Evaluating Novel Organic Substrates for Sulfate-Reducing Biochemical Reactors Treating Mine Water

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Abstract

Sulfate-reducing biochemical reactors (SRBRs) have been used for decades to passively treat mining-influenced water. SRBRs rely on microbially-mediated processes such as fermentation and sulfate-reduction to remove metals and sulfate from water and increase pH. Passive SRBRs typically consist of a biodegradable organic medium through which mine water passes. The complex community of microbes formed in SRBRs include cellulolytic bacteria responsible for initial breakdown of lignocellulose that provide more readily bioavailable carbon to fermenters and sulfate-reducing bacteria. A wide range of organic media have been evaluated in the literature for effectiveness in SRBRs and identification of effective lignocellulosic media is critical for short- and long-term SRBR performance.

In this study, we conducted screenings of SRBR media, as well as collaborated with universities in the U.S. and Indonesia. Media evaluated included coconut trees and coconut shells, rice husk, cassava, sugarcane bagasse, and spent brewer's grains. Using lab-scale SRBRs and synthetic mine water, media were screened for their ability to support sulfate-reduction via monitoring of the oxidation-reduction potential, dissolved O_2 concentration, and pH, and metal removal and sulfate reduction rates. A subset of media was evaluated for treatment performance, lignocellulosic composition (cellulose, hemicellulose, and lignin before and after operation), available carbon sources, and microbial community composition. As expected, results varied among organic materials evaluated. A small number of substrates evaluated (coconut husk, coconut palm wood, and merbau) failed to achieve conditions necessary to support sulfate

reduction. Remaining substrates (*Phragmites*, corn stalk, date palm, coconut husk, sweet potato, vegetable waste, king grass, spent brewing grains, and sugarcane bagasse) successfully supported conditions necessary for sulfate reduction. Metal removal rates for these substrates ranged from 37-98%. Results stemming from these screenings can provide insights into the type of lignocellulosic media with potential for sustaining essential SRBR microbial processes in short- or long-term operation. Variation in microbial communities among SRBRs was attributed to organic composition, with sulfate reduction proportional to the amount of readily available carbon (e.g., cellulose, hemicellulose).

Introduction

A multitude of historical mine sites exist throughout the western United States with a portion of these releasing mining-influenced water (MIW) into the environment. Passive, biologically-based water treatment strategies can be a cost-effective and low maintenance technology for treating MIW, particularly for remotely-located legacy mine sites. One such technology is sulfate-reducing biochemical reactors (SRBRs), which have been used for decades to treat MIW (see Kleinmann et al. 2021 for a historical perspective). SRBRs rely on microbially-mediated processes such as fermentation and sulfate-reduction to improve water quality by increasing pH and removing metals and sulfate from water (see review by Sheoran et al. 2010). Under anoxic conditions, sulfate-reducing bacteria (SRB) utilize a carbon source to complete dissimilatory sulfate reduction, producing sulfide and bicarbonate alkalinity in the process. The sulfide reacts with various cations to form relatively insoluble metal sulfides. Secondary metal removal processes include the formation of hydroxides, carbonates, and sorption mechanisms (Sheoran et al. 2010). The alkalinity produced during sulfate reduction increases pH of the water. Passive versions of SRBRs primarily consist of a biodegradable organic media as the carbon source. The complex community of microbes within SRBRs include lignocellulolytic bacteria responsible for initial breakdown of lignocellulosic components such as hemicellulose, cellulose and lignin that provide more readily bioavailable carbon to fermenters and SRB. These microbial processes also create the anoxic environment essential for sulfate reduction.

A wide range of organic media have been evaluated in the literature for effectiveness in SRBRs and identification of effective SRBR lignocellulosic media is critical for short- and long-term performance (Sheoran et al. 2010). Some characteristics of organic matter that are particularly important for SRBRs are organic fraction, nutrient content, and composition (Kuyucak and St-Germain 1994, Sheoran et al. 2010, Cao et al. 2012). In this paper, we will discuss the characteristics of novel, regionally-available lignocellulosic media to identify candidates for potential use in large-scale applications. We collaborated with universities in the U.S. and Indonesia to conduct screenings of regionally-available SRBR media for potential use in large-scale SRBRs. Results stemming from these screenings can provide insights into the

type of lignocellulosic media with potential for sustaining essential SRBR microbial processes in short- or long-term operation.

