VI) and V(V) at pH 6 (Millero et al., 1995). Thus, the high concentration of dissolved Fe(II) observed in this study could be due mainly to the increase in the solubility of Fe(III), caused by citric acid. Moreover, in the traditional biological process, the IRB can transfer the electron to insoluble Fe(III) directly, or it can enhance the solubility of Fe(III) through its metabolites (Roden and Urrutia, 2002; Weber et al., 2006). The IRB in the biocathode may also promote in different ways electron transfer between the cathode electrode and Fe(III) (Lovley, 2011; Zhao et al., 2018). Besides, bacterial interactions with the electrode surface may decrease the energy required for metal reduction (Huang et al., 2015). Moreover, citric acid, as easily biodegradable organic matter, can also provide an electron donor for Fe(III) during decomposition. On the one hand, compared to traditional electrochemical cathodes (pH <2.5), the dissolved Fe(II) at the biocathode with citric acid was regenerated under near-neutral conditions (pH 6), which greatly reduces the consumption of reagents for pH adjustment (Kishimoto et al., 2013). Furthermore, no energy supply was required for this process, thus reducing energy consumption, whilst actual wastewaters have been used successfully as fuel in the anode, thereby providing the possibility of wastewater treatment at the same time (An et al., 2016; Li et al., 2014). On the other hand, compared to the traditional biological process using organic matter as a means of reducing power, the regenerated Fe(II) solution in the biocathode was purely driven by electrons derived from the anode, which could benefit its utilisation in the following Fenton reaction.
3.2. The effect of initial Fe(III) concentration and the molar ratio of citric acid and Fe(III)

The effect of initial concentrations found in Fe(III) on Fe(II) regeneration was investigated with a fixed citric acid dosage (3.6 mM). As shown in Fig. 3a, the regenerated concentration of dissolved Fe(II) increased gradually, in line with the increase in the initial concentration of Fe(III). Specifically, when the initial concentration of Fe(III) increased from 200 to 1600 mg/L, the dissolved Fe(II) increased from 200 to 703 mg/L. However, the ratio of regenerated Fe(II) and initial Fe(III) decreased gradually, from 100% to 43.9%, in line with the increase in initial Fe(III) concentration from 200 to 1600 mg/L (Fig. 3a). The initial concentrations of Fe(III) determined the number of electrons required for Fe(II) regeneration, so the above result could be due to the limited electron donor at a fixed sodium acetate concentration, which makes it impossible to meet the increasing demand of electrons for Fe(II) regeneration.

**Fig. 3 is here**

In addition to the initial concentration of Fe(III), the molar ratio of citric acid to initial Fe(III) may also affect the regeneration of Fe(II). As shown in Fig. 3b, when the ratio was 1 (the dosage of citric acid was 3.6 mM), the concentration of dissolved Fe(II) reached an equilibrium concentration of 200 mg/L at 32 h. When the molar ratio of citric acid to Fe(III) was 0.01 (the dosage of citric acid was 36 μM), the maximum concentration of regenerated Fe(II) decreased to 120 mg/L. In a previous study, Cu(II) was reduced in the MFC biocathode at pH 5.8 and at a rate of 1 mg/(L*h) (Huang et al., 2015). The reduction rate was the same as identified in this study (1 mg/(L*h)) when the molar ratio of citric acid to Fe(III) was 0.01.
3.3. The contribution of anodic oxidation to Fe(II) regeneration

