Passive treatment test of acid mine drainage from an abandoned coal mine in Kaili, Guizhou, China

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ABSTRACT

Discharge of acid mine drainage (AMD) from abandoned coal mines of the YuDong catchment in Kaili City, Guizhou Province, China, has severely damaged local ecological environments. In this study, a laboratory-scale dispersed alkaline substrate (DAS) was studied for the treatment of simulated AMD. The experimental conditions and reaction mechanisms were preliminarily explored. The treatment effect and variation law of vertical effluent water quality of the experimental conditions were thoroughly analysed. The results indicated that small-sized limestone (diameter 5–7 mm) having a 20:1 mixture ratio with shavings and minimum HRT of 20 hours result in increasing effluent pH from 3.5 to 6.6, achieving 66.2% and 99.1% removal of Fe and Al, respectively. There were obvious differences in each reaction layer for the removal of various pollutants from AMD along the depth by DAS, the main reaction zone was first 20–30 cm of the reaction column. The removal process of metal ions and sulfate was accompanied by bio-mineralization reaction. This test provided a valuable support for the local practical engineering applications, enriched the AMD processing technology experimental cases, and provided reference for the treatment technology of similar polluted areas.

Key words: acid mine drainage, dispersed alkaline substrate, heavy metal, packing optimization, passive treatment technique

HIGHLIGHTS

- A dispersed alkaline substrate was designed to treat the acid mine drainage from abandoned coal mines of YuDong valley, which provides a reference for the further design of site device and other similar contaminated areas.
- Combining the physical and chemical parameters of the effluent, and mineralogical characterization of the filler along with the microbial diversity of the system, the mechanism of DAS treatment of AMD was analyzed.
- Under this experiment conditions, DAS can raise the effluent pH to 6.6, and the average removal rate of Fe and Al was 66.2% and 99.1%, respectively. Daily decrease rate of Fe was 16–18 g/m2/d.

INTRODUCTION

Oxidation of metallic sulfide minerals in active or abandoned mines results in the formation of Acid Mine Drainage (AMD) with the characteristics of low pH, presence of heavy metals and sulfate in large quantity (Rakotonimaro et al. 2016; Kefeni et al. 2018). Almost all the major coal-producing countries in the world such as the United States, Great Britain, Germany, Spain, South Africa and Canada have severe AMD problems (Aaron et al. 2014). In China, the distribution of AMD is mainly in the southern mining areas, such as Guangdong, Fujian, Zhejiang, Jiangsu, Guizhou, and Hunan (Olías et al. 2019). AMD can lead to serious soil and water pollution of the surrounding environment (Chen et al. 2007; Zhou et al. 2007). Yudong River basin is located in the northwest of Kaili city, Guizhou province, China. The Yudong River basin saw large-scale mining activities of the coal resources with the operation of more than 80 coal mines beginning in the early 1980s and at present all of them are out of production. There has been severe damage to the ecological environment of Yudong River basin with many related water bodies and their bottom mud becoming yellowish or even reddish-brown. The water quality in the lower reaches of the river has been significantly deteriorated and it has been evaluated as a heavily polluted water body (Feng et al. 2014).

Various AMD treatment technologies can be divided into active and passive methods. Traditionally, the treatment of AMD mainly involves the active treatment methods. Due to scattered drainage of the abandoned mines, unstable water flow,
restricted sites, and other reasons, the passive treatment techniques are more suitable for the AMD of abandoned mines. Low energy and chemical consumption, less operational control and maintenance requirements make the passive treatment particularly attractive for remote sites or abandoned mines and also during the closing phase of the projects (Neculita et al. 2008; Schwarz et al. 2020). Passive treatment techniques only use naturally available energy (e.g., gravity flow systems, such as wetlands or underground flow bioreactors) for the purification of water. The design of these treatment techniques is to ensure regular operation during their design life as a consequence of reduced maintenance (Younger et al. 2003). Dispersed Alkaline Substrate (DAS) is a combination of passive treatment technology and biological physico-chemical method and is based on the traditional alkali preparation systems such as Anoxic Limestone Drains (ALD) and Reducing and Alkalinity Producing Systems (RAPS). In the DAS, a matrix of wood chips is mixed with adhesive limestone fine particles in order to improve the reactivity of limestone and reduce the passivation and blockage problems; the high permeability and large pores of the wood particles prevent the problems of system blockage (Rötting et al. 2008). A pilot-scale DAS system has been set up at a mine in the Iberian Peninsula of Spain based on DAS1 (limestone)-sedimentation, tank-DAS 2 (limestone)-sedimentation, and tank-DAS3 (magnesium oxide). The final effluent after treatment was observed to be alkaline, reaching the World Health Organization drinking water limits as well as the U.N. Food and Agriculture Organization’s irrigation water standards. The treatment methodology showed good removal for the heavy metals such as As and Cd with ten times higher acidity removal effect compared to that of the traditional passive processing system designs (Caraballo et al. 2011).

