

# High Efficacy Two-Stage Metal Treatment Incorporating Basic Oxygen Furnace Slag and Microbiological Sulfate Reduction

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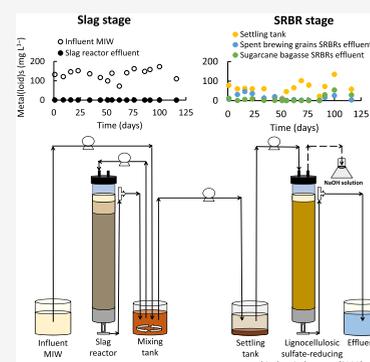
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**ABSTRACT:** Lignocellulosic sulfate-reducing biochemical reactors (SRBRs) can be implemented as passive treatment for mining-influenced water (MIW) mitigating the potentially deleterious effects of MIW acidic pH, and high concentrations of metal(loid)s and  $\text{SO}_4^{2-}$ . In this study, a novel two-stage treatment for MIW was designed, where basic oxygen furnace slag (slag stage) and microbial  $\text{SO}_4^{2-}$  reduction (SRBR stage) were incorporated in series. The SRBRs contained spent brewing grains or sugarcane bagasse as sources of lignocellulose. The slag reactor removed >99% of the metal(loid) concentration present in the MIW ( $130 \pm 40 \text{ mg L}^{-1}$ ) and increased MIW pH from  $2.6 \pm 0.2$  to  $12 \pm 0.3$ . The alkaline effluent pH of the slag reactor was mitigated by remixing slag effluent with acidic MIW before SRBR treatment. The SRBR stage removed the bulk of  $\text{SO}_4^{2-}$  from MIW, additional metal(loid)s, and yielded a circumneutral effluent pH. Cadmium, copper, and zinc showed high removal rates in SRBRs ( $\geq 96\%$ ) and likely precipitated as sulfide minerals. The microbial communities developed in SRBRs were enriched in hydrolytic, fermentative, and sulfate-reducing taxa. However, the SRBRs developed distinct community compositions due to the different lignocellulose sources employed. Overall, this study underscores the potential of a two-stage treatment employing steel slag and SRBRs for full-scale implementation at mining sites.

**KEYWORDS:** acid mine drainage, sulfate-reducing bioreactor, lignocellulose, heavy-metal remediation, metal-bearing wastewater



## 1. INTRODUCTION

A pertinent environmental challenge at mining sites worldwide is the formation of mining-influenced water (MIW), such as rock/mine drainage. The excavation of pyrite ( $\text{Fe}_2\text{S}$ )-bearing rocks and their exposure to water and oxic conditions leads to abiotic and microbial oxidation of  $\text{Fe}_2\text{S}$ . MIW streams are often acidic (pH 2–3) and commonly contain elevated concentrations of heavy metals and metalloids, such as iron (Fe), aluminum (Al), copper (Cu), zinc (Zn), lead (Pb), arsenic (As), among others.<sup>1,2</sup> MIW may pose a threat to human health, wildlife ecology, and water resources,<sup>3,4</sup> requiring treatment to limit metal(loid) mobility and exposure.

Lignocellulosic sulfate-reducing biochemical reactors (SRBRs) are a type of passive treatment for MIW most often used at remote and/or abandoned mining sites.<sup>5,6</sup> The development of lignocellulosic SRBRs began in the late 1970s based on observations and microbiological principles from anaerobic wetlands containing plant material.<sup>7,8</sup> The first field-scale SRBRs were implemented in the early 1990s at an underground mining site and a smelting residue dumping site in Pennsylvania, USA.<sup>9</sup> These early SRBRs used spent mushroom compost as the lignocellulosic packing material, demonstrating effective removal of Al, cadmium (Cd), Fe,

manganese (Mn), nickel (Ni), and Zn from acidic and circumneutral MIW streams.<sup>9</sup>

The appeal of lignocellulosic SRBRs for MIW treatment stems from their relatively low cost, minimal maintenance, and installation suitability—especially when needed at the most remote mining locations.<sup>10,11</sup> SRBRs can be constructed as packed-bed bioreactors in columns, tanks, or lined dugouts, depending on the scale. Many SRBR designs continue to employ lignocellulose. Lignocellulose is the major component in plant cell walls and is mainly composed of cellulose (glucose polymers), hemicellulose (xylose and other sugar polymers), and lignin (monolignol polymers).<sup>12,13</sup> Examples of materials used in SRBRs include alfalfa hay, straw, or other grasses, which are high in cellulose/hemicellulose content, and sawdust, wood chips, or nut shells, which are high in lignin content. The selection of the packing materials reflects a

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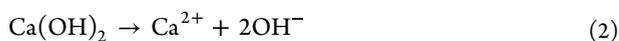
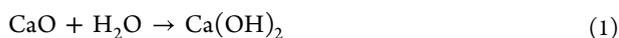


balance between the lignocellulose biodegradation potential. For example, hemicellulose and cellulose are more readily biodegradable than lignin under anoxic conditions.<sup>14</sup> Selection is also dependent on the porosity of the material (for effective MIW flow and clogging prevention) and the ability of the lignocellulose material to sustain treatment long term.<sup>11,15,16</sup>

Treatment of MIW via lignocellulosic SRBRs relies on an anaerobic syntrophic microbial community with specific functions. Hydrolytic and fermenting bacteria break down lignocellulose polymers to monomers and simple products of fermentation, such as short-chain organic acids, alcohols, and H<sub>2</sub>.<sup>17,18</sup> These fermentation products are then utilized by sulfate-reducing bacteria as electron donors to reduce SO<sub>4</sub><sup>2-</sup> (terminal electron acceptor) to sulfides (H<sub>2</sub>S, HS<sup>-</sup>, S<sup>2-</sup>).<sup>19–21</sup> SO<sub>4</sub><sup>2-</sup> reduction increases pH via bicarbonate production and removes dissolved metal(loid)s from MIW primarily as relatively insoluble sulfide precipitates.<sup>22,23</sup>

Sulfate-reducing bacteria pertinent to MIW treatment require a pH above 4 for growth.<sup>16,24,25</sup> One strategy to provide a source of alkalinity and manage the acidic pH of MIW is to pack SRBRs with a mix of lignocellulosic material and limestone (CaCO<sub>3</sub>). Limestone can account for 10–80% of the total weight of the packed material in an SRBR.<sup>9,26–30</sup> Limestone can also be used as a chemical pretreatment step for MIW (e.g., limestone reactor) before MIW is bioremediated in an SRBR.<sup>29,31–33</sup> However, limestone's ability to increase MIW pH declines over time due to passivation/armoring.<sup>34</sup> Given that pH controls both microbial activity and metal(loid) precipitation in an SRBR, there is a need for novel or effective alkalizing materials to support SRBR longevity and treatment efficacy.