Two low molar ratios of citric acid to Fe(III) (0.1 and 0.01) with an open circuit and a closed circuit were used to investigate the contribution of anodic oxidation to the regeneration of Fe(II) in the biocathode. As shown in Fig. 4, when the molar ratio of citric acid to Fe(III) was 0.1, the enhancement of dissolved Fe(II) concentration in the closed circuit was limited compared with that of the open circuit. This could be due to the fact that citric acid at high concentrations can provide sufficient electrons for iron reduction in an open circuit, even if no electrons are transferred from the anode. When the molar ratio of citric acid to Fe(III) was 0.01 (the dosage of citric acid was 36 µM), the theoretically maximum concentration of regenerated Fe(II) can only reach 36 mg/L if the 36 µM of citric acid was used as the electron donor. However, the maximum concentration of regenerated Fe(II) reached 120 mg/L in the closed circuit. And the maximum concentration of regenerated Fe(II) in the open circuit decreased by 68% compared to the closed circuit (Fig. 4). Meanwhile, the removal efficiency of the COD in the anode for the closed circuit was 96%. This demonstrates that the electrons generated from synthetic wastewater at the anode were the main drivers of the Fe(III) reduction at the biocathode when the availability of citric acid was limited. In other words, the mixed culture IRB in the cathode can use the cathode electrode as the main electron donor for Fe(III) reduction. In a previous study, the cathode electrode was used as an electron donor for *Geobacter sulfurreducens*, in order to reduce fumarate (Gregory et al., 2004). The result showed that 50% of the fumarate reduction used the cathode electrode as the electron donor, and some of the organic acids in the cathode were metabolised. These results are similar to those in the present study when the citric acid/Fe(III) ratio was 0.01.

Fig. 4 is here
The contribution at the anode was significantly increased when the molar ratio of citric acid to Fe(III) was 0.01 (the dosage of citric acid was 36 μM) in the above results. An increase in anode contribution may facilitate the adhesion of bacteria to the cathode electrode, which in turn could be beneficial to the future development of a continuous flow system. As a result, this was also beneficial to the utilisation of the regenerated Fe(II) from the biocathode in the Fenton reaction, so the regenerated Fe(II) from the biocathode under initially operational conditions of 200 mg/L Fe(III) and 36 μM citric acid was used in the following neutral Fenton.

3.4. Comparison of pharmaceutical removal by regenerated and commercial Fe(II) in a neutral Fenton

To verify that the Fe(II) solution from the biocathode was as good a source of Fe(II) as possible for Fenton treatment, the regenerated Fe(II) from the biocathode was used to activate H₂O₂ in the neutral Fenton for the removal of pharmaceuticals from deionised water and municipal wastewater effluent. A commercial FeCl₂ was also used for comparison. As shown in Fig. 5a, the removal efficiencies of atenolol, iomeprol and diclofenac increased in line with an increase in Fe(II) dosage. The highest removal rate for most pharmaceuticals was observed when the Fe(II) and H₂O₂ dosages were 15 mg/L and 5 mg/L, respectively. Both regenerated-Fe(II) and commercial-Fe(II) can efficiently catalyse the Fenton process. Efficiencies relating to the removal of pharmaceuticals (including clarithromycin, bicalutamide, citalopram, azithromycin and sertraline) from deionised water were all above 90%. The removal performance of the Fenton process using the regenerated-Fe(II) was basically the same as when using commercial FeCl₂; thus, the Fe(II) solution regenerated from the ferric sludge had the potential to replace FeCl₂ in the Fenton application. Furthermore, efficiencies relating to the removal of pharmaceuticals in deionised water were higher than in the effluent wastewater, because the latter contained 15.7 mg/L of DOC, which
consumed partial HO• and H2O2. The different initial reaction pH in deionised water (pH 6.5) and municipal wastewater effluent (pH 7.5) could be another reason for this outcome. In a neutral photo-Fenton process, the concentrations of DOC and pharmaceuticals were usually close to or lower than this study (De la Cruz et al., 2012). Although the best dosage of Fe(II) in this study (15 mg/L) was higher than that of the previous study (5 mg/L), the dosage of H2O2 (5 mg/L) in this study was nine times lower than for the neutral photo-Fenton (50 mg/L). Besides, homogeneous Fenton systems can also effectively remove micro-pollutants at near-neutral pH by adding iron complexes (Clarizia et al., 2017). However, the molar concentration of the ferrioxalate complexes (e.g. citrate and oxalate) were 1 to 20 times higher than the iron dose required to remove over 70% of pollutants (Clarizia et al., 2017), which subsequently increased reagent costs.