Herein, according to the local conditions of Yudong River basin, we designed a similar treatment process for the AMD pollution based on the principle of non-excessive treatment. DAS showed improvement in the water quality of AMD and discharge characteristics of the abandoned coal mines in the Yudong River Basin. After preliminary exploration of the experimental conditions and reaction mechanisms, detailed analysis of the treatment effect and variation law of vertical effluent water quality was carried out.

METHODS

Experimental materials

The experimental scheme of materials for the optimization of DAS are shown in Table 2. The materials used for this experiment included limestone (CaCO₃ > 90%) with different particle sizes, shavings and quartz sand. The shavings were incorporated to improve the overall porosity and permeability of the filler (Macías et al. 2012a, 2012b; Ayora et al. 2013) as well as delay the system blockage. All the inflows in this study were configured based on the water quality characteristics of one drainage point AMD in Yudong River Basin, Kaili, Guizhou, China. The main components are shown in Table 1.

Experiment

Optimization of experimental parameters

In order to explore the best packing ratio, five experimental columns were set up using the same weight of limestone and were numbered as I, II, III, IV, and V. The Hydraulic Retention Time (HRT) was controlled by the peristaltic pump and the column operation for each column was carried out for 24 h. In the experimental columns I, II, and III, the particle size of the limestone was in the middle range (diameter of 10–15 mm). The respective weight ratio of limestone to shavings in the columns was 20:1, 11:1, and 8:1. The limestone in columns IV and V had small (diameter 5–7 mm) and large particle size (diameter 20–30 mm), respectively, while the weight ratio of limestone to the inert material for both the columns was 8:1. The influence

| Parameter | Concentration (mg/L, except pH) | Source          |
|-----------|---------------------------------|-----------------|
| pH        | 3.5 ± 0.5                       | HCl (AR)        |
| Fe²⁺      | 100 ± 10                        | FeSO₄·7H₂O (AR)  |
| Mn²⁺      | 5 ± 0.5                         | MnSO₄·2H₂O (AR)  |
| Al³⁺      | 25 ± 1                          | Al₂(SO₄)₃·18H₂O (AR) |
| SO₄²⁻     | 1,000 ± 50                      | NaSO₄ (AR)      |

AR: The purity specification for chemical agents is Analytical Reagent.
of different packing mixture ratios on the treatment effect was analyzed by comparing the results of AMD treatment by column I, II, and III; the influence of different particle sizes on the treatment effect were analyzed by comparing the results of column III, IV, and V. The other five experimental columns, numbered VI, VII, VIII, IX, and X, were used to explore the most appropriate HRT. The same weight of limestone with small particle size was filled into each experimental column when the weight ratio of limestone to the inert material was fixed at 8:1 for all the columns. The filling height of column I and column II were 65 and 50 cm, respectively, while for the other experimental columns it was 30 cm. The total volume of the experimental columns was about 17.66 L. Ten groups of columns were tested for 15 days to select the best operating conditions. Schwarz et al. (2020) observed that a DAS reactor required short HRT by evaluating the dispersive alkaline matrix and diffusion exchange system for passive treatment of acidic wastewater from copper mines. Therefore, HRT for experimental columns VI, VII, VIII, IX, X were set at 8 h, 12 h, 16 h, 20 h, and 24 h, respectively.

Inlet flow of each experimental column, calculated by determining the porosity of the filler via the drainage method, was controlled by the peristaltic pump. The diagram of the experimental device is shown in Figure 1. Simulated AMD flowed into the experimental setup from the top and discharged at the bottom outlet through a connected pipe. The DAS system consisted

| NO. | Limestone: shavings (weight ratio) | Limestone particle size | Hydraulic Retention Time (h) |
|-----|-----------------------------------|-------------------------|-----------------------------|
| I   | 20:1                              | Middle (10–15 mm)       | 24                          |
| II  | 11:1                              | Middle (10–15 mm)       | 24                          |
| III | 8:1                               | Middle (10–15 mm)       | 24                          |
| IV  | 8:1                               | Small (5–7 mm)          | 24                          |
| V   | 8:1                               | Large (20–30 mm)        | 24                          |
| VI  | 8:1                               | Middle (10–15 mm)       | 8                           |
| VII | 8:1                               | Middle (10–15 mm)       | 12                          |
| VIII| 8:1                               | Middle (10–15 mm)       | 16                          |
| IX  | 8:1                               | Middle (10–15 mm)       | 20                          |
| X   | 8:1                               | Middle (10–15 mm)       | 24                          |

**Figure 1** | Schematic diagram of experimental column.
of a water layer, packing layer, and quartz layer from top to bottom. The water layer (1–2 cm) at the top was to distribute water and reduce the low flow occurrence evenly. The quartz layer mainly played the role of preventing the filler from blocking the water outlet.