Basic oxygen furnace slag, a type of steel slag, is one such alkalizing material with the potential for MIW treatment. Basic oxygen furnace slag is created when molten iron ores are blasted with oxygen gas (O<sub>2</sub>) and then combined with lime or dolomite to remove impurities.<sup>35</sup> The generated liquid steel sinks to the bottom of the furnace, while the lime or dolomite combined with impurities floats to the top.<sup>35</sup> Steel slags are porous and composed of crystal lattices, which include metal oxides of calcium (Ca), magnesium (Mg), Fe, and silicon (Si).<sup>36</sup> Coupling slag with lignocellulosic SRBRs can offer major advantages for MIW treatment. First, similar to SO<sub>4</sub><sup>2-</sup> reduction, slag can provide alkalinity to increase MIW pH and precipitate metal(loid)s. The alkalinity of steel slag leachate is 500–2000 mg L<sup>-1</sup> as CaCO<sub>3</sub> equivalents, whereas limestone leachate has an alkalinity of 60–80 mg L<sup>-1</sup>.<sup>37</sup> Slag has been reported to increase the pH of MIW from 2 to 12.<sup>36</sup> Second, slag can remove metal(loid)s from MIW and, by doing so, can decrease the overall metal(loid) loading in an SRBR, potentially extending its operation lifetime. Previous studies have shown that slag is effective at precipitating and adsorbing Al, beryllium, Cd, Cu, Fe, Ni, Pb, thallium, and Zn.<sup>38</sup> When metal oxides in slag are hydrated (eq 1), hydroxide ions (OH<sup>-</sup>) are produced (eq 2). Released OH<sup>-</sup> increases the pH of MIW and metal(loid)s are precipitated as shown in eq 3, where M is a metal(loid) and x is the metal(loid) charge (e.g., 2+ or 3+) and the stoichiometric coefficient of OH<sup>-</sup>.



The effectiveness of metal(loid) removal from MIW by slag is highest as the pH increases (e.g., pH 12).<sup>36,39–41</sup> Thus, treatment designs for MIW combining steel slag and SRBRs must balance the abiotic and microbiological reactions to achieve an effluent MIW with low metal(loid) concentrations and a circumneutral pH appropriate for discharge.

While steel slag has great potential in MIW treatment, research efforts to combine slag with microbial SO<sub>4</sub><sup>2-</sup> reduction in SRBRs are extremely limited and have only recently emerged.<sup>42</sup> In this study, we designed a novel two-stage treatment for MIW where basic oxygen furnace slag (slag stage) and microbial SO<sub>4</sub><sup>2-</sup> reduction (SRBR stage) were incorporated in series to achieve a synergistic MIW treatment. We used the two-stage design to assess MIW treatment effectiveness by quantitatively defining the specific contributions of slag and SRBRs to metal(loid) removal from MIW. We also analyzed the microbial community composition of SRBRs receiving MIW pretreated by basic oxygen furnace slag and determined the relative changes in lignocellulose composition. Operation of the two-stage treatment with MIW showed that the slag stage removed the bulk of the metal(loid) concentration. However, the very alkaline pH (~12) had to be managed by remixing a fraction of the slag effluent with acidic MIW prior to MIW entering further treatment by SRBRs. At the SRBR stage, additional metal(loid) removal occurred as a polishing step *via* microbial SO<sub>4</sub><sup>2-</sup> reduction and yielded a circumneutral effluent pH. This novel two-stage treatment underscores the potential of slag and lignocellulosic SRBRs for implementation at the field scale.

## 2. MATERIALS AND METHODS

**2.1. Materials and Preparation of Synthetic MIW.** The basic oxygen furnace slag used in this study was provided by Phoenix Services LLC, Indiana-Burns Harbor, Indiana. The slag was sieved, retaining particles in the 9–20 mm diameter range. Spent brewing grains and sugarcane bagasse were used as the lignocellulosic substrates in this study because of their documented potential in stimulating SO<sub>4</sub><sup>2-</sup> reduction in SRBRs.<sup>15,30,42</sup> Spent brewing grains (after the mashing process) were obtained from SanTan Brewery in Chandler, Arizona, USA, and were transported to Arizona State University on the same day. Sugarcane bagasse was provided by Cajun Sugar Company in New Iberia, Louisiana. The lignocellulosic materials were stored at 4 °C in airtight plastic containers until they were used to pack the SRBRs. Pertinent properties of the spent brewing grains and sugarcane bagasse can be found in Table 1.

Synthetic MIW (referred henceforth as MIW) was prepared as described in Miranda et al.<sup>30</sup> The composition of the MIW from this study, including the type and concentration of metal(loid)s and concentration of SO<sub>4</sub><sup>2-</sup>, was based on the characteristics of an actual MIW from a legacy hard rock mine (see Table S1 in the Supporting Information). The MIW had the following composition per liter: 50 mg Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>·H<sub>2</sub>O, 0.18 mg NaAsO<sub>2</sub>, 0.01 mg BaSO<sub>4</sub>, 0.30 mg CdSO<sub>4</sub>, 330 mg CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.01 mg K<sub>2</sub>CrO<sub>4</sub>, 0.07 mg CoCl<sub>2</sub>·6H<sub>2</sub>O, 1.8 mg CuCl<sub>2</sub>·5H<sub>2</sub>O, 290 mg Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>·H<sub>2</sub>O, 100 mg Fe(SO<sub>4</sub>)·H<sub>2</sub>O, 0.15 mg PbCl<sub>2</sub>, 160 mg MgCl<sub>2</sub>·6H<sub>2</sub>O, 300 mg MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.04 mg NiSO<sub>4</sub>, 1.1 mg K<sub>2</sub>HPO<sub>4</sub>, 0.7 mg KCl, 0.2 μg AgNO<sub>3</sub>, 0.06 mg VCl<sub>3</sub>, 380 mg NH<sub>4</sub>Cl, 175 mg ZnCl<sub>2</sub>, 63 mg Na<sub>2</sub>SO<sub>4</sub>, and 0.16 mL H<sub>2</sub>SO<sub>4</sub>. The final pH was 2.5, and the total SO<sub>4</sub><sup>2-</sup> concentration was 6.5 mM (650 mg L<sup>-1</sup>).