Methods

This paper summarizes three separate but complementary lab-scale evaluations of potential SRBR media. An initial screening of a range of regionally-available organic substrates was conducted in the U.S. and Indonesia using lab-scale SRBRs and synthetic MIW. Organic substrates evaluated in the U.S. included coconut (*Cocos nucifera*) wood and husk, common reed (*Phragmites* sp.), date palm (*Phoenix dactylifera*) wood, merbau/ironwood (*Intsia* sp.) wood, and corn (*Zea mays*) stalk. Substrates were individually screened. Six SRBRs were evaluated using a 50:50 mixture of either coconut husk and palm wood or coconut husk and ironwood. Substrates individually evaluated at the Institut Teknologi Bandung (ITB), Indonesia included merbau/ironwood (*Intsia* sp.) sawdust, rice husk, cassava (*Manihot esculenta*) tuber, sweet potato (*Ipomoea batatas*), corn (*Zea mays*) stalk, vegetable waste, and king grass (*Pennisetum* sp.). These substrates were screened for their ability to support sulfate reduction as indicated by changes in oxidation-reduction potential (ORP), dissolved oxygen (DO), and pH. The SRBRs operated at ITB were also evaluated for metal removal and sulfate reduction rates. Finally, four SRBRs were evaluated at Arizona State University (ASU), USA using a mixture of 30% limestone and 70% spent brewing grains or sugarcane (*Saccharum* sp.) bagasse, by-products from the beer brewing and sugar industries, respectively. Water chemistry parameters such as pH, ORP, and DO were measured throughout operation. Additionally, metal removal, sulfate reduction, and available volatile fatty acid (VFA) concentrations were also measured. The lignocellulosic composition (pre- and post-study) of the changes in available hemicellulose, cellulose and lignin were evaluated, including an analysis of the available microbial community capable of degrading lignocellulosic material.

Feed solution

Synthetic feed solutions (Tables 1 and 2) were used for the lab-scale SRBR screening projects and their composition is loosely based on actual MIW chemistry. The feed solution in Table 1 also mimics water that has received limestone pre-treatment to remove Al and Fe and increase pH. The SRBR lab-scale studies conducted at ASU used a synthetic feed solution containing the properties listed in Table 2.

Table 1. Synthetic MIW chemistry used for SRBR substrate screening.

		Feed solution	Chemicals required per 1 L deionized water
pH (s.u.)		4.5-5.0	
Sulfate (mg/L)		235	
Metals	Cu	100	386 mg $CuSO_4 \cdot 5H_2O$

(mg/L)	Mn	13	38 mg $MnSO_4 \cdot H_2O$
	Zn	40	199 mg $ZnSO_4 \cdot 7H_2O$

Table 2. Synthetic MIW chemistry used for SRBRs in the ASU lab-scale study.

		Feed solution	Chemicals required per 1 L deionized water
pH (s.u.)		2.6	
Sulfate (mg/L)		500	
Metals (mg/L)	Cd	0.1	0.3 mg $CdSO_4$
	Co	0.02	0.07 mg $CoCl_2 \cdot 6H_2O$
	Cu	0.7	1.8 $CuCl_2 \cdot 5H_2O$
	Fe	59	290 mg $Fe_2(SO_4)_3 \cdot H_2O$
			100 mg $Fe(SO_4) \cdot H_2O$
	Ni	0.01	0.04 mg $NiSO_4$
	Pb	0.04	0.15 mg $PbCl_2$
	Zn	57	175 mg $ZnCl_2$

Lab-scale SRBRs

SRBRs were filled with individual organic substrates to assess their capability to support sulfate-reducing bacteria and sulfate reduction. Limestone chips and/or pellets (30% dry wt.) were also mixed into the substrate to help neutralize MIW acidity while the sulfate-reduction activity ramped up. Once the SRBR reaches steady-state, then bicarbonate alkalinity generation is sufficient to neutralize influent acidity. A small amount of cow manure (1% dry wt.) was added as an inoculum in some SRBRs while others were inoculated with the SRB *Citrobacter freundii*. The SRBRs operated at ITB also included a small amount of compost as an inoculant. Organic substrates evaluated were identified following a desk-top screening of regionally-available plant species, agricultural by-products, and local cultural knowledge. Organic materials were then gathered locally from a variety of locations, chopped into smaller sizes (2-6 cm), and dried to constant temperature prior to use.