Simulation experiment for the treatment of AMD

A laboratory-scale DAS with 1 m height having sampling ports every 5 cm was set up as per the schematic diagram shown in Figure 1. Simulated AMD was allowed to flow into the experimental setup from the top and discharged through a pipe connected at the bottom outlet. The composition of the DAS system from top to bottom consisted of a water layer, packing layer, and quartz layer. The packing, having a height of 65 cm, was composed of a mixture of limestones (small particle size) and wood shavings with a mixing-ratio of 20:1. The HRT was controlled by a peristaltic pump and was run for 24 h and total duration of the experiment was 75 days. Cellulose was the main component of the shavings used in this experiment to prevent system clogging. During long experimental operation time, a large number of bacteria including iron oxidizing bacteria and sulfate-reducing bacteria will breed in the system and load on the shavings, which may affect the experimental treatment. Therefore, under the same experimental conditions, another control column was established for which the filler was only shavings. The effect of shavings and the microbial load on the treatment was reflected by measuring the change of iron content in the effluent.

Water sampling and analyses

In the experiments for optimization of the parameters, the effluent was collected only from the outlet at the end of the device every day for testing. For the simulation experiment, daily effluent samples were collected from every sample outlet. The changes in the sulfate, alkalinity, Fe$^{2+}$, Fe$^{3+}$, total iron (Fe$\text{t}$), Al$^{3+}$ and Ca$^{2+}$ concentrations of every sample were monitored following the methods by the Standard Methods for the Examination of Water and Wastewater (APHA 2012): 4500-SO$_4$2-, 2320-alkalinity, 3500-Fe, 3500-Al, 3500-Mn, 3500-Ca. Measurement of pH was carried out by a pH meter (PX5J-216F, Rex, Shanghai, China). The ORP was measured by using the ORP meter (SX630, Sanxin, Shanghai, China). The calculation and measurement of acidity and alkalinity is a simple method to assess the quality of coal mine drainage analyses. It is used to determine its potential environmental impact, and to plan appropriate treatment methods to remove metals and produce drainage close to neutral pH value. (Hedin et al. 1994; Kirby & Cravotta 2005a) In this study, net acidity (mg/L in CaCO$_3$ equivalents) was calculated according to Equation (1) (Hedin 2006):

$$\text{Net acidity (mg/L in CaCO}_3\text{)} = 50 \times \left[ 2 \times \frac{[\text{Fe}^{2+}]}{56} + 3 \times \frac{[\text{Fe}^{3+}]}{56} + 2 \times \frac{[\text{Mn}^{2+}]}{55} + 3 \times \frac{[\text{Al}^{3+}]}{27} + 10^3 \times 10^{-\text{pH}} \right] - \text{Alkalinity} \quad (1)$$

where, [Fe$^{2+}$], [Fe$^{3+}$], [Mn$^{2+}$], [Al$^{3+}$] in the equation represent the concentrations of Fe$^{2+}$, Fe$^{3+}$, Mn$^{2+}$, Al$^{3+}$, and all are represented in mg/L. Alkalinity is measured as gross alkalinity (mg/L as CaCO$_3$).

Relative metal removal r (%) at the drain pipe was calculated as per Equation (2) (Rötting et al. 2008):

$$r = \frac{(C_{\text{in}} - C_{\text{out}})}{C_{\text{in}}} \times 100\% \quad (2)$$

where, C$_{\text{in}}$ is the metal concentration of inflow (mg/L), and C$_{\text{out}}$ is the concentration in the drain pipe (mg/L).

Daily decrease rate (R$_d$) of Fe and Net acidity (g/m$^2$/day), normalized by the system area, was calculated as per Equation (3):

$$R_d = \frac{Q (C_{\text{in}} - C_{\text{out}})}{1000 \times A} \quad (3)$$

where, Q is the flow rate (L/day), C$_{\text{in}}$ and C$_{\text{out}}$ are the inflow and drain pipe of total iron, and A is the horizontal area of the treatment system (m$^2$).

Solid sampling and characterization

After the simulation experiment was over, we collected samples from the packing layers at different depths (0–1 cm, 1–5 cm, 5–15 cm, 15–30 cm, 30–45 cm, and 45–65 cm) in the column for the analysis of phase and mineral compositions, and mineral
saturation was also calculated. Samples were collected and air-dried in the laboratory. After dust removal, the samples were grounded and screened for subsequent analysis. The phases and ingredients of the collected samples were determined by X-ray diffraction (XRD) using a Bruker D8 ADVANCE X-ray diffractometer, Germany, with Cu Kα radiation operated at 40 Kv and 30 Ma. A scan rate of 0.02°/s was applied to record the pattern in the 2θ range of 3°–105°. The samples' mineral composition was determined by a Bruker Senterra Laser Confocal Roman Spectrometer, Germany, with an excitation wavelength of 523 nm and spectral resolution of <1.5 cm⁻¹, horizontal resolution <1 mm, vertical resolution <2 mm. The calculation of mineral saturation corresponding to known elements was implemented by phreeqc software, version 2.18.00, and the saturation was calculated using the water4f database. The morphologies of three representative limestone samples (a, b, and c), selected from the shallow packing layer after the experiment, were examined by scanning electron microscope (SEM, Quanta-250, FEI) equipped with an EDS (energy-dispersive X-ray spectroscopy) detector. The images were taken at accelerating voltages of 20 and 18 kV.