**Table 1. Characteristics of the Lignocellulosic Materials Used to Pack the SRBRs in This Study**

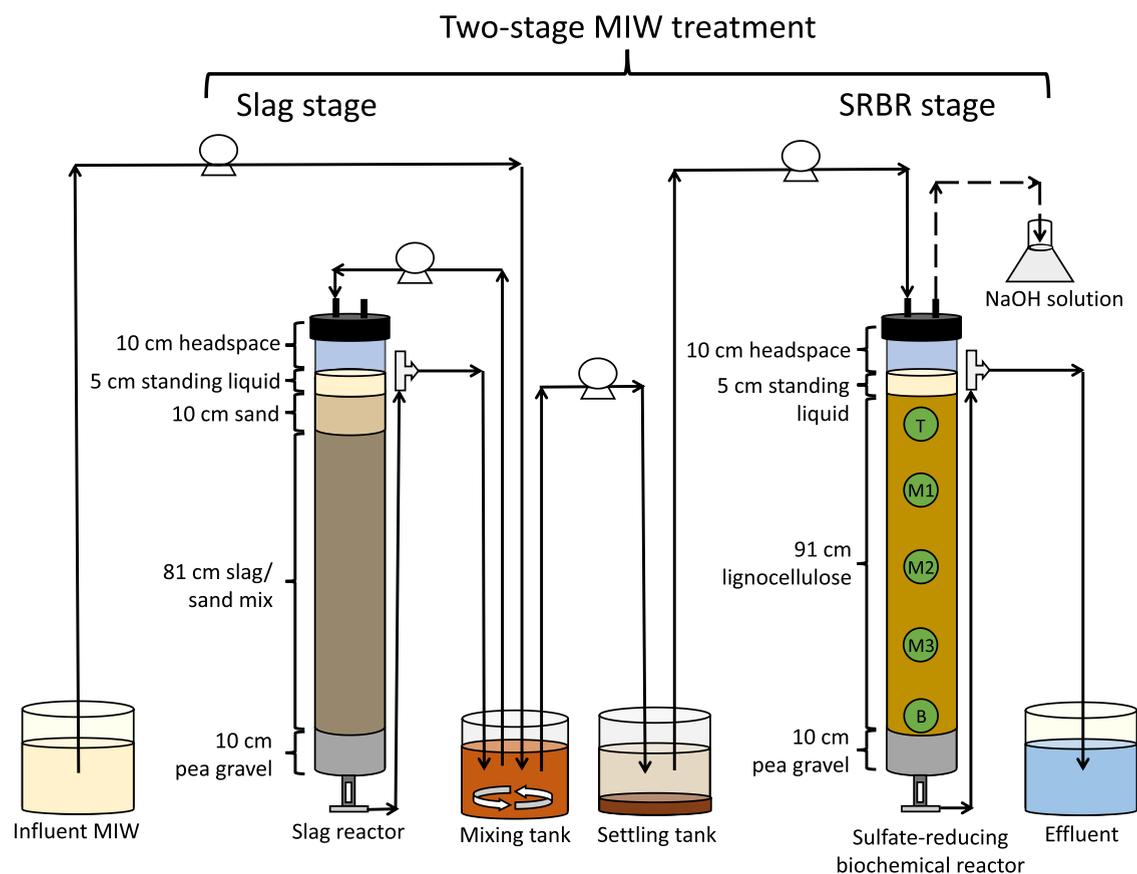
parameter	spent brewing grains	sugarcane bagasse
moisture (%)	75.0 ± 0.7	60 ± 2
density (kg L <sup>-1</sup> )	1.1	0.68
porosity	0.40	0.79
particle size length (mm)	1–4 <sup>a</sup>	1–20 <sup>b</sup>
total organic carbon (TOC) (mg kg <sup>-1</sup> )	(4.8 ± 0.2) × 10 <sup>5</sup>	(4.47 ± 0.05) × 10 <sup>5</sup>
pH (s.u.) <sup>c</sup>	4.09 ± 0.01	6.44 ± 0.03
total dissolved solids (ppm) <sup>c</sup>	260 ± 3	160 ± 4
conductivity (μS cm <sup>-1</sup> ) <sup>c</sup>	580 ± 5	350 ± 5
SO <sub>4</sub> <sup>2-</sup> (mg kg <sup>-1</sup> )	36 ± 3	19 ± 2

<sup>a</sup>Spent brewing grains particle size length was obtained from Alonso-Riaño et al.<sup>43</sup> <sup>b</sup>Sugarcane bagasse particle size length was obtained from Zadrazil et al.<sup>44</sup> and Aghcheh et al.<sup>45</sup> <sup>c</sup>pH, total dissolved solids, and conductivity measurements were obtained from 20 g spent brewing grains + 25 mL DI water or 10 g sugarcane bagasse + 45 mL DI water. The data are averages with standard deviations of triplicate samples.

**2.2. Design and Operation of a Two-Step Slag and SRBR Treatment for MIW.** The schematic of the two-stage treatment from this study is shown in Figure 1. A photograph of the system is shown in Figure S1. The components of the slag stage were a slag reactor, a settling tank, and a mixing tank.

The SRBR stage consisted of two sets of SRBRs packed with either spent brewing grains or sugarcane bagasse. The slag reactor and SRBRs were constructed from clear, schedule 40 PVC pipes ( $L = 116$  cm,  $ID = 10$  cm,  $V = 9.1$  L) and were mounted using strut channel clamps to a shelf. SRBRs had outflow PVC clear flexible tubing connected to a three-way tee placed 10 cm below the top and 5 cm above the packed material to control the water level. A remaining port guided the effluent of each reactor to its next destination through PVC flexible tubing, while another port remained open to the atmosphere to maintain 5 cm of standing liquid in the reactors. Liquid from the settling tank (end of the slag stage) was then pumped into the SRBRs.

**2.2.1. Slag Stage.** The slag reactor contained a 10 cm thick pea gravel drainage layer at the bottom (Vigro, Illinois), 81 cm of a mixture of 70% Ottawa 20–30 silica sand (U.S. Silica, Ottawa, Illinois), and 30% slag (basic oxygen furnace slag), and a top layer of 10 cm of Ottawa 20–30 silica sand. Once packed, the slag reactor was filled with MIW from the bottom to top to achieve material saturation. Peristaltic pumps (Masterflex C/L Dual Channel, Cole-Parmer, Vernon Hills, Illinois) were used to flow synthetic MIW into a 5 L mixing tank at 2.9 L day<sup>-1</sup>. The mixing tank was stirred at 250 rpm on a stir plate (Thermo Fisher Scientific, Waltham, Massachusetts). The liquid from the mixing tank flowed at the same flow rate (2.9 L day<sup>-1</sup>) into a settling tank where metal(loid) precipitates settled. The slag reactor was in a recycle loop



**Figure 1.** Configuration of the two-stage treatment in this study employing a basic oxygen furnace slag reactor and sulfate-reducing biochemical reactors (SRBRs). The solid arrows show liquid flow, while the dashed arrows indicate the flow of excess gas to a concentrated solution of NaOH. The green circles show approximate locations where the SRBR contents were sampled at the end of operation: T = top of SRBR (closest to influent); M1, M2, and M3 = locations in the middle of the SRBR; B = bottom of SRBR (closest to effluent).

(~20% recycle capacity per day) with the mixing tank, where liquid from the mixing tank flowed to the top of the slag reactor and back into the mixing tank at a recycle flow rate of 0.6 L day<sup>-1</sup>. The continuous mixing of MIW and slag effluent served to maintain the pH at ~5.

**2.2.2. Sulfate-Reducing Biochemical Reactor (SRBR) Stage.** Duplicate SRBRs were packed with pea gravel (Vigoro, 10 cm bottom layer) and either spent brewing grains or sugarcane bagasse (91 cm layer) (Figure 1). The lignocellulosic substrate was added to the SRBRs with their respective “as arrived” moisture content (Table 1). Because the lignocellulosic substrate had different densities (Table 1), the total material added to the spent brewing grains and sugarcane bagasse SRBRs varied. Specifically, each SRBR was packed with either 3.5 kg of spent brewing grains (2.6 kg dry weight) or 1.45 kg of sugarcane bagasse (0.87 kg dry weight). The SRBRs were filled from bottom to top with MIW from the settling tank until there was a 5 cm standing liquid above the lignocellulosic substrate (Figure 1). The SRBRs were inoculated with 125 mL of anaerobic digester sludge from the Mesa Northwest Water Reclamation Plant, Mesa, Arizona, and 25 mL of a culture enriched on either spent brewing grains or sugarcane bagasse and SO<sub>4</sub><sup>2-</sup>. The enrichment process and the composition of the microbial community of these cultures were reported in a previous study.<sup>30</sup>

MIW from the settling tank entered the SRBRs at the top. The SRBRs were operated directly in continuous mode (no batch operation) as previously done<sup>30</sup> to acclimate the microbial community to MIW. The flow rate for the spent brewing grains SRBRs during the acclimation period (day 0 to 38) was 0.6 L day<sup>-1</sup> (corresponding to an HRT of 8 days). On day 38, the operational phase commenced at a flow rate of 1.1 L day<sup>-1</sup> (4.6-day HRT) and 13.5 HRTs were completed until the end of the experiment (day 121). The flow rate for the sugarcane bagasse SRBRs during acclimation was 1.1 L day<sup>-1</sup> (7.3-day HRT) for the first 35 days. Then for the operational phase, the flow rate was increased to 1.8 L day<sup>-1</sup> (3.3-day HRT) for a total of 19.7 HRTs until the end of the experiment (day 121). The slag and SRBRs stages were frequently sampled during operation and analyzed for a variety of parameters.