Fig. 5 is here

Figs. 5 b and c show the reaction parameter (pH and H2O2) changes before and after reaction in deionised water and municipal wastewater effluent, respectively. The initial pH in deionised water was set at about 6.5, and the pH after the reaction was between 6.8 and 7.2. The municipal wastewater effluent used in this study was no different from its natural counterpart (pH 7.5). No significant changes in the pH were observed after the reaction, which provides the advantage of avoiding the acidification and neutralisation of wastewater in the real application. In some studies, municipal wastewater effluent was adjusted to acidic conditions (2.5 <pH ≤3) or filtered before the Fenton reaction, which not only improved reaction efficiency, but also increased operation costs (Li et al., 2012; Miralles-Cuevas et al., 2014). Besides, the residual H2O2 showed a downward trend with the increase in Fe(II) dosage in both the deionised water and the wastewater system (Figs. 5 b and c). When the dosage of Fe(II) was 15 mg/L, the residual H2O2 was lower than the detection limit (0.3 mg/L). This low residual H2O2 effectively avoids the problem of
excessively high residual H$_2$O$_2$ in the bio-electro-Fenton system which therefore requires subsequent treatment (X. Li et al., 2018). Moreover, low residual H$_2$O$_2$ may also be beneficial in the reuse of iron sludge in the biocathode. Importantly, the concentration of dissolved Fe(II) after the reaction was below detection limits (0.2 mg/L), which helped control the loss of iron during the discharge of treated wastewater. The biocathode-Fenton process may offer insights into the development of the neutral Fenton. On the other hand, the application of dissolved Fe(II) is not limited to the typical Fenton reaction; for example, it can also activate persulfate or peroxymonosulfate to produce sulfate radicals (Li et al., 2020).

3.5. The proposed reaction process and chemical cost calculation

The MFC biocathode was proved for the first time to be able to regenerate dissolved Fe(II) at pH 6 for a neutral Fenton. The proposed reaction equations can be found below. The classic Fenton reaction process is well known (Equations 1-3) (Neyens and Baeyens, 2003), but it is worth mentioning that the Fe(III) was almost insoluble in neutral conditions, due to its poor solubility (Equation 4) (Millero et al., 1995). This indicates that Fe(III) can be separated by precipitation in a neutral Fenton (Muñiz et al., 2009). The solubility of Fe(II) was much higher than that of Fe(III), due to the different K$_{sp}$ (Millero et al., 1995). In the MFC biocathode, Fe(III) can be reduced to dissolved Fe(II) by using the electron harvested from the anode whereby organic wastewater is the source of the main electron donor at pH 6 (Equations 5-6); moreover, we can still obtain some Fe(II) in an open circuit. The citric acid in the cathode can not only increase the solubility of Fe(III) under neutral conditions, but it can also serve as an electron donor for Fe(III) reduction (Equation 7). Furthermore, the regenerated dissolved Fe(II) from the MFC biocathode can activate H$_2$O$_2$ to degrade pharmaceuticals once again.
Fe(II) + H₂O₂ → Fe(III) + HO• + OH⁻ (Neutral Fenton) (1)
HO• + pollutants → oxidation products (Neutral Fenton) (2)
Fe(II) + O₂ + 4H⁺ → Fe(III) + 2H₂O (Neutral Fenton) (3)
Fe(III) + 3OH⁻ → Fe(OH)₃ (Neutral Fenton) (4)
C₂H₃NaO₂+2H₂O → 2CO₂ + 7H⁺ + 7e⁻ (Anode) (5)
Fe(III)+e⁻ → Fe(II) (Cathode) (6)
C₆H₈O₇ + 5H₂O + 18Fe(III) → 6CO₂ + 18H⁺ + 18Fe(II) (Cathode) (7)

Tab. 3 shows the key chemical input and output calculations for the regeneration of Fe(II).