**Sampling, DNA extraction, PCR amplification and Illumina MiSeq sequencing**

We also collected samples from different depths of the packing layer (0–1 cm, 1–30 cm, and 30–65 cm) for each column experiment and tested for their microbial diversity after the experiment. These samples were named as I-1, I-2, I-3, II-1, II-2, II-3, III-1, III-2, III-3, respectively. Microbial diversity was determined by using 16S rRNA sequencing, which includes DNA extraction, PCR amplification, and Illumina MiSeq sequencing.

The total DNA was extracted from 1.0 g samples using E.Z.N.A.™ soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) by following the manufacturer’s protocols. The final DNA concentration and purification were determined by Nanodrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% Agarose Gel Electrophoresis. The bacteria 16S DNA gene were amplified 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWT CTAAT-3') by thermocycler PCR system (GeneAmp 9700, ABI, USA). Purified amplicons were pooled in equimolar concentration and sequenced (300 bp paired) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd (Shanghai, China).

Sequence data were processed to produce Raw Fastq files. And then Raw Fastq files were demultiplexed, and quality filtered by Trimmomatic, and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window. (ii) Primers were exactly matched, allowing 2 nucleotide mismatching, and reads containing ambiguous bases were removed. (iii) Sequences whose overlap was longer than 10 bp were merged according to their overlap sequence. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using USEARCH (version 7.0). The taxonomy of each 16S DNA gene sequence was analyzed by RDP Classifier (version 2.2) Algorithm against the Silva database using confidence threshold of 70%.

**RESULTS AND DISCUSSION**

**Optimization of parameters**

The packing optimization experiment (Figure 2) shows that for each group, when the pH of the treated AMD was 3.2–3.3, the effluent pH can increase to more than 6.4. Among I, II and III, no significant difference was observed for the increase of pH value. However, pH of the effluent from column V was found to be significantly lower than that of the other groups. This is mainly due to the large particle size and low specific surface area of limestone in group V, which results in a low reaction rate for the acid-base neutralization and thus having a weak effect on the increase of pH.

The concentration of Fe³⁺ in the effluent of each group was low, while Fe²⁺ and total Fe was similar and showed the trend: V > IV ≈ III > II > I. This trend shows that in terms of Fe removal, group I was the best with Fe removal efficiency of more than 50% while group V was the worst. This is mainly due to the solubility product constant K_sp of Fe(OH)₃ being 8.0 × 10⁻³⁸ leading to hydrolysis and precipitation of Fe³⁺ in a solution having a pH higher than 2.3 and thus Fe³⁺ in the effluent of each group was relatively low. The packing height of each experimental column was different due to the different mixing proportions of each group of fillers. In the case of same HRT, the increased limestone proportion results in a decrease of the vertical flow velocity leading to longer contact time of the surface water layer with air. Thus, the dissolved oxygen content increased, resulting in better oxidation of Fe²⁺ and better removal effect of Fe. In terms of the removal effect of Mn and Al, there was no significant difference between the experimental groups. There was no significant removal of Mn but the removal of Al was pronounced. The concentration of Al in the effluent was less than 1 mg/L with a removal rate of more than 96%.
HRT is one of the main parameters affecting the processing efficiency of DAS. The effective release of alkaline materials in the system can be promoted by optimization of the hydraulic characteristics of the reaction mixture (Ayora et al. 2013). Figure 3 depicts the influence of hydraulic retention time on the DAS performance. Under varying HRT conditions, the effluent pH reached above 6.5 for all the groups. The effluent pH for groups VII – X is slightly higher than that of group VI, indicating that low HRT is not conducive for the complete neutralization reaction between the alkaline materials and simulated AMD. After reaching dynamic equilibrium, further extension of HRT did not have any noticeable effect on the increase of pH. The prolongation of HRT was beneficial for the removal of Fe. More than 76% removal rate of Fe was observed for group IX (HRT = 20 h) and group X (HRT = 24 h). All the experimental groups showed no visible removal effect of Mn but had excellent Al removal.

The mixing ratio of packing was found to have little influence on the increment of pH of the system, but larger particle size was found to be non conducive for the increment of pH. The mixing ratio and particle size was observed to have a visible effect on the removal of Fe. The increase of limestone proportion and the decrease of particle size of limestone showed an enhancement of Fe removal of the system. Therefore, the small-sized limestone having a 20:1 mixture ratio with shavings was found to be more conducive for the removal of acidity and Fe from the AMD. The removal of Fe, Al and acidity reached equilibrium when the HRT was more than 20 h.