**2.3. Chemical and Microbiological Analyses.** pH, oxidation–reduction potential (ORP), and dissolved oxygen (DO) were measured using an Orion Versa Star Pro benchtop multipurpose meter fitted with a ROSS Ultra pH/ATC Triode Refillable Electrode (Thermo Fischer Scientific, Chelmsford, Massachusetts). Conductivity and total dissolved solids were measured using an Oakton Multi-Parameter PCSTestr 35 probe (Eutech Instruments Pte LTD, Vernon Hills, Illinois). All probes were calibrated according to the manufacturers’ instructions. Total organic carbon in spent brewing grains and sugarcane bagasse was measured by a Lotix Total Organic Carbon analyzer with a Lotix Solids Sampler Boat Module (Teledyne Tekmar, Mason, Ohio) using a method previously reported in Miranda et al.<sup>30</sup>

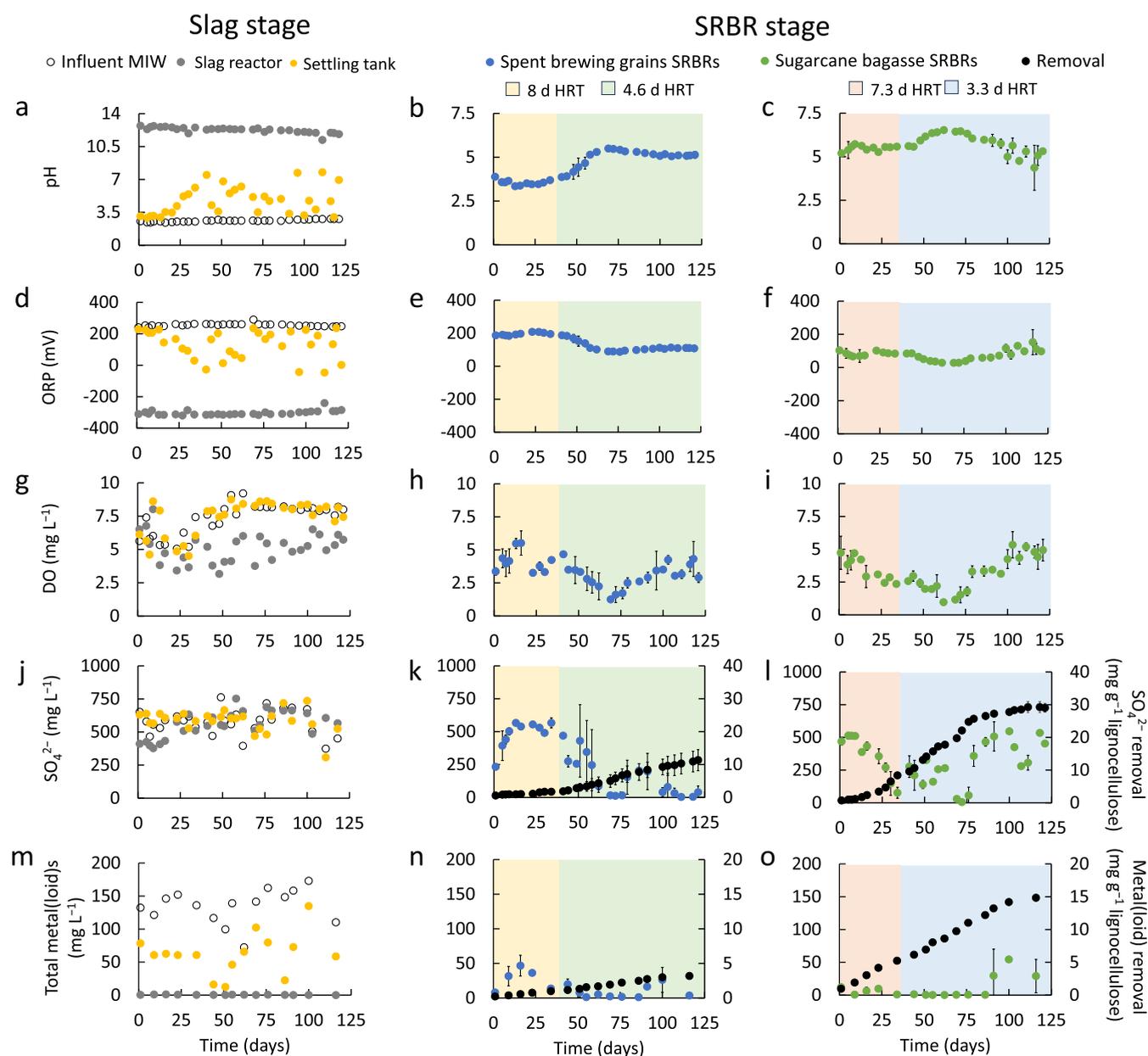
The concentration of SO<sub>4</sub><sup>2-</sup> was analyzed using an ion chromatograph (Metrohm 930 Compact Flex, Riverview, Florida) as described in detail in the Supporting Information (Section S1). SO<sub>4</sub><sup>2-</sup> concentrations in the lignocellulosic substrates were obtained from triplicate samples containing 1 g of material and 10–20 mL of DI water (≥18 mΩ). The concentration of total dissolved sulfide (S<sup>2-</sup>) was measured using a HACH color test kit (Loveland, Colorado) according

to the manufacturer’s protocol. The detection range of the kit was 0.4–10 mg L<sup>-1</sup> S<sup>2-</sup>.

The concentrations of silver (Ag), Al, As, barium (Ba), Ca, Cd, chromium (Cr), cobalt (Co), Cu, Fe, Mg, Mn, Ni, Pb, vanadium (V), and Zn were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) by the Metals and Environmental and Terrestrial Analytical Laboratory, Chemical and Environmental Characterization Facility, Arizona State University. Concentrations of fermentation products (formic acid, acetic acid, ethanol, propionic acid, lactic acid, butyric acid, valeric acid, and caproic acid) were measured with a Shimadzu high-performance liquid chromatograph (HPLC, LC-20AT, Columbia, Maryland) from 1 mL of liquid samples filtered through a 0.2 μm PVDF membrane filter. The methodology for this analysis was described in detail previously.<sup>30,46,47</sup> The composition of cellulose, hemicellulose, and lignin in the spent brewing grains and sugarcane bagasse was determined using an Ankom Technology A2000 fiber analyzer (Macedon, New York). Hemicellulose, cellulose, and lignin composition of the spent brewing grains and sugarcane bagasse before use in SRBRs (on day 0) and at the end of SRBR operation (on day 121) at locations T, M1, M2, M3, and B locations (Figure 1) was determined using the recommended protocols by Ankom Technology.<sup>30</sup> Additional method details can be found in the Supporting Information (Section S2 and Tables S2–S3).

