Production of Hydroxyl Radicals From Oxygenation of Simulated AMD In Presence of Extracellular Polymers Substances

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Abstract

While the reaction mechanisms Fe(II) abiotic oxidation produce $\cdot\text{OH}$ by CaCO_3 -induced in AMD are well-documented, little is known about the influence of extracellular polymeric substances (EPS) secreted by microorganisms on Fe(II) oxidation in AMD. Given the recent finding, this study experimentally measured the cumulative concentrations of $\cdot\text{OH}$ produced from oxygenation of simulated AMD in the presence of EPS. Results of this study show that the cumulative $\cdot\text{OH}$ increased from 56.75 to 158.70 μM within 24 h at pH 3 with the increase in EPS concentration from 0 to 12 mg/L. An appropriate pH (about 6) and EPS (6 mg/L) concentration were required for the moderate rate of Fe(II) oxidation, corresponding to the maximum production of $\cdot\text{OH}$. The presence of EPS enhanced the $\cdot\text{OH}$ production from Fe(II) oxidation in simulated AMD under acid conditions. In the presence of EPS, $\cdot\text{OH}$ production is attributed mainly the complexation of Fe(II) with EPS, of which is rich of carboxyl and hydroxyl groups. Besides, the yield of $\cdot\text{OH}$ increased remarkably with the addition of Fe^{3+} . It is most likely that EPS can contribute to reduce $\text{Fe}(\text{III})$ to Fe(II), which is beneficial to the production of $\cdot\text{OH}$. The findings reveal from this study supplement the fundamental of $\cdot\text{OH}$ production from Fe(II) oxidation by microorganisms in natural AMD.

Introduction

Acid mine drainage (AMD) is characterized by the high iron concentrations, the low pH values and amount of various toxic elements. The widely distributed carbonate react with AMD in karst area, which increases the pH. An appropriate pH existed for the oxidation of Fe(II) and generates reactive oxygen species (ROSs, eg; $\cdot\text{OH}$) through the Haber-Weiss mechanism (Koppenol, 2001). $\cdot\text{OH}$ is a strongly ROSs, which can rapidly oxidize redox-sensitive elements such as As(III) (Dutta et al., 2005; Zhang et al., 2016; Zhu et al., 2017) and is identified as an important product in the abiotic oxidation of Fe(II) by O_2 in AMD (Zhu et al., 2017). The diverse sources of $\cdot\text{OH}$ generation by the oxidation of Fe(II) in AMD. Under low pH conditions, the mechanisms of $\cdot\text{OH}$ production have been clarified (Borda et al., 2003; Schoonen et al., 2010; Zhang et al., 2016). $\cdot\text{OH}$ that is produced from ionically bound species of Fe(II) by mechanism of Fenton (Hug and Leupin, 2003). However, the yield of $\cdot\text{OH}$ production from oxidation of inorganic dissolved Fe(II) by O_2 or H_2O_2 is low under acidic conditions (Miller et al., 2013; Remucal and Sedlak, 2011). The adsorption Fe(II) benefits the production of $\cdot\text{OH}$ by enhancing the reducibility of Fe(II) (Ai et al., 2013). It was documented that $\cdot\text{OH}$ production from iron-bearing minerals, for example, magnetite (Fe_3O_4) (Ardo et al., 2015), siderite (FeCO_3) (Guo et al., 2013), and pyrite (FeS_2) (Pham and Waite, 2008; Zhang et al., 2016) and so on, are activated by O_2 . The presence of citrate (Zhang and Yuan, 2017), ethylenediamine tetraacetic acid (EDTA) (Christina et al., 2008) or dissolved organic matter (DOM) (Page et al., 2013; Rose and Waite, 2002) can increase the yield of $\cdot\text{OH}$ markedly via complexation, which accelerates $\text{Fe}(\text{III})$ oxidation in AMD. The complexation of Fe(II) can reduce the redox of $\text{Fe}(\text{III})/\text{Fe}(\text{II})$ couple and accelerate the oxidation rate (Christina et al., 2008; Keenan and Sedlak, 2008).