Changes of the physicochemical index of effluent

During the experiment, we collected daily effluent samples from the sample outlets at different depths of the experimental device to test the physical and chemical indexes, with the test results being depicted in Figure 4. The $\text{Ca}^{2+}$ concentration...
and alkalinity in the effluent increased continuously and showed the same change pattern from shallow to deep. Net alkalinity (negative net acidity) of effluent appeared in the middle and lower layer (30–65 cm) of reaction column, which indicated that the middle and lower layer of this reaction column was in anoxic state and was not conducive to the removal of various metal ions, which was also consistent with the phenomena of this experiment. The ORP of the effluent at this area of the experimental column was mostly negative (Kirby & Cravotta 2005b). The pH of effluent suffered gradual increment to reach around 6.6, while the content of Fe$^{2+}$, Al$^{3+}$, and net acidity continued to decrease and finally decreased to 0. The average removal rates of total Fe and Al from the AMD by DAS were found to be 65% and 99.1%, respectively. The daily decrease rate of Fe was 16–18 g/m$^2$/d. This indicates gradual removal of the pollutants from AMD during the experiment. The increase of pH and decrease of net acidity are mainly due to the chemical reactions taking place between limestone and acid.

In the horizontal comparison, decrease of pH of the shallow effluent (first 30 cm) took place with progress of the experiment, together with the decrease of Ca$^{2+}$ content and alkalinity. This indicates gradual decrease of the reactivity of the shallow limestone. One of the possible reasons is the consumption of limestone due to reaction. The other possible reason is the blockage of contact between limestone and AMD due to the coverage of the limestone surface by calcium, aluminum sulfate, and other precipitates produced in the solution.

**Figure 4** | The changes in effluent quality.
Over the period of experiment, the reaction zone of DAS was found to be gradually moving down. At the initial stage of reaction, partition point for the typical reaction zone of DAS was 20 cm, which was the stable area of each index, indicating that the reaction had been stabilized. At the end of the experiment, deepening of the reaction zone takes place from the first 20 cm to the first 30 cm, which can provide a relevant reference for the designing of reaction tank height in the field engineering application. If the whole DAS is covered by the reaction zone, it signifies that the service life of DAS is nearing the end. Therefore, whether the design life of the filler can be determined depends mainly on whether the change of the reaction zone can be predicted well.

The formation of mineral precipitation in the device after the experiment is shown in Figure 5. Major precipitation of Fe$^{2+}$ and Al in this DAS takes place in the region of 0–5 cm and 0–20 cm, respectively. There is a strong correlation between the pH and change of Al$^{3+}$ as significant decrease of the concentration of Al$^{3+}$ takes place when pH > 4. Decrease of the pH of shallow effluent with time led to gradual deterioration of the Al$^{3+}$ removal effect in the shallow effluent, and Al$^{3+}$ was already at a low level (<1 mg/L) when pH > 5.

With the increase of depth, the solution became specifically anaerobic due to the gradual decrease of ORP; water in the deeper area cannot re-oxygenate as it is not directly coming in contact with the atmosphere. Moreover, consumption of oxygen due to the oxidation of Fe$^{2+}$ and the action of microorganisms can also reduce ORP (Ayora et al. 2013). However, it can be observed that the REDOX conditions inside the DAS are gradually improving, the transition from anaerobic to aerobic environment may be influenced by the drop-in temperature and pH (the temperature change before and after the experiment was above 10 °C).

The experimental results indicate that the system has no obvious effect on sulfate removal, so for the system with pure shavings only the changes of Fe$^{2+}$ are shown in Figure 6. Gradual decrease of the content of Fe$^{2+}$ took place during the experiment with a maximum reduction of 32.13 mg/L, while the removal quantity was mainly in the range of 15 mg/L–20 mg/L in most cases. This may be due to the role played by iron-oxidizing bacteria in the DAS system.

Figure 5 | Distribution of Fe and Al precipitates at the end of the experiment.
Characterization of the solid samples

Figure 7. shows the SEM images of the limestones in DAS after the experiment. Figure 7(a) shows the SEM characterization results of the limestone surface in DAS prior to the experiment. Deposits on limestone surfaces can be divided into two distinct groups: one is the loose structure of the upper right. The elemental composition of this mainly includes O, Al, and S, with the molar ratio of Al/S being 4:1. From the energy spectrum analysis, the precipitate was mainly Basaluminite \( \text{Al}_4(\text{SO}_4)(\text{OH})_{10}\cdot4\text{H}_2\text{O} \) \cite{Carrero et al. 2017}. The other group is the rough structure of the lower left, its elemental composition is mainly composed of O, and Fe, besides having a small amount of Si, K, Na, and S; the main component is supposed to be Schwertmannite \( \text{Fe}_8\text{O}_8(\text{OH})_6\text{SO}_4 \) \cite{Burton et al. 2010}. Both of them are iron sulfate secondary minerals which are often found in AMD and areas affected by AMD.

Figure 7(b) shows the SEM images of limestone after the experiment. We can find that CaCO\(_3\) is the interior of this particulate, while a shell composed of sediments of Fe and Al forms the outer layer. The molar ratio of Fe/S is 6.34, which is close to the Schwertmannite \cite{Zhou et al. 2007}.