Genomic DNA for 16S rRNA amplicon sequencing was extracted using a DNeasy PowerFood Microbial Kit (QIAGEN, Germantown, Maryland). DNA was extracted from 1.8 mL of a liquid sample taken from anaerobic digester sludge (ADS) and from each lignocellulose material enrichment culture. DNA was also extracted from 0.2 g of sample taken from the initial spent brewing grains, the initial sugarcane bagasse, and duplicate SRBRs post operation from locations T (top of bioreactor, closest to influent), M2 (middle of bioreactor), and B (bottom of bioreactor, closest to effluent) (Figure 1). DNA yield and purity were determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts).

Amplicon sequencing was performed at the Center for Fundamental and Applied Microbiomics at the ASU KED Genomics Core Facility, Arizona State University, Tempe, Arizona, using a MiSeq instrument (Illumina, Inc., San Diego, California). The V4 hyper-variable region of the 16S rRNA gene for Bacteria and Archaea was targeted using the original Earth Microbiome Project primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806 R (5'-GGACTACHVGGGTWTCTAAT-3').<sup>48,49</sup> CASAVA 1.8 paired-end demultiplexed sequences were imported into Quantitative Insights into Microbial Ecology (QIIME 2, v. 2020.8.0).<sup>50</sup> The sequences were truncated to 250 base pairs for quality control and then denoised using the DADA2 pipeline.<sup>51</sup> Taxonomy was assigned to the amplicon sequence variants (ASVs) by referencing the SILVA database (v.138)<sup>52–55</sup> as described in previous studies.<sup>30,56–58</sup> Bray–Curtis diversity analyses, Faith’s phylogenetic diversity indices, and Pielou’s evenness indices were generated via the q2-diversity plugin core-metrics-phylogenetic method. For these analyses, the sampling depth was 3872, which is the lowest sequence number in the samples from this study. The raw sequences were deposited in the National Center for Biotechnology (NCBI) Sequence Read Archive (SRA) under project number PRJNA699925 with accession numbers SAMN17816982–SAMN17816998.



**Figure 2.** (a–c) pH, (d–f) oxidation–reduction potential (ORP), (g–i) dissolved oxygen (DO) concentrations, (j–l)  $\text{SO}_4^{2-}$  concentrations and removal, and (m–o) total metal(loid) concentrations and removal in the slag stage and the SRBR stage of treatment. Empty symbols show influent MIW concentrations while filled symbols show effluent concentrations. The SRBR data are averages with standard deviation of duplicates. BOF slag reactor: days 0–121: HRT = 5 days, flow rate = 2.9 L day<sup>-1</sup>. Spent brewing grains SRBRs: days 0–35: HRT = 8 days, flow rate = 0.6 L day<sup>-1</sup>; days 35–121: HRT = 4.6 days, flow rate = 1.1 L day<sup>-1</sup>. Sugarcane bagasse SRBRs: days 0–38: HRT = 7.3 days, flow rate = 1.1 L day<sup>-1</sup>; days 38–121: HRT = 3.3 days, flow rate = 1.8 L day<sup>-1</sup>.

### 3. RESULTS AND DISCUSSION

**3.1. Slag Stage Provided Appropriate Conditions for Downstream  $\text{SO}_4^{2-}$  Reduction and Served as the Major Mechanism for Metal(loid) Removal from MIW.** In the two-stage treatment process evaluated in this study, a slag reactor, a mixing tank, and a settling tank comprised the slag stage (Figure 1). The mixing tank received fresh MIW, was in recirculation with the slag reactor, and fed a settling tank. The SRBR stage received pretreated MIW from the slag stage and was composed of two sets of lignocellulosic SRBRs (in parallel) packed with either spent brewing grains or sugarcane bagasse. Figure 2 compiles the measured parameters during 121 days of operation at the slag and SRBR stages of

treatment. The most notable effects on MIW by the slag reactor were increases in pH, decreases in ORP and DO, and extensive removal of metal(loid)s. The pH of the influent MIW averaged  $2.6 \pm 0.1$ , and the average pH in the slag reactor effluent was  $12.3 \pm 0.3$  (Figure 2a). A pH ranging from 10 to 13 has been previously reported in MIW treated abiotically by slag.<sup>59–61</sup> pH is a driver of microbial community development and activity, but the activity of sulfate-reducing bacteria is limited below pH 4 and above pH 9.<sup>16,24,25,62</sup> An average pH of  $5.0 \pm 1.0$  (Figure 2a) was achieved by mixing the slag reactor effluent with influent MIW in the mixing tank before MIW entered the SRBRs. Thus, the influent pH in the

Table 2. Metal(loid) Removal by the Two-stage MIW Treatment<sup>a</sup>

metal(loid)	MIW influent (mg L <sup>-1</sup> )	slag reactor removal (%)	SRBR influent (settling tank) (mg L <sup>-1</sup> )	spent brewing grains		sugarcane bagasse	
				SRBR stage removal (%)	two-stage treatment removal (%)	SRBR stage removal (%)	two-stage treatment removal (%)
Al	2.8 ± 0.9	>99	1 ± 2	95 ± 3	98 ± 2	70 ± 42	89 ± 29
As	0.05 ± 0.04	>99	0.0	N/A	96 ± 11	N/A	≥99
Cd	0.08 ± 0.04	>99	0.08 ± 0.03	>99	99 ± 3	99.6 ± 0.9	98 ± 23
Co	0.008 ± 0.008	>99	0.006 ± 0.006	70 ± 50	80 ± 42	88 ± 35	88 ± 40
Cr	0.03 ± 0.02	>99	0.01 ± 0.01	67 ± 38	90 ± 15	65 ± 39	89 ± 24
Cu	0.6 ± 0.2	>99	0.5 ± 0.4	97 ± 8	99 ± 3	98 ± 6	99 ± 24
Fe	60 ± 20	>99	10 ± 20	20 ± 36	88 ± 14	43 ± 48	77 ± 33
Ni	0.02 ± 0.02	>99	0.01 ± 0.01	96 ± 11	88 ± 32	89 ± 40	92 ± 34
Pb	0.01 ± 0.01	>99	0.003 ± 0.005	23 ± 44	58 ± 45	37 ± 49	74 ± 32
V	0.06 ± 0.04	>99	0.02 ± 0.02	9 ± 19	41 ± 46	12 ± 20	55 ± 41
Zn	70 ± 20	>99	60 ± 20	>99	>99	99.4 ± 0.9	99 ± 24
Total	130 ± 40	>99	80 ± 40	91 ± 9	95 ± 6	86 ± 30	89 ± 16

<sup>a</sup>The data tabulated show the average (±SD) of metal(loid) concentrations in MIW influent and settling tank (influent for SRBRs). The data tabulated also show the average % removal (±SD) of metal(loid)s by the slag reactor, the SRBR stage, and two-stage treatment system. The analyses in this table are for days 62–121 (at least 5 HRTs after starting the operation phase) when the spent brewing grains SRBRs were operated at an HRT of 4.6 days and the sugarcane bagasse SRBRs were operated at an HRT of 3.3 days. For removal calculation details, see Supporting Information, Section S3. N/A = not applicable.