In the studies conducted at ASU, two types of inocula were used: one was 25 mL of an enrichment culture grown for 1 month on the respective substrate and sulfate-rich medium. The other was 125 mL of anaerobic digester sludge from the Mesa Northwest Water Reclamation Plant, a local municipal wastewater treatment plant in Mesa, AZ. The spent brewing grains were gathered from San Tan Brewery, a Phoenix-area brewery, and the sugarcane bagasse was obtained from Cajun Sugar Company in New Iberia, Louisiana. The organic substrates were mixed in a 7 to 3 ratio of organic substrate and limestone by weight and then packed into the SRBRs.

Lab-scale SRBRs were fabricated using PVC columns having approximate dimensions of 10 cm in diameter and 117 cm tall. The base of the column was capped with a standard PVC pipe cap, which is tapped in the center to thread in a drain valve assembly. A plastic mesh screen was placed in the base of the column prior to filling with substrates to prevent contents from obstructing the drain valve. Columns were filled from bottom to top, first with 10 cm-depth of pea gravel for drainage, followed by 91 cm-depth of substrate (Figure 1). Mass of substrates used to fill the SRBRs was recorded to quantify sulfate reduction rates on a mass basis. Water level within SRBR columns was maintained at 5 cm above the top of substrate during operation. The top of the SRBRs was capped with a rubber lid to prevent oxygen intrusion. The SRBR columns were operated in continuous, down-flow mode with feed solution introduced through the cap at the top of the column. Flow rates varied among projects, ranging from 0.6-2 L/d and controlled using peristaltic pumps. The length of operation varied from approximately 60 to 130 days depending on the project.

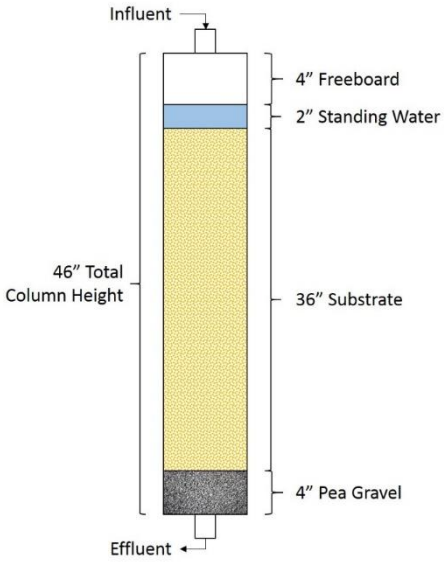

Figure 1. Lab-scale SRBR column schematic.

Water quality monitoring

During dissimilatory sulfate reduction by SRBs, hydrogen ions are consumed, and bicarbonate alkalinity is produced. These reactions are only possible in an anoxic and reducing environment. Therefore, changes in redox conditions within SRBRs may be used as evidence for sulfate reduction. Aqueous samples were collected on a weekly basis. Physico-chemical parameters measured varied from project to project but included pH and ORP, at a minimum. Optimal conditions for SRBs to thrive and reduce sulfate include a pH in the range of 5 to 8 s.u. and ORP in the range of -100 to -300 mV (Sheoran et al. 2010). Dissolved oxygen (DO), electrical conductance (EC), and temperature was also recorded for some SRBRs. Analysis

for total and dissolved metals was conducted at the outset to check for elements leaching out of the substrates. Weekly analysis of solely Cu, Mn, and Zn on both total and filtered samples occurred after the columns were no longer leaching other metals. Sulfate, free sulfide, and alkalinity/acidity were quantified weekly in some SRBRs to assess the extent of sulfate reduction. For the ASU SRBRs, a measurement for pH, ORP and DO were collected at least twice a week and the analysis of sulfate, sulfide and metals such as Cd, Co, Ni, Pb, Fe, Cu, and Zn, were conducted weekly. A weekly quantification of VFAs was analyzed using high performance liquid chromatography (HPLC) and included analysis of formic, acetic, propionic, butyric, valeric, and caproic acids.

Lignocellulosic and microbial composition analysis

Hemicellulose, cellulose, and lignin were characterized for the ASU lab-scale studies. Samples of the organic material analyzed were taken before the start of SRBR operation and after completion of the trials. Substrates samples from 3 equidistant locations (top to bottom) of each duplicate SRBR were retrieved for lignocellulosic and microbial analysis. Lignocellulosic analysis was characterized using Ankom Technology A2000 fiber analyzer. DNA extractions were performed for each sample using a DNeasy PowerFood Microbial Kit from Qiagen as directed by the protocol. After DNA extraction, the samples were sent for high throughput DNA sequencing at the Center for Fundamental and Applied Microbiomics at the ASU KED Genomics Core Facility. Quantitative Insights into Microbial Ecology (QIIME 2) pipeline was used to assign taxonomy for the amplicon sequence variants (ASVs) using the SILVA database (Bolyen et al. 2019, Glöckner et al. 2017, Quast et al. 2012, Yarza et al. 2014, Yilmaz et al. 2014). For the ASU study, select genera of microbes known to have lignocellulytic activity were specifically identified.