The XRD and Raman spectra of the DAS samples are shown in Figures 8 and 9, respectively. According to the ICPD database, it can be seen in Figure 8 that in every sample, calcite is the dominant species together with a small amount of Dolomite, Quartz, Goethite and Jarosite. Calcite is an alkaline substance used in this experiment. Goethite decreased gradually with increasing of depth, which is a series product of Fe(III), and also produced by dehydration of Fe(OH)\(_3\). In this system, the content of Jarosite \([\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6]\), which is a sulfate mineral produced by the reaction of Fe(III) with \(\text{SO}_4^{2-}\) and \(\text{OH}^-\), increased with increasing depth of DAS \cite{Daoud & Karamanev 2006}. With the limestones dissolved, \(\text{CO}_3^{2-}\) could be released and formed Dolomite. With the progress of experiment, the reaction zone of limestone responsible for generation of alkali gradually moved down. The effective region responsible for the increase of pH was 15–30 cm at the end of experiment, so the diffraction peaks of Calcite and Dolomite become sharper with larger peak area.

This can be confirmed from the Raman spectra as shown in Figure 9. The Raman spectra of each sample were basically the same, but the difference lay in the peak size. Each sample had obvious Raman peaks at 148 cm\(^{-1}\), 276 cm\(^{-1}\), 713 cm\(^{-1}\), 402 cm\(^{-1}\) and 1,086 cm\(^{-1}\). \cite{Andreas et al. 2011}. mentioned that the presence of Calcite can be identified from its main peak at 1,083 cm\(^{-1}\) along with the secondary peaks at 276, 707 and 146 cm\(^{-1}\), which mainly corresponds to Calcite and a small amount of Dolomite in this study. Previous studies have shown that the characteristic Raman peak of Goethite at about 391 cm\(^{-1}\), Ferrihydrite is at 700 cm\(^{-1}\), and Schwertmannites is at 712 cm\(^{-1}\) and 420 cm\(^{-1}\), which are close to each other \cite{Ciobota et al. 2013}. Therefore, the corresponding minerals in this study may be one or more of Goethite, Ferrihydride and Schwertmannites.
According to the phreeqc simulation results, the supersaturated state in DAS consists of Basaluminite, Boehmite, Diaspore, Gibbsite, Goethite, Hematite, Jurbanite, Maghemite, Magnetite, and Siderite. Saturation of the related minerals of Al and Fe$^{2+}$ mainly occurred in the middle and deep parts of the column. With increasing pH, the Al-related minerals tend to supersaturate, while the supersaturation of Fe$^{2+}$ needs certain pH and alkalinity. Fe$^{3+}$ related minerals' saturation occurs at different depths of the system, and supersaturation was related to the presence of Fe$^{3+}$ in the water. We also observed some supersaturations of alum minerals at some sampling points in the shallow parts of the designed system. The above discussions indicate that these mineral deposits might be formed in the system during the DAS operation.

**Microbial community composition in DAS**
According to Miseq high-throughput sequencing, statistical analysis was carried out at the gate level according to the biodiversity (Figure 10). The experimental results revealed that the abundant bacteria in DAS accounting for more than 99% bacteria were *Proteobacteria* (69.8%), *Bacteroidetes* (9.99%), *Actinobacteria* (7.16%), *Firmicutes* (6.49%), *Verrucomicrobia* (3.61%) and *Acidobacteria* (1.72%). *Proteobacteria* are commonly found in DAS, and its richness tends to decrease from shallow to deep. *Bacteroidetes* and *Verrucomicrobia* were mainly distributed in the middle and deep layers of the device,
with the deep layer being richer than the middle layer. *Actinobacteria* and *Firmicutes* could be detected in the whole system, and there were no obvious changes between the levels. *Acidobacteria* was mainly distributed in the shallow and middle layers of the system. The genera in the samples were analyzed, and the results are shown in Figure 11. With the increase of depth, the richness of the bacterial community visibly increased, and the change of dominant species was undeniable. In the surface (I-1, I-2, and I-3), the dominant species were mainly *Acidithiobacillus* (16.06%–32.32%), *Thiomonas* (11.50%–31.14%) and *Acidocella* (5.31%–27.04%). In addition, the richness of *Acidisphaera* in I-1 and I-2 was higher, accounting for 22.77% and 6.88%, respectively. In the middle layer (II-1, II-2, and II-3), the proportion of *Gallionella*, *Rhizomicrobium*, *Sphingomonas*, *Opitutus*, and *Novosphingobium* increased significantly and became the dominant

**Figure 8** | XRD pattern of DAS solid samples.

**Figure 9** | Raman pattern of DAS solid samples.
In the deep layer (III-1, III-2, and III-3), dominant strains became Oppitutus, Pedobacter, Sporobacter, Propionibacteriaceae, Desulfovibrio, Cellulomonas, Dechloromonas, Cloacibacterium, and Aquabacterium, but the majority of these accounted for less than 10%.