SRBRs was expected to promote the growth of many strains of sulfate-reducing bacteria.

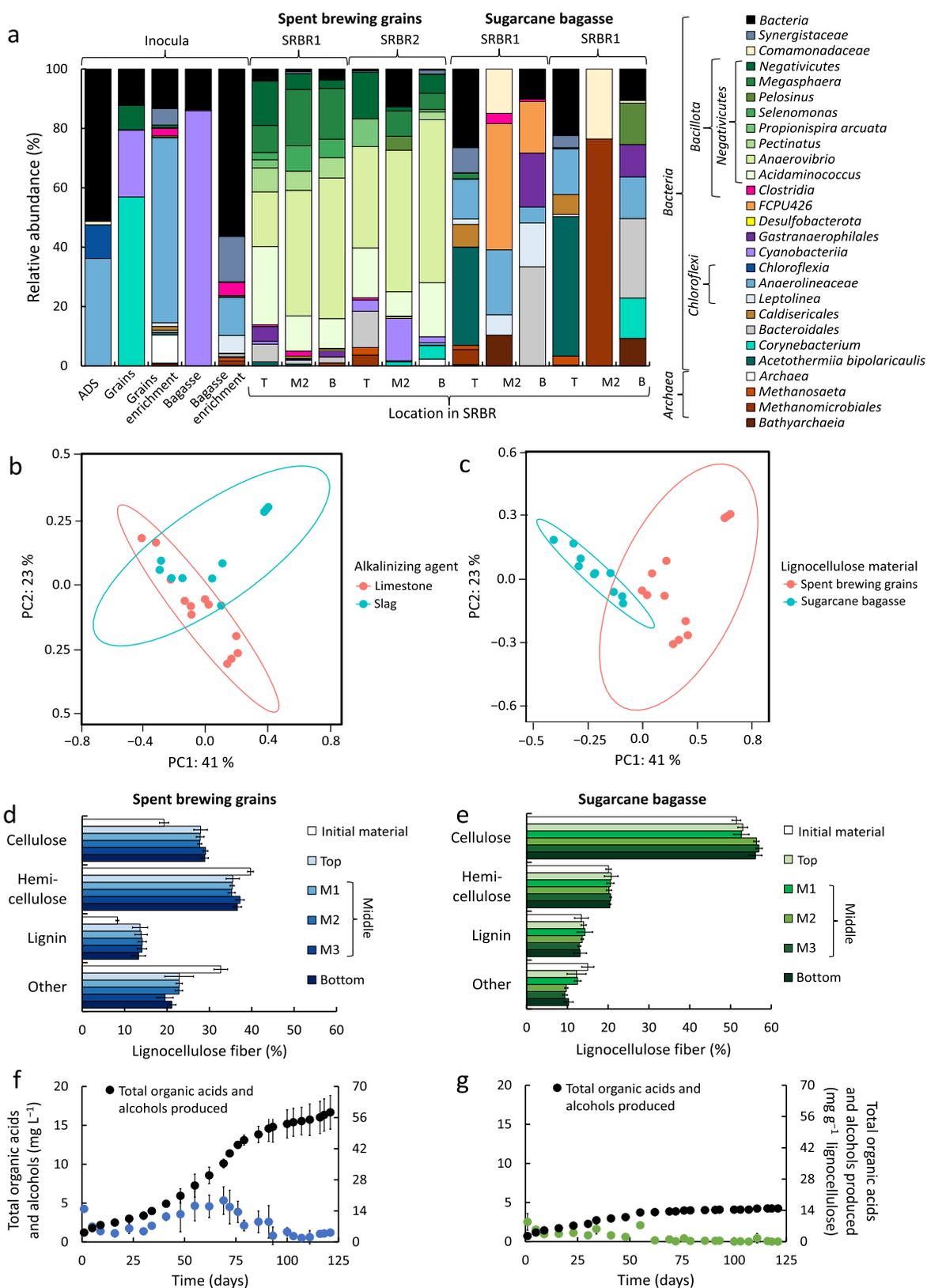
Anoxic conditions and ORP ≤100 mV are characteristics of an environment suitable for SO<sub>4</sub><sup>2-</sup> reduction.<sup>63</sup> The ORP of the influent MIW was ~250 mV and decreased to an average of -300 ± 10 mV in the slag effluent (Figure 2d). The drastic decrease in ORP was due to the elevated concentration of Ca<sup>2+</sup> ions in the slag effluent, a type of reducing agent.<sup>64</sup> Ca<sup>2+</sup> and OH<sup>-</sup> were released from slag after hydrolysis of CaO.<sup>36</sup> The Ca<sup>2+</sup> concentration in the slag effluent was on average 3.3 times higher (330–1200 mg L<sup>-1</sup>) than in the influent MIW (70–160 mg L<sup>-1</sup>) (Table S4). Similarly, the DO decreased from 8.0 ± 0.6 mg L<sup>-1</sup> in the influent MIW to 5 ± 1 mg L<sup>-1</sup> in the slag effluent (Figure 2g). Overall, the slag reactor configuration from this study increased the MIW pH and decreased the ORP and DO, showing the potential of the slag stage to promote the required conditions for SO<sub>4</sub><sup>2-</sup> reduction in SRBRs.

The slag reactor also provided extensive metal(loid) removal from MIW. The total metal(loid) concentration in the slag reactor effluent was <0.02 mg L<sup>-1</sup> throughout operation (Figure 2m), with a total metal(loid) removal of >99.9% (Table 2). Slag has been reported to remove 99% of Fe<sup>59,65</sup> and 98% of Al<sup>59</sup> concentrations from MIW streams containing 290–1000 mg L<sup>-1</sup> Fe and 130 mg L<sup>-1</sup> Al, respectively. In the slag stage, Al and Fe were likely precipitated as hematite (Fe<sub>2</sub>O<sub>3</sub>), goethite (FeO(OH)), gibbsite (Al(OH)<sub>3</sub>), amorphous Al(OH)<sub>3</sub>, and basaluminite (Al<sub>4</sub>(OH)<sub>10</sub>SO<sub>4</sub>) based on previous studies using slag to treat MIW.<sup>59,65</sup> Cr, Co, Cd, Cu, Pb, Ni, and Zn were likely coprecipitated or adsorbed by these Fe and Al minerals.<sup>59,66</sup> The extensive metal(loid) removal of the slag reactor in this study should prove advantageous to reduce the metal(loid) loading in the SRBRs and thus increase the overall SRBR longevity.