Results and Discussion

SRBR substrate screening

As expected, results varied among organic materials evaluated (Table 3). SRBRs filled with coconut husk or merbau/ironwood (*Intsia* sp.) failed to achieve reducing conditions after 15 weeks of operation, with ORP remaining > 0 mV. pH did increase between 0.3 and 1.0 s.u. but that was most likely due to the added limestone. All SRBRs containing mixtures that included ironwood failed to exhibit reducing conditions even after 15-27 weeks of operation, suggesting that the *Intsia* sp. used contains phytocompounds that grant it anti-microbial and/or anti-mold properties (cf. Reinprecht et al. 2020). The coconut husk was, however, able to support reducing conditions when used in a mixture with date palm wood. These SRBRs achieved reducing conditions (ORP < −200 mV) within 2-4 weeks and maintained the reducing environment for at least 22 weeks, exhibiting an average ORP of −222 mV. This observation suggests that coconut husk does not directly inhibit SRBs but perhaps did not contain sufficient readily available organic carbon to support

the necessary microbial communities, which was provided by date palm wood. However, coconut husk has been documented to inhibit common human oral microbial pathogens (Jose et al. 2014), as well as other microbes (e.g., Viju et al. 2013). Coconut palm wood also failed to support reducing conditions, though ORP did decline towards 0 mV at the end of 10 weeks of operation. Alternatively, *Phragmites* and corn stalk supported reducing conditions within 2 and 5 weeks, respectively, of start-up and maintained conditions throughout the 23 weeks of operation with a mean ORP of -184 and −173 mV, respectively. The SRBRs supporting sulfate reduction also exhibited the largest increases in pH providing evidence of bicarbonate alkalinity production. The SRBRs with date palm, *Phragmites*, and corn stalk showed an increase in pH from 5.3 s.u. in the synthetic MIW to 6.3-6.6 s.u. The increase in pH was even more pronounced in the SRBRs with strongly reducing conditions (e.g., the date palm wood mixtures), which exhibited effluent pH ranging from 6.4-6.9 s.u. These results from this screening indicate that coconut husk, date palm, *Phragmites*, and corn stalk should be retained for further consideration.

Table 3. SRBR substrates screened in the United States. Mean effluent ORP and pH are shown. Mean influent MIW ORP was 207 mV and pH was 5.3 s.u.

	Effluent	
Substrate	**ORP (mV)**	**pH (s.u.)**
Coconut shells	112	5.4
Phragmites	-184	6.4
Date palm	-232	6.6
Merbau/Ironwood	65	6.2
Coconut palm	92	5.1
Corn stalk	-173	6.3

SRBRs evaluated at ITB containing locally-available substrates—sweet potato, corn stalk, vegetable waste, or king grass—all supported reducing conditions in their respective SRBRs (Table 4), with effluent ORP ranging from -16 to -572 mV during the 60-day trial. Vegetable waste and king grass SRBRs exhibited the most strongly reducing conditions (ORP = -429 and -572 mV, respectively). Alternatively, SRBRs containing rice husk and cassava tubers exhibited the lowest effluent sulfate concentrations, decreasing from 347 mg/L to <30 mg/L by the end of a 28-day trial, despite exhibiting weakly or moderately oxidizing conditions (ORP = 0 and 206 mV, respectively). Sulfate reduction has also been observed in other SRBRs despite effluent ORP values > 0 mV. This is because effluent values may not accurately reflect water quality in microhabitats within the SRBR substrate matrix. SRBRs with cassava tubers and sweet potato exhibited decreases in pH to 4.0 s.u., possibly due to their naturally-occurring cyanide (Padmaja 1995). During the final 4 weeks of the ~10-week trials, SRBRs containing merbau sawdust, rice husk, cassava, sweet potato,

corn stalk, vegetable waste, or king grass removed 84-98% of Cu and 37-90% of Zn. The generally lower removal of Zn is not surprising since Zn particulates are relatively small and often are not fully filtered out by SRBR substrates. The ability of some substrates to remove metals despite not supporting a strongly reducing environment, such as cassava, suggests that mechanisms other than sulfide formation (e.g., adsorption, carbonate formation) are also acting on the cations within SRBRs. Combing ORP, pH, and metal removal results to infer the degree of sulfate reduction suggests that merbau, rice husk, sweet potato, corn stalk, and king grass were the best candidates to be retained for further consideration.