The mass of the Fe(II) in the regenerated Fe(II) solution from the biocathode was the same as that for FeCl₂. The main chemical input was the consumption of HCl for maintaining the pH of the cathode at 6. The pH increase in the MFC cathode is commonplace (Rossi et al., 2019). The dissolution of Fe(OH)₃ was accompanied by acid consumption. Compared with the electrochemical reduction (pH <3), this study not only reduced chemical consumption, due to the near-neutral operating pH and the electrons harvested by bacteria in the anode (Wang et al., 2017), but some dissolved Fe(II) also existed in the form of Fe(OH)⁺ at pH 6, which further reduced acid consumption (Morgan and Lahav, 2007). Besides, ferric sludge can be obtained from a neutral Fenton reaction, which was not included in the cost assumption. It is noteworthy that in an actual Fenton treatment case, the unit price of conventional ferric sludge treatment is much higher than the purchase price of FeCl₂ (Rodríguez et al., 2016). With the process proposed in this study, the output can still reach 30.6 times the input, without considering the benefits of iron sludge reuse.

In the latest research, boron crystals (C-Boron) have been used to accelerate the reduction of Fe(III) at pH 3 (Zhou et al., n.d.). However, this strict pH condition results in the large consumption of acids and bases in the Fenton reaction, and it is accompanied by the leaching of boron. In this
study, soluble Fe(II) was successfully regenerated at pH 6 in the cathode, while synthetic wastewater was degraded in the anode. The concentration of regenerated dissolved Fe(II) reached 120 mg/L with 36 µM citric acid dosing at the biocathode. The dosage of Fe(II) in the neutral Fenton was usually around 1 to 20 mg/L upon pollutant concentration (Clarizia et al., 2017; Xiao et al., 2020), whilst the dissolved Fe(II) regenerated by the novel biocathode was sufficient for the neutral Fenton. Overall, this novel Fe(II) regeneration technology provides a low-cost and green method for a neutral Fenton, which may offer an efficient alternative for the iron cycle.

**Tab. 3 is here**

4. Conclusions

This study proposed a new Fe(II) regeneration concept for iron recycling in the neutral Fenton process. The key findings are recapped as follows:

(1) The regenerated concentration of dissolved Fe(II) in the novel biocathode was much higher than in systems either without citric acid or with an abiotic cathode.

(2) Electrons generated from wastewater in the anode were the main drivers of iron reduction when the availability of citric acid was limited.

(3) Diverse pharmaceuticals were successfully eliminated by the Fenton reaction, using the regenerated Fe(II) at a neutral pH, which was comparable to the commercial FeCl₂.

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Fig 1. Diagram of the two-chambered MFC with iron-reducing bacteria in the cathode.
Fig. 2 Fe(II) regeneration under different conditions. (a) The effect of citric acid on Fe(II) regeneration in the serum bottle. Experiment conditions: pH 6, the concentration of initial Fe(III): 200 mg/L, the concentration of sodium acetate: 1.0 g/L, the concentration of citric acid: 3.6 mM. (b) Comparison of Fe(II) regeneration under different cathode conditions in the MFC. Experiment conditions: pH 6, initial Fe(III): 200 mg/L, citric acid: 3.6 mM.
Fig. 3 (a) The effect of initial Fe(III) concentrations on Fe(II) regeneration at the MFC biocathode. Experiment conditions: pH 6, citric acid: 3.6 mM; (b) The effect of the molar ratio of citric acid to initial Fe(III) on Fe(II) regeneration at the MFC biocathode. Experiment conditions: pH 6, initial Fe(III): 200 mg/L, citric acid: 3.6 mM or 36 μM.
Fig.4 Contribution of anodic oxidation to Fe(II) regeneration at different Fe(III) and citric acid ratios. Experiment conditions: citric acid/Fe(III) = 0.1 (800 mg/L Fe(III) and 1.8 mM citric acid), or citric acid/Fe(III) = 0.01 (200 mg/L Fe(III) and 36 μM citric acid).
Fig. 5 Comparison of regenerated-Fe(II)/H$_2$O$_2$ and commercial FeCl$_2$/H$_2$O$_2$ on pharmaceutical removal. (a) The removal efficiencies of nine pharmaceuticals in deionised water and municipal wastewater effluent; (b) pH and (c) H$_2$O$_2$ changes in deionised water and municipal wastewater effluent; N.D. refers to not detected. Final Fe(II) concentrations were all below detection levels.
Table 1. Description of initially operational conditions in different reactors.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>IRB</th>
<th>MFC</th>
<th>citric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRB and sodium acetate in the bottle</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IRB, sodium acetate and citric acid in the bottle</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MFC with an abiotic cathode</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MFC with an abiotic cathode and citric acid</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MFC with a biocathode</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MFC with a biocathode and citric acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