**3.2. Microbial SO<sub>4</sub><sup>2-</sup> Reduction at the SRBR Stage Contributed to Additional Metal(loid) Removal from MIW to Achieve Low Effluent Concentrations.** The second stage of our MIW treatment process consisted of receiving MIW from the settling tank (end of the slag stage). Operation of the SRBRs commenced directly in continuous

mode and the SRBRs were considered acclimated when S<sup>2-</sup> was detected in the effluent as previously documented.<sup>30</sup> Acclimation due to excess S<sup>2-</sup> detection was achieved by day 23 in the sugarcane bagasse SRBRs and by day 50 in the spent brewing grains SRBRs (Figure S2a,b). As seen in Figure 2j–l, SO<sub>4</sub><sup>2-</sup> removal mainly occurred in the SRBRs and not in the slag stage of the treatment. It was not the goal of this study to achieve specific water quality criteria. However, to benchmark the SO<sub>4</sub><sup>2-</sup> effluent concentrations in the SRBRs, we used the United States Environmental Protection Agency (US EPA) National Secondary Drinking Water Regulations nonmandatory SO<sub>4</sub><sup>2-</sup> standard of 250 mg L<sup>-1</sup>.<sup>67</sup> SRBRs are often employed as part of a full-scale MIW treatment system; therefore, a comparison to specific water quality criteria can be scientifically useful but not necessarily applicable. Nonetheless, in this study, the SRBRs with spent brewing grains and sugarcane bagasse receiving MIW from the slag stage had average effluent SO<sub>4</sub><sup>2-</sup> concentrations of 150 and 300 mg L<sup>-1</sup>, respectively, during the operation phase (Figure 2k,l). When normalized to the initial mass of lignocellulose, the sugarcane bagasse removed ~3 times more SO<sub>4</sub><sup>2-</sup> per dry material mass than the spent brewing grains throughout operation (Figure 2k,l, day 121). These data demonstrate that a two-stage system incorporating slag and SRBRs has the potential to achieve SO<sub>4</sub><sup>2-</sup> effluent concentration benchmarks, if applicable, at full-scale systems.

Although the slag reactor removed the bulk concentration of metal(loid)s from MIW, the SRBRs provided additional metal(loid) removal from the mixture of the slag reactor effluent and fresh MIW. Theoretically, 42 mg L<sup>-1</sup> S<sup>2-</sup> was required to remove the metal(loid) concentration entering the SRBRs from the settling tank (Table S5). The SRBRs from this study fulfilled and exceeded this requirement as there was an average effluent S<sup>2-</sup> concentration (free/unbound S<sup>2-</sup>) of 8 ± 13 mg L<sup>-1</sup> in the spent brewing grains SRBRs and 10 ± 21 mg L<sup>-1</sup> in the sugarcane bagasse SRBRs (Figure S2a,b). Operation for 3–5 HRTs is typically considered enough time for a bioreactor to achieve steady state.<sup>68</sup> In our study, we used day 62 to delineate when a steady state was achieved in the SRBRs (Table 2). During this steady state, the average pH in the spent



**Figure 3.** (a) Microbial community composition at various taxonomic levels in the inocula and SRBRs at the end of operation. (b, c) Weighted Unifac diversity analysis using samples from SRBRs packed with spent brewing grains or sugarcane bagasse receiving MIW pretreated by slag (this study) and samples from SRBRs packed with spent brewing grains or sugarcane bagasse and 30% limestone<sup>30</sup>. (d, e) Lignocellulose composition before (day 0) and at the end of operation (day 121) in 5 sections of the SRBRs pretreated by slag. (f, g) Production of organic acids and alcohols throughout SRBR operation. Abbreviations: SRBR = sulfate-reducing biochemical reactor; ADS = anaerobic digester sludge; grains = spent brewing grains, bagasse = sugarcane bagasse, enrichment = microcosm enrichment culture, T = SRBR top (influent side), M2 = SRBR middle location #2, B = SRBR bottom (effluent side). Data in (d–g) are averages ( $\pm$  SD) of duplicate SRBRs.

brewing grains and sugarcane bagasse SRBR effluents was  $5.2 \pm 0.2$  and  $5.7 \pm 0.7$ , respectively (Figure 2b,c). The resulting pH is in line with previous studies where MIW was treated by lignocellulosic SRBRs.<sup>30,69–72</sup> Also at steady state, the SRBRs removed between  $86 \pm 30$  and  $91 \pm 9\%$  of the total metal(loid) concentration from the settling tank influent during the operation phase (Table 2). The SRBR stage showed particularly robust removal of Cd, Cu, and Zn (at  $\geq 96\%$ , Table 2). In the slag reactor effluent at pH 12 (Figure 2a), Zn, Cd, and Cu were likely precipitated as metal oxides. At a pH of 7 or less, the solubility of Zn, Cu, and Cd hydroxides is  $>10 \text{ mg L}^{-1}$ .<sup>22</sup> Therefore, Zn, Cd, and Cu hydroxides formed in the slag reactor likely resolubilized in the settling tank, which was at an average pH of  $5 \pm 1$  (Figure 2a). In the SRBRs, Zn, Cd, and Cu were likely immobilized as sulfide complexes, which have a solubility of  $<0.01 \text{ mg L}^{-1}$  at pH 5–12.<sup>22</sup> Through geochemical digests, electron microscopy, and energy-dispersive X-ray spectroscopy, Drennan et al. showed that Zn removal from an actual MIW was both temporal and spatial in lignocellulosic SRBRs.<sup>73</sup> Zn removal was initially dominated by carbonate precipitation and adsorption to lignocellulose but later transitioned to sulfide precipitation which grew from the outlet end toward the inlet.<sup>73</sup> It is possible that such a dynamic removal for Zn and other metals also occurred in the SRBRs from this study, but the mechanism was not directly probed. Results from this work clearly demonstrate the essential role of SRBRs for  $\text{SO}_4^{2-}$  reduction and a complementary role to the slag stage for metal(loid) immobilization. Overall metal(loid) removal by the two-stage system ranged from  $89 \pm 16\%$  to  $95 \pm 6\%$  (Table 2), leading to low metal(loid) effluent concentrations and circumneutral pH.

### 3.3. Microbial Community Composition in SRBRs Was Most Heavily Influenced by the Type of Lignocellulose Material.

In a lignocellulosic SRBR, a microbial community capable of hydrolysis and fermentation of lignocellulose and sustained  $\text{SO}_4^{2-}$  reduction is essential for the continuous removal of metal(loid)s from MIW. A few studies have used slag to enhance certain microbial processes, such as fermentation, denitrification, or even  $\text{SO}_4^{2-}$  reduction.<sup>42,74–76</sup> While one study has evaluated slag and lignocellulosic SRBRs to treat MIW,<sup>42</sup> none have characterized the microbial community composition of these systems. In this study, the SRBR microbial community composition and diversity were analyzed as a function of the lignocellulosic material (Figures 3a–c and S3). *Desulfobacterota*, a phylum containing sulfate-reducing species,<sup>77</sup> was common to both sets of SRBRs (Figure 3a). The fermentative and hydrolytic bacterial taxa varied between the spent brewing grains and sugarcane bagasse SRBRs (Figure 3a). *Negativicutes* made up 70–94% of total sequences in the spent brewing grains SRBRs, with genus *Anaerovibrio* at 18–55% of the total sequences by the end of operation (Figure 3a). *Anaerovibrio* spp. can ferment lactate, ribose, and fructose to acetate, propionate,  $\text{CO}_2$ ,  $\text{H}_2$ , and succinate<sup>78</sup> and was one of the major identified ASVs in SRBRs packed with a mixture of spent brewing grains and limestone.<sup>30</sup> As shown in Figure S4, acetic and propionic acids were among the most abundant fermentation products in the effluent of the spent brewing grains SRBRs. In the sugarcane bagasse SRBRs, *Anaerolineaceae* made up 14–30% of the total sequence abundance, with genus *Leptolinea* at 15% (Figure 3a). *Leptolinea* spp. are capable of fermenting sugars and proteins<sup>79</sup> and have been detected in waste activated sludge containing high concentrations of lignocellulose.<sup>80</sup> In a previous study,

limestone (at 30 wt %) was used as the alkalizing agent in SRBRs packed with spent brewing grains or sugarcane bagasse to treat the same MIW as used in this study.<sup>30</sup> The SRBRs with limestone were also operated at similar HRTs (between 3 and 12 days).<sup>30</sup> To understand if the alkalizing agent (slag versus limestone) plays a role in the resulting microbial communities in SRBRs, we combined the sequences from this study and from Miranda et al.<sup>30</sup> in Figure 3b,c. According to the weighted UniFrac analysis (Figure 3b,c), the type of lignocellulose material (spent brewing grains or sugarcane bagasse) was a stronger driver of the microbial community composition than the type of alkalizing agent. This outcome, combined with the evidence for the essential microbial metabolisms ( $\text{SO}_4^{2-}$  reduction, anaerobic lignocellulose biodegradation) found in typical lignocellulosic SRBRs strongly suggests that slag is a viable alternative to limestone as the alkalizing agent.