Under acidic conditions, there are rich of microorganisms acid-philic $\text{Fe}(\text{III})$ -oxidizing bacteria in AMD (Schrenk and M., 1998) which can broadly classified into directly or indirectly to promote $\text{Fe}(\text{III})$ oxidation

(Boon, 2001; Rodriguez et al., 2003). Recently researches have discovered microorganisms that are likely to produce extracellular ROSs, including fungi, phytoplankton or heterotrophic bacteria as potentially significant sources of ROSs in natural waters (Diaz et al., 2013; Zhang et al., 2016). Extracellular Polymeric Substances (EPS) are organic polymers secreted by microbial cells, a complex high-molecular-weight mixture of polymers, of which 70%~80% are proteins and polysaccharides (Houghton and Stephenson, 2002; Kinzler et al., 2003). EPS are a major component of biofilms and play a key role in cell surface attachment (Vu et al., 2009). Keenan and Sedlak reported that the yield of $\cdot\text{OH}$ production is increased drastically in the presence of organic ligands such as oxalate or EDTA, which can accelerate the chemical oxidation of Fe(II) by O_2 and inhibit the precipitation of Fe(III) (Keenan and Sedlak, 2008). Fe(III)-DOM complexes in water are oxidized to promote the production of $\cdot\text{OH}$ (Page et al., 2013). In addition, it is play a role in H_2O_2 -mediated Fe(II) oxidation can produce $\cdot\text{OH}$ when dissolved Fe(II) is complexed by Suwannee River fulvic acid (SRFA) (Miller et al., 2013). The presence of Low-molecular-weight organic acids (LMWOAs) or natural organic matter (NOM) can improve the efficiency of $\cdot\text{OH}$ production dramatically because of complexation (Peiffer and Stuber, 1999; Rose and Waite, 2002). EPS contains abundant negatively-charged functional groups, for example, carboxyl, phosphoric, sulfhydryl, phenolic and hydroxyl groups, and efficiently adsorb irons, and then form Fe(II)-EPS complexes. (Abzac et al., 2010; Guibaud et al., 2005a; Gutnick and Bach, 2000; Pagnanelli et al., 2009). Fe(II)-EPS complexes may effect $\cdot\text{OH}$ production via affecting Fe(II) oxidation by O_2 . However, little reports on the production of $\cdot\text{OH}$ by the Fe(II)-EPS complex. which is rich in organic functional groups. Therefore, the objective of this study was to ascertain the mechanism of Fe(II) chemical oxidation on the production of $\cdot\text{OH}$ in the presence of EPS, which is of great scientific significance for understanding the migration and transformation process of As/Sb and other pollutants in the chemical oxidation.

In this study, We extracted the EPS of microorganisms secretion and simulated AMD to carry out static experiments to explore the mechanism of the production of $\cdot\text{OH}$ in the presence of EPS on Fe(II) chemical oxidation. It is noted that Fe(IV) is likely to generate at neutral conditions (Remucal and Sedlak, 2011; Wiegand et al., 2017), but is not emphasized in our study. The oxidation reaction is stimulated by the addition of CaCO_3 powder. To achieve this goal, sodium benzoate (BA) was added as a probe compound to quantify the cumulative concentrations of $\cdot\text{OH}$ produced from oxygenating simulated AMD (Joo et al., 2005). In batch experiments, the EPS concentration and suspension pH by the addition of different dosages of CaCO_3 powder were varied to explore the mechanisms. The influence of ionically Fe^{3+} was ultimately tested. The main components of EPS characterized by Three-dimensional excitation-emission matrix (EEM) fluorescence spectra or Fourier transform infrared spectroscopy (FT-IR). The solid particles after oxygenation were characterized X-ray powder diffraction (XRD).

Materials And Methods

2.1 Chemicals

CaCO_3 (99.9%), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (99.9%), BA (99.5%), *p*-HBA (99%), $\text{Fe}_2(\text{SO}_4)_3$ (99.9%), All the solutions were prepared using 18 M Ω ·cm deionized water (Milli-Q water from a Millipore system). Simulated AMD (500 mg/L Fe^{2+} at pH 3) was prepared by adding a specific quantity of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ to 250 mL of deionized water, followed by pH adjustment to 3 by 1 M H_2SO_4 .

microorganisms were obtained from acid mine drainage in Xingren country, Guizhou province. It was cultivated for 9K liquid medium (Wang et al., 2009).