"+" means involved; "-" means not involved.
Table 2. Initial concentrations of pharmaceuticals in municipal wastewater effluent and deionised water.

<table>
<thead>
<tr>
<th></th>
<th>Atenolol</th>
<th>Iomeprazole</th>
<th>Diclofenac</th>
<th>Erythromycin</th>
<th>Clarithromycin</th>
<th>Bicalutamide</th>
<th>Citalopram</th>
<th>Azithromycin</th>
<th>Sertraline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deionised water</strong></td>
<td>75.1 ± 1.6</td>
<td>57.6 ± 0.2</td>
<td>11.3 ± 0.2</td>
<td>28.4 ± 0.5</td>
<td>22.6 ± 1.1</td>
<td>13.4 ± 0.1</td>
<td>11.3 ± 0.3</td>
<td>31.2 ± 1.3</td>
<td>9.4 ± 0.1</td>
</tr>
<tr>
<td><strong>Wastewater effluent</strong></td>
<td>56.6 ± 0.1</td>
<td>38.5 ± 1.3</td>
<td>11.7 ± 0.1</td>
<td>26.1 ± 0.7</td>
<td>27.5 ± 1.3</td>
<td>13.5 ± 0.1</td>
<td>12.2 ± 0.3</td>
<td>27.7 ± 1.2</td>
<td>9.4 ± 0.2</td>
</tr>
</tbody>
</table>
Table 3. Key chemical input and output calculations for regenerating Fe(II).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Mass (kg)</th>
<th>Unit price ($/kg)</th>
<th>Total price ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid (&gt;99.5%)</td>
<td>0.015</td>
<td>0.6</td>
<td>0.009</td>
</tr>
<tr>
<td>HCl (37 %)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Ferric sludge</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FeCl₂ (&gt;98%)</td>
<td>1</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Output/Input = 30.6

*aThe price of the regenerated Fe(II) solution was calculated as FeCl₂, and the Fe(II) mass of the two was the same.
Highlights:

- A neutral Fenton can be amended, to avoid Fe(II) consumption and iron sludge waste
- Dissolved Fe(II) was regenerated from ferric sludge, using a biocathode at pH 6
- Wastewater, used as an electron donor, separated from Fe(II) into two chambers
- Citric acid in the cathode enhanced the regeneration of Fe(II) by solubilising Fe(III)
- Regenerated and commercial Fe(II) performed the Fenton reactions in equal measure
Fe(II) regeneration at biocathode for neutral Fenton application

Anode

Biocathode

Fe(III)

Electrogenic bacteria

Iron-reducing bacteria

CO₂ + H⁺ + e⁻

Fe(II)

Synthetic wastewater

Neutral Fenton

Fe(III) (insoluble)

OH⁻

Degradation products

Fe³⁺ + OH⁻

H₂O₂

Fe²⁺ (soluble)

pharmaceuticals
CRediT authorship contribution statement

Guan Wang: Conceptualization, Investigation, Methodology, Validation, Formal analysis, Writing - original draft. Kai Tang: Pharmaceutical analysis, Writing – review & editing. Yufeng Jiang: Investigation, Writing – review & editing. Henrik Rasmus Andersen: Conceptualization, Supervision, Resources, Funding acquisition, Writing – review & editing. Yifeng Zhang: Conceptualization, Supervision, Resources, Funding acquisition, Writing – review & editing.