Figure 10 | Bacterial composition of the samples at phylum level.

Figure 11 | Bacterial composition of the DAS samples at genus level.
The first 20 dominant species in the samples were selected for further analysis and the results are shown in Figure 12. It can be seen that *Acidithiobacillus Ferrooxidans* has the highest relative abundance in the system, followed by *unclassified-g-Acidocella* and *Thiomonas-sp.-FB-6*, which are mainly distributed in the shallow layer of the device. They were mainly distributed in the shallow layer of the device. They were mainly acidophilic and aerobic bacteria, which can oxidize Fe$^{2+}$ or S$^{2-}$ (Hallberg & Johnson 2005; Coupland & Johnson 2008; Hedrich et al. 2011). This is mainly due to the pH of the surface water being only 3–4, and the ORP being high under open conditions at the later stage of the experiment. In the middle, there was visible fluctuation of the water quality with pH of the water body increasing from 3.5 to 6.5 and ORP suffering significant decrease (the maximum decrease was 300 mv). At this level, the community acidophilic aerobic bacteria decreased, and the neutral facultative anaerobic/anaerobic bacteria increased. Most of these neutrophils were related to the degradation and fermentation of organic matter. For example, *Rhizomicrobium* and *Novosphingobium* were reported to be related to the degradation of hydrocarbons such as cellulose and aromatic compounds (Zhi et al. 2005; Kodama & Watanabe 2011; Zhen et al. 2014). In the deep layer, the water quality changed slightly, the pH of the water body was basically stable between 6 and 7, and ORP was maintained at a relatively low level. The community was dominated by anaerobes at this level; for example, *unclassified-g-Opitutus*, *Pedobacter-toumenirensis*, *uncultured-bacterium-g-Sporobacterium*, *uncultured-f-Propionibacteriaceae*, *uncultured-g-Desulfuviibrio* and *uncultured-bacterium-g-Aquabacterium* are often found in composting systems, anaerobic reactors or soil, and other places. They have the ability to degrade different types of organic substances (Behnami et al. 2018; Gao et al. 2018; Viana et al. 2018). The *uncultured-g-Desulfuviibrio* is one kind of Sulfate Reducing Bacteria (SRB) with the ability to reduce sulfate (Devereux et al. 1990). A sulfate-reducing passive bioreactor constructed by sulfate-reducing bacteria is a good choice for long-term treatment of acid mine wastewater (Serrano et al. 2019). The existence of *Acidithiobacillus Ferrooxidans* and SRB in the reaction column indicated the next research direction for us. Combined treatment device using chemical and biological methods can be designed to improve the efficiency of AMD, and SRB can be added into the deep layer of reaction column to improve the treatment effect of SO$_4^{2-}$ in AMD.

Figure 12 | The heat map of the relative abundance of the top 20 strains in DAS.
Mechanism analysis of DAS processing AMD

Based on the combined result of the above analysis and previous studies (Offeddu et al. 2015; Zhou 2017), we speculated that the observed precipitation during the experimental process was generated mainly through the following processes. Firstly, reaction of limestone with the H\(^+\) in AMD produced alkalinity, which resulted in the increase of pH. When pH < 2.8, Fe\(^{2+}\) could react with SO\(_4^{2-}\) and some metal ions to form Schwertmannite and Ihleite, MFe\(_3\)(SO\(_4\))\(_2\)(OH)\(_6\), with some microbes promoting this reaction. The specific processes are shown in Equation (6), where M\(^+\) are the cations (K\(^+\), Na\(^+\), NH\(_4^+\), H\(_2\)O\(^+\), etc.). Iron sulfate secondary minerals are unstable (Bao et al. 2017), hence dissolution and phase transition will occur when environmental conditions change, especially pH change.

\[
\begin{align*}
\text{Fe}^{2+} + \frac{1}{2} \text{O}_2 + \text{H}^+ & \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O} \quad (4) \\
8\text{Fe}^{3+} + 14\text{H}_2\text{O} + \text{SO}_4^{2-} & \rightarrow \text{Fe}_8\text{O}_8(\text{OH})_8\text{SO}_4 + 22\text{H}^+ \quad (5) \\
3 \text{M}^+ + 3\text{Fe}^{3+} + 2\text{SO}_4^{2-} + 6\text{H}_2\text{O} & \rightarrow \text{MFe}_3(\text{SO}_4)\_2(\text{OH})_6 + 6\text{H}^+ \quad (6)
\end{align*}
\]

when pH = 2.8–4.5, Schwertmannite and Ihleite might react with the relatively more stable goethite, and the adsorbed heavy metals could be released, along with H\(^+\) as per Equations (7) and (8) (Offeddu et al. 2015). The main sedimentary species of Fe are Schwertmannite and a small amount of goethite (FeOOH) along with pyrite at the same time.