2.2 EPS extraction and characterization

microorganisms were initially cultured in 9K medium about 7–14 days to reach the stable growth phase. EPS were extracted from the bacterial suspension, as described in the literature (Kang et al., 2017), with a slight modification. The bacterial suspension was first separated from the medium by centrifugation (4500 g at 4 °C, 30 min) and the supernatant was discarded followed by repeated washing with deionized water for the precipitate. The precipitate was collected and added a little deionized water, and then processed by ultrasound with an intensity of 45 W at a frequency of 25 KHz for 9 min to separate EPS from the pure cells and then centrifugation at 12000 g at 4 °C for 20 min to separate the cells. The supernatant was collected, followed by pH adjustment to 8 by 1 M NaOH for 12 h of exposure, and then filtered through a 0.22 μm membrane to remove $\text{Fe}(\text{OH})_3$ precipitation. The filtrate (aqueous EPS) pH was controlled by the 1 M H_2SO_4 and stored at 4 °C for later batch reaction experiments within 1 week.

The concentration of EPS was quantified by the total organic carbon (TOC) (Zhang et al., 2021). Three-dimensional excitation-emission matrix (3D-EEM) fluorescence spectra (HORIBA aqualog, American) for measuring EPS components, as fluorescence characteristics are greatly related to their structure and functional groups in molecules.

2.3 Batch experiments

All Oxygenation experiments were conducted in a 500-mL glass beaker at the room temperature ($28 \pm 1^\circ\text{C}$) that were wrapped in tinfoil to avoid light. The beaker was exposed to air through several small pores in the top. In a typical reaction, 0.36 g of BA was dissolved in 250 mL simulated AMD to produce 10 mM BA for trapping the $\cdot\text{OH}$ produced. The suspension pH not conditioned during the process. Stirring at 300 r/min was applied using a Teflon-coated magnetic bar.

A series of experiments were conducted to explore the effect of EPS on $\cdot\text{OH}$ production of $\text{Fe}(\text{OH})_3$ aerobic oxidation. (1) To explore the influence of EPS on $\cdot\text{OH}$ production, EPS at 0, 3, 6 or 12 mg/L was added to simulated AMD. (2) To simulate different pH conditions from oxygenation of AMD, different dosages (0.69 g/L, 1.04 g/L or 1.39 g/L) of CaCO_3 was added. (3) $\text{Fe}_2(\text{SO}_4)_3$ (0, 25 or 50 mg/L) was added to evaluate impact of Fe^{3+} on $\text{Fe}(\text{II})$ oxidation and $\cdot\text{OH}$ production.

All the experiments were carried out at least in duplicate. At predetermined time intervals, suspensions were withdrawn for analysis of pH, *p*-HBA and iron species. In all figures, the points and error bars represent the average values and standard deviations for duplicate experiments, respectively.

2.4 Chemical analysis

Approximately 1-mL sample was filtered through a 0.22µm membrane and the filtrate was mixed rapidly with 1-mL methanol. The concentration of *p*-HBA was measured by an HPLC as described previously (Zhang et al., 2016). A conversion factor of 5.87 was used to estimate the cumulative ·OH from the *p*-HBA concentration (Joo et al., 2005). The detection limit of *p*-HBA is 0.1 µM, which corresponds to 0.59 µM ·OH.

Fe(II) was measured by the 1,10-*o*-phenanthroline analytical method at 510 nm using a UV–visible spectrophotometer. The total iron was assayed by reducing Fe(III) to Fe(II) by using hydroxylamine-HCl. The Fe(III) concentration was calculated from the difference between the Fe(II) and total Fe concentrations (Tamura et al., 1974).

2.5 Characterization

Fluorescence spectroscopy, FTIR and XRD absorption spectroscopy were used to probe the interactions between EPS and Fe(II).

Three-dimensional excitation-emission matrix (EEM) fluorescence spectra were obtained using a luminescence spectrometry (HORIBA aqualog, American) with subsequent scanning emission spectra from 220 to 650 nm by varying the excitation wavelength from 220 to 550 nm. Excitation and emission slits were set at 5 nm and the scanning speed was 1200 nm/min. 3D-EEM fluorescence spectra for measuring EPS components, as fluorescence characteristics are greatly related to their structure and functional groups in molecules. Upon the completion of complexation (24 h), the suspension was transferred into a 50 ml centrifuge tube and separated at 4500 g centrifugation and then dried in freeze dryer. The particles were mixed gently with 1% KBr in an agate mortar and analyzed by using a Nicolet IS50 FT-IR (32 scans of accumulation, DLaTGS detector, 4 cm⁻¹ resolution). The structure of the Fe(III)-EPS complex was examined by small-angle powder X-ray diffraction (XRD) using Cu Kα radiation (Panalytical Advance X-ray diffractometer). The mineral phases were identified qualitatively using the MDI Jade 6.0 software.