Table 4. SRBR substrates screened at ITB. Mean effluent water quality parameters during final four weeks of SRBR operation are shown.

				Copper		Zinc	
	Eh	pH (s.u.)	Sulfate (mg/L)	Cu (mg/L)	Removal	Zn (mg/L)	Removal
Merbau sawdust	64	8.1	9	4.3	97%	8.04	89%
Rice husk	0	7.6	30	7.5	94%	7.28	90%
Cassava tuber	206	4.0	20	13.7	90%	18.52	75%
Sweet potato	-16	4.1	157	7.7	96%	41.2	44%
Corn stalk	-102	6.1	176	4.2	98%	34.3	53%
Vegetable waste	-429	6.7	238	6.4	96%	33.1	55%
King grass	-572	7.8	211	28.4	84%	46.3	37%

SRBR substrate and lignocellulolytic microbial community characterization

The spent brewing grains and sugarcane bagasse SRBRs evaluated at ASU were operated for a total of 135 days. The spent brewing grains and sugarcane bagasse SRBRs began operation at a 3-day hydraulic retention time (HRT) on day 50. SRBRs containing spent brewing grains continued at a 3-day HRT until the end of operation, however, the sugarcane bagasse SRBRs maintained the 3-day HRT from day 50 to 100 (additional details can be found in Miranda et al. 2022). The HRT for the sugarcane bagasse SRBRs was then increased to 6 days until the end of operation. SRBR performance was the greatest during the 3-day HRT and the average effluent water chemistry, sulfate, and total VFAs are presented in Table 5. The pH, ORP, and DO values during this time were well within the values needed for sulfate reduction to take place. Sulfate reduction rate during the 3-day HRT was 92% for the spent brewing grains SRBRs and 17% for the sugarcane bagasse SRBRs. A possible explanation for the observed difference in sulfate reduction between the two substrates stems from the differences in concentration of available VFAs, which is partly determined by the lignocellulosic material utilized. Sulfate-reducing bacteria are ubiquitous in their ability to utilize carbon sources, which are commonly sugars and VFAs derived from the breakdown of lignocellulosic material. During the 3-day HRT, VFAs found in the effluent of the spent brewing grains

SRBRs included acetic, propionic, butyric, valeric and caproic acids. Sulfate-reducing bacteria can utilize acetic, propionic, and butyric acids as carbon sources for energy (Liamleam et al. 2007). VFAs were not quantifiable in the sugarcane bagasse SRBRs, suggesting there was minimal availability of VFAs for the microbial community. It was hypothesized, that the sugarcane bagasse SRBR's flow rate (2 L/day) to achieve the 3-day HRT was too high (spent brewing grains had a 1 L/day flow-rate to achieve the 3-day HRT), therefore, the microbial community was suffering the effects of washout. Washout effects include the physical removal of biofilm formation and readily available carbon sources. For this reason, the flow-rate for the sugarcane bagasse SRBRs was decreased, achieving an HRT of 6-days (1 L/day flow-rate) from day 100 to day 135. However, this only increased the sulfate-reduction rates to an average of 27%.

Table 5. Average performance of duplicate SRBRs containing spent brewing grains and sugarcane bagasse during a 3-d HRT. VFAs = Volatile fatty acids, BDL= Below detection limit

	pH (s.u.)	ORP (mV)	DO (mg/L)	Sulfate (mg/L)	Total VFAs (mM)
Spent brewing grains	5.9	–260	1.3	41	20
Sugarcane bagasse	6.3	–100	2.5	414	BDL

SRBR removal of metals during the 3-day HRT are summarized in Table 6. Metals that had excellent removal in SRBRs containing spent brewing grains with ~95% removal or greater were Cd, Co, Cu Fe, and Zn. In the sugarcane bagasse SRBRs, metals with removal >95% were Cd, Cu, Fe, and Zn. Spent brewing grains exhibited better metal removal than sugarcane bagasse and this may be related to differences in sulfate reduction rates