\[
\begin{align*}
\text{Fe}_8\text{O}_8(\text{OH})_8\text{SO}_4 + 2\text{H}_2\text{O} & \rightarrow 8\text{FeOOH} + \text{SO}_4^{2-} + 2\text{H}^+ \quad (7) \\
\text{MFe}_3(\text{SO}_4)\_2(\text{OH})_6 & \rightarrow 3\text{FeOOH} + 2\text{SO}_4^{2-} + 3\text{H}^+ + \text{M}^+ \quad (8)
\end{align*}
\]

when pH > 4, Al\(^{3+}\) will hydrolyze into Al(\text{OH})\(_3\) colloidal precipitate, with the critical pH for the formation of Al\(^{3+}\) hydrolyzed precipitate being affected by the sulphate content of the water. When pH is between 4.5 and 6.5, Fe\(^{3+}\) is oxidized by Fe\(^{2+}\) in the hydrolyzed water to form Ferrihydrite, and the primary mineral phases of iron are Ferrihydrite (Fe(\text{OH})\(_3\)) and Schwertmannite. Ferrihydrite is a kind of red-brown spherical nanoparticle with large specific surface area and high surface activity. After the experiment, a large area of reddish-brown precipitation was observed in the experimental device (shown in Figure 5), which confirmed the presence of Ferrihydrite. It can interact with the pollutants in water through adsorption and co-precipitation. For pH > 6.5, Ferrihydrite and goethite become the primary mineral phases of iron in water.

When the concentration of SO\(_4^{2-}\) in water is high, Al\(^{3+}\) might react with SO\(_4^{2-}\) to form Basaluminite Al\(_4\)(SO\(_4\))(\text{OH})\(_{10}\)·36H\(_2\)O, Equation (9). Besides, the production of Schwertmannite can also take place by the reaction of Basaluminite and Fe\(^{3+}\), Equation (10), which leads to the re-release of Al and SO\(_4^{2-}\) from Basaluminite.

\[
\begin{align*}
\text{Al}^{3+} + \text{SO}_4^{2-} + \text{CaCO}_3 + \text{H}_2\text{O} & \rightarrow \text{Ca}^{2+} + \text{CO}_2 + \text{Al}_4(\text{SO}_4)(\text{OH})_{10} \cdot 36\text{H}_2\text{O} \quad (9) \\
\text{Fe}^{3+} + \text{Al}_4(\text{SO}_4)(\text{OH})_{10} \cdot 36\text{H}_2\text{O} & \rightarrow \text{Fe}_8\text{O}_8(\text{OH})_8\text{SO}_4 + \text{Al}^{3+} + \text{SO}_4^{2-} + \text{H}_2\text{O} \quad (10)
\end{align*}
\]

CONCLUSION

Under the set experimental conditions, small-sized limestone having 20:1 mixture ratio with shavings was found to be more conducive for the removal of acidity and Fe in AMD than others. Effective removal of acidity and heavy metals from the AMD by DAS was possible only when HRT was more than 20 hours. The pH of effluent increased to 6.6, while the average removal rate of Fe and Al was 66.2\% and 99.1\%, and daily decrease rate of Fe was 16–18 g/m\(^2\)/d. According to the SEM, XRD and Raman analysis results, it can be concluded that the increase of effluent alkalinity is mainly due to the dissolution of limestones. A series of minerals, such as Schwertmannite, Goethite, Jarosite and Basaluminite, can be also produced during the removal process of iron and aluminum from AMD. The removal of metal ions and sulfate were accompanied by microbial mineralization taking place at all the depths of the reaction column, which points out the direction for further research in new treatment technology. The future work can focus on the screening and cultivation of mineralized microorganisms, and the treatment technology can be a combination of chemical and biological methods to improve the treatment efficiency. The thickness of the reaction layers of DAS for the removal of various pollutants in AMD was significantly different. The
increase of pH occurred mainly in the shallow layer (upper 30 cm). The precipitation of Fe mainly occurred in the surface layer of the device (0–5 cm), while the main precipitation area of Al was the shallow layer (0–30 cm), the main reaction zone was the first 20–30 cm of the column, and the reaction stabilization time was 10–12 h. These data can provide support for the design of height and operation time of the field test equipment, enrich the experimental cases, and provide reference for AMD treatment in similar polluted areas.

AUTHOR CONTRIBUTIONS
Wenbo Li performed the experiment, data analyses and wrote the manuscript; Qiyan Feng contributed significantly to analysis and manuscript preparation; Haoqian Liang performed the experiment and data analyses; Di Chen helped perform the analysis with constructive discussions; Xiangdong Li contributed to the conception of the study.

CONFLICTS OF INTEREST
There are no conflicts to declare.

ACKNOWLEDGEMENTS
This research is supported by the National Natural Science Foundation of China: Mechanism and kinetics of microbial acid inhibition in acid mine drainage of abandoned coal mine (No. 41977159).

DATA AVAILABILITY STATEMENT
All relevant data are included in the paper or its Supplementary Information.

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First received 8 March 2021; accepted in revised form 7 September 2021. Available online 20 September 2021