2.6 Calculation of reaction rate constant

The reaction rate constant is calculated to fit the removal rate of the aqueous Fe(II). The reaction rate constant is obtained by calculating the degradation rate of different sampling time under the optimum concentration ratio and pH condition of reaction about Fe²⁺ and EPS. Fe²⁺ degradation process accords with pseudo-first-order reaction kinetics equation (Eq. 1). The calculations were carried out using origin 2018 software.

$$-\frac{d[Fe^{2+}]}{dt} = k_i[Fe^{2+}] \quad (1)$$

Where $[\text{Fe}^{2+}]$ (mg/L) is the concentration of free Fe^{2+} , t is different sampling times, and k_i is the rate of Fe(II) degradation.

Results And Discussion

3.1 $\cdot\text{OH}$ production from simulated AMD oxygenation in presence of EPS

Upon oxygenation of simulated AMD with addition of 1.04 g/L CaCO_3 , the cumulative concentration of $\cdot\text{OH}$ increased gradually, indicating that EPS could significantly promote $\cdot\text{OH}$ generation (Fig. 1a). The suspension pH rose quickly from initially 3 to about 6 within 1 h, and then decreased slowly and stabilized at about 5 (Fig. 1b). The variation of pH was ascribed to the neutralization of H^+ by the CaCO_3 . The increase of pH accelerated the Fe(II) oxidation (Fig. 1c) in AMD. During the AMD oxygenation, significant concentration of $\cdot\text{OH}$ were accumulated, showing a rapid increase in 1 h and a gradual increase within 1–24 h (Fig. 1a) because of the oxidation Fe(II) by O_2 . In the process of AMD oxygenation, the cumulative $\cdot\text{OH}$ increased dramatically, and were 56.75 μM , 84.97 μM , 102.71 μM , or 158.70 μM at 0 mg/L, 3 mg/L, 6 mg/L, or 12 mg/L EPS (Fig. 1a), respectively. This result clearly suggests that EPS facilitated the produce of $\cdot\text{OH}$ during Fe(II) oxidation.

The 3D-EEM spectra of EPS showed that the EPS are mainly composed of humic acid (A, B) and tryptophan (C) (Fig. 2a), which is consistent with previous studies (Coble, 1996; Li et al., 2013; Xu et al., 2013; Xu et al., 2013; Yamashita and Jaffe, 2008; Yan et al., 2019). The functional groups ($-\text{OH}$ and $-\text{COOH}$) in humic acid can complex Fe(II) to produce complexed Fe(II) (Daugherty et al., 2017; Strouse et al., 1977). FT-IR spectra of EPS were used to explore the functional groups which complex Fe(II) (Fig. 2b). The shifting and becoming spikes of the peaks at about 3441–3221 cm^{-1} region in FT-IR spectra indicated that Fe(II) could be also bonded onto the O-H groups. The decrease of the peak at 1400 cm^{-1} indicated that the carboxyl group (COO^-) in the EPS forms coordination compounds with metal ions (Fe/Ca/Mg , etc.), which leads to the decrease of the absorption intensity of the peak (Guibaud et al., 2005b; Guibaud et al., 2003; Li and Yu, 2014). This result also evidenced that the hydroxyl and carboxyl groups in EPS complexed with Fe(II) . Fe(II) -EPS complex can retarded oxidation rate of Fe(II) (Table 1), which can increase production of $\cdot\text{OH}$ (Zhang and Yuan, 2017; Zhu et al., 2017).

Meanwhile, the absorption peak at 1116 cm^{-1} after complexation by EPS with Fe(II) can indicated that the oxidation of Fe^{2+} favored the generation of lepidocrocite (Gomes et al., 2007). But XRD patterns of solid particles produced from oxygenation of simulated AMD only measured the production of lepidocrocite after 24 h oxidation in the absence or presence of EPS (Fig. 3). The adsorbed Fe(II) by lepidocrocite can accelerate the apparent oxidation of Fe(II) (Ai et al., 2013; Park and Dempsey, 2005). The adsorption alters the coordination of Fe(II) , which benefits the production of $\cdot\text{OH}$ from Fe(II) -catalyzed decomposition of H_2O_2 . This result suggests that the presence of EPS can facilitate the yield of $\cdot\text{OH}$ via Fe(II) oxidation under acidic conditions.