Given that SRBR treatment heavily relies on lignocellulose biodegradation, we analyzed the components of lignocellulose (cellulose, hemicellulose, and lignin) before SRBR operation and at the end of operation (Figure 3d,e). Nonlignocellulosic components (e.g., sugars, starches, proteins) were grouped into the “other” category in Figure 3d,e. Before operation, hemicellulose made up most of the spent brewing grains’ composition ( $40 \pm 1\%$ , Figure 3d) and decreased to  $36 \pm 1\%$  at the end of operation (Figure 3d). The nonlignocellulosic category “other” decreased by  $\sim 10\%$  by day 121 in the spent brewing grains SRBRs (Figure 3d). While cellulose had the highest abundance in sugarcane bagasse (initially at  $51 \pm 1\%$ ), only the nonlignocellulosic “other” had a measurable decrease of  $\sim 5\%$  at the end of operation in the middle (sections M1 and M2) and bottom of the sugarcane bagasse SRBRs (from  $15 \pm 1\%$ , Figure 3e). In the sugarcane bagasse SRBRs, fermentation products were also in lower concentrations throughout operation (Figure 3g). Previous studies have associated the higher availability of organic acids and alcohols to higher extents of  $\text{SO}_4^{2-}$  reduction in SRBRs treating MIW.<sup>30,31,81,82</sup> High concentrations of propionic acid have also been correlated with inadequate  $\text{SO}_4^{2-}$  reduction in an up-flow anaerobic sludge blanket bioreactor treating  $\text{SO}_4^{2-}$ -rich water.<sup>82</sup> While a variety of organic acids and alcohols from the biodegradation of lignocellulose were detected in our SRBR effluents, acetic acid was consistently higher in concentration compared to propionic acid (Figure S4a,b), supporting good performance for  $\text{SO}_4^{2-}$  reduction to treat MIW.

## 4. CONCLUSIONS

Our study is the second to combine basic oxygen furnace slag with microbial  $\text{SO}_4^{2-}$  reduction to treat MIW. The first study was conducted by Naidu et al.,<sup>42</sup> where a configuration involving 6 vessels in series was employed for MIW treatment: two slag vessels, a sedimentation vessel (receiving fresh MIW), two separate  $\text{SO}_4^{2-}$ -reducing vessels (the first containing sugarcane bagasse), and a collection reservoir from which partial liquid (60–95%) was redirected to the first slag vessel and passed again through the entire system. The configuration in Naidu et al.<sup>42</sup> demonstrated versatility and treatment potential by successfully removing Al, Fe, Mn, and Mg in MIW collected from two mining sites. In our two-stage treatment (Figure 1), the slag stage featured a single slag reactor in recirculation with a mixing tank, which received MIW and flowed into a settling tank. Settling of precipitates in the settling tank alleviated metal(loid) loading to the SRBRs,

mitigating sludge buildup, and reducing possible clogging (pipes, SRBR, etc.). Given that full-scale SRBR systems are designed for operation on the order of years, our design should ultimately help the lifetime and operation of SRBRs for MIW treatment.

The single pass through each stage in this study was a key treatment design. The partial recycling of the effluent through the entire system in the configuration from Naidu et al.<sup>42</sup> made it difficult to determine the specific treatment capacities of the slag and SRBRs. The distinct two-stage configuration from our study facilitated the assessment of treatment by its individual components (pH, metal(loid)s, and  $\text{SO}_4^{2-}$  at the slag and SRBR stages). Each stage demonstrated its capacity to treat MIW, where the slag stage was exceptional at removing metal(loid)s (as hydroxides and oxides), increasing MIW pH, and decreasing ORP and DO. The SRBR stage was focused on removing  $\text{SO}_4^{2-}$  and immobilizing the remaining metal(loid)s as more stable sulfide precipitates. A future two-stage configuration targeting MIW treatment may consider a design where anoxic conditions are more tightly controlled at the slag stage or at the SRBR stage (e.g., by increasing the HRT in SRBR to allow anaerobic conditions to prevail) to mitigate the possibility of  $\text{S}^{2-}$  reoxidation. Overall, treatment of MIW by the two stages in series was effective and complementary.

The two types of lignocellulosic materials used in this study had distinct advantages when treating the MIW. The spent brewing grains SRBRs reached an average  $\text{SO}_4^{2-}$  effluent concentration within the range of  $250 \text{ mg L}^{-1}$  (nonmandatory standard), while the sugarcane bagasse SRBRs removed more  $\text{SO}_4^{2-}$  and metal(loid)s per mass of dry lignocellulosic material. Based on these observations, a combination of the two materials may be beneficial to meet any applicable strict metal(loid) and  $\text{SO}_4^{2-}$  effluent concentrations in an SRBR system designed for years or decades of operation in the field. In our work, the slag stage was decoupled from the SRBRs to more tightly control the SRBR influent pH. Configurations where slag is directly combined with the lignocellulosic material (similar to limestone) may also be feasible. Such SRBR designs will require preliminary studies to fine-tune the amount of slag needed to maintain a circumneutral pH within the SRBR. In conclusion, our study supports the feasibility of using basic oxygen furnace slag in combination with SRBRs for full-scale treatment of MIW at remote mining sites.

## ■ ASSOCIATED CONTENT

### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsestengg.3c00381>.

Calculations of density and porosity for the lignocellulosic materials (S1); details on analyses for  $\text{SO}_4^{2-}$ , metal(loid)s, organic acids and alcohols, and lignocellulose (S2); calculations of metal(loid) removal (S3); synthetic and hard rock legacy mine MIW compositions (Table S1); ICP-OES analyte detection limits (Table S2); HPLC analyte detection limits (Table S3); concentrations of metal(loid)s in the slag reactor and the SRBRs (Table S4); theoretical  $\text{S}^{2-}$  required to precipitate metal(loid)s (Table S5); photograph of the two-stage treatment (Figure S1);  $\text{S}^{2-}$  concentrations and production throughout SRBR operation (Figure S2); Bray–Curtis, Faith's phylogenetic diversity indices, and Pielou's evenness indices of the microbial community in

the SRBRs (Figure S3); and concentrations of organic acids and alcohols throughout SRBR operation (Figure S4) (PDF)

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### Notes

The authors declare no competing financial interest.

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