3.2 Effect of CaCO_3 dosage on $\cdot\text{OH}$ production from AMD oxygenation in the presence of EPS

Because pH influences Fe(II) oxidation and $\cdot\text{OH}$ production, the suspension pH was varied by adding different dosages of CaCO_3 . In theory, 1.39 g/L CaCO_3 exactly neutralized the simulated AMD. So, 0.69, 1.04 or 1.39 g/L of CaCO_3 dosages were added to alter the suspension pH in experiments. Considerable influence of CaCO_3 dosage on the accumulation of $\cdot\text{OH}$ was observed, being 71.21, 95.91 or 27.37 μM within 60 h for 0.69, 1.04 or 1.39 g/L (Fig. 3a). The patterns of $\cdot\text{OH}$ accumulation were quite different within different dosages of CaCO_3 , respectively. The cumulative concentration of $\cdot\text{OH}$ increased continuously and then slowed down in the presence of 0.69 g/L CaCO_3 . In the presence of 1.04 g/L CaCO_3 , the cumulative concentration of $\cdot\text{OH}$ increased rapidly and reached 95.91 μM within 60 h, which was the maximum concentration for all groups. The cumulative $\cdot\text{OH}$ concentration show a moderate increase in the initial 2 h and a later stagnation within 1.39 g/L CaCO_3 (Fig. 3a).

For all the dosages tested, the suspension pH rapidly rose to about 6 within 2 h, and then varied differently due to addition of different dosages of CaCO_3 (Fig. 3b). pH effect significantly Fe(II) oxidation (Hug and Leupin, 2003; Zhu et al., 2017), in which $\cdot\text{OH}$ was produced. As shown in Fig. 3c, in the presence of 6 mg/L EPS, the oxidation of Fe(II) was consistent with the pH, being fast in the initial 2 h, and then slowed or halted later on (Fig. 3c). The Fe(II) oxidation percentage was only about 36.4% with addition of 0.69 g/L CaCO_3 (Fig. 3c), the cumulative $\cdot\text{OH}$ concentration was 14.3 μM within 2 h (Fig. 3a). Fe(II) oxidation percentage was increased to 60.5% with addition 1.04 g/L CaCO_3 (Fig. 3c), the cumulative concentration of $\cdot\text{OH}$ was 13.7 μM , and then increased linearly to 95.9 μM , eventually (Fig. 3a). Fe(II) oxidation percentage was increased to 74.5% and reached to nearly 100% within 8 h with addition 1.39 g/L CaCO_3 (Fig. 3c), and then a later nearly stagnation and the cumulative $\cdot\text{OH}$ concentration increased slightly from 23.7 to 27.4 μM , and finally approached stagnation (Fig. 3a). This result suggests that in presence of 6 mg/L EPS, the rise of pH could promote the oxidation rate of Fe(II) in simulated AMD (Zhu et al., 2017), but the rapid oxidation was not conducive to the production of $\cdot\text{OH}$ (King et al., 1995; Stumm and Lee, 1961). As a consequence, the efficiency of $\cdot\text{OH}$ production depends on suitable pH.

3.3 Effect of Fe^{3+} on $\cdot\text{OH}$ production from AMD oxygenation in the presence of EPS

To further evaluate the influence of Fe^{3+} concentration on $\cdot\text{OH}$ production, Fe^{3+} at 0, 25 or 50 mg/L were added into the simulated AMD in the presence of 6 mg/L EPS at pH 3, separately. The cumulative $\cdot\text{OH}$ concentration in AMD with addition of Fe^{3+} were much higher than that without Fe^{3+} within 24 h, and increased significantly with the increase of Fe^{3+} concentration (Fig. 5a). When the Fe^{3+} concentration increased from 0 to 50 mg/L, the cumulative concentration $\cdot\text{OH}$ at 24 h was boosted from 40.41 to 82.98 μM (Fig. 5a).

The yield of $\cdot\text{OH}$ increased remarkably with increasing Fe^{3+} concentration (Fig. 5a). It is most likely that EPS could contribute to reduce Fe^{3+} to Fe^{2+} (O'Loughlin et al., 2010), and promote the generation of the adsorption $\text{Fe}(\text{II})$ (Park and Dempsey, 2005; Shu-Sung et al., 1998) by iron (oxy)hydroxide to produce $\cdot\text{OH}$. The results were consistent with previous finding that NOM is able to reduce $\text{Fe}(\text{III})$ to $\text{Fe}(\text{II})$, resulting in oxidation of the reductants and low concentrations of $\text{Fe}(\text{II})$ (Cohn et al., 2006; Pullin and Cabaniss, 2003). It is documented that the EPS could reduce $\text{Cr}(\text{VI})$ (Long et al., 2019), and reduce Ag^+ to silver nanoparticles (AgNPs) by the hemiacetal groups of sugars in EPS (Kang et al., 2014). The adsorption of $\text{Fe}(\text{II})$ can accelerate the apparent oxidation of $\text{Fe}(\text{II})$ because of the increase in its reducing ability (Park and Dempsey, 2005), and increased the production of $\cdot\text{OH}$.

3.4 Environmental significance

$\text{Fe}(\text{II})$ chemical oxidation in AMD is dominated by microbial action under low pH conditions ($\text{pH} < 4$) (Singer and Stumm, 1970). Microorganisms can secrete EPS dominated by polysaccharides and proteins with functional groups such as carboxyl and hydroxyl groups, which may affect the oxidation of $\text{Fe}(\text{II})$, thus affecting $\cdot\text{OH}$ production. Our findings suggest that the $\text{Fe}(\text{II})$ -EPS complexes in the simulated AMD facilitate $\cdot\text{OH}$ production. The observed $\text{Fe}(\text{II})$ -EPS complexes are likely important forms of bioavailable $\text{Fe}(\text{II})$ for micro-organisms, thereby influencing Fe cycling and production of ROS (eg. $\cdot\text{OH}$), and then affecting transfer and transformation of heavy metals, such as As or Sb (Sun et al., 2020).

Conclusions

Our findings clarify the mechanism of EPS-mediated $\cdot\text{OH}$ production by $\text{Fe}(\text{II})$ chemical oxidation in acidic conditions. Oxidation experiments results substantiate that $\text{Fe}(\text{III})$ complexation by EPS increases the $\cdot\text{OH}$ yield from the influence of carboxyl and hydroxyl groups in EPS. $\cdot\text{OH}$ production increases with increase of in EPS concentration at low pH. $\cdot\text{OH}$ maximum yield depends on suitable pH and EPS concentrations. $\text{Fe}(\text{III})$ -EPS complex can increase oxidation rate of $\text{Fe}(\text{II})$ with H_2O_2 , which can increase production of $\cdot\text{OH}$. The $\cdot\text{OH}$ that is produced from $\text{Fe}(\text{III})$ oxidation in AMD could cause the oxidative transformation of redox-active substance (such as $\text{As}(\text{III})$ pollutant) in acid environments, particularly in the presence of high concentration of EPS.

Declarations

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Conflict of interest

On behalf of all authors, the corresponding author declare that there is no conflict of interest.

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Tables

Experimental conditions	$K_{Fe(II)} [mM/h]$	R^2
0 mg/L EPS	0.0948 ± 0.009	0.96
3 mg/L EPS	0.0945 ± 0.011	0.94
6 mg/L EPS	0.0894 ± 0.012	0.91
12 mg/L EPS	0.0838 ± 0.014	0.88

Figures

Figure 1

Upon oxygenation of simulated AMD with addition of 1.04 g/L CaCO_3 , the cumulative concentration of $\cdot\text{OH}$ increased gradually, indicating that EPS could significantly promote $\cdot\text{OH}$ generation (Fig. a). The suspension pH rose quickly from initially 3 to about 6 within 1 h, and then decreased slowly and stabilized at about 5 (Fig. b). The variation of pH was ascribed to the neutralization of H^+ by the CaCO_3 . The increase of pH accelerated the Fe(II) oxidation (Fig. c) in AMD

Figure 2

The 3D-EEM spectra of EPS showed that the EPS are mainly composed of humic acid (A, B) and tryptophan (C) (Fig. a), which is consistent with previous studies (Coble, 1996; Li et al., 2013; Xu et al., 2013; Xu et al., 2013; Yamashita and Jaffe, 2008; Yan et al., 2019). The functional groups (-OH and -COOH) in humic acid can complex Fe(III) to produce complexed Fe(II) (Daugherty et al., 2017; Strouse et al., 1977). FT-IR spectra of EPS were used to explore the functional groups which complex Fe(III) (Fig. b).

Figure 3

XRD patterns of solid particles produced from oxygenation of simulated AMD only measured the production of lepidocrocite after 24 h oxidation in the absence or presence of EPS fig.

Figure 4

0.69, 1.04 or 1.39 g/L of CaCO₃ dosages were added to alter the suspension pH in experiments

Figure 5

Fe³⁺ at 0, 25 or 50 mg/L were added into the simulated AMD in the presence of 6 mg/L EPS at pH 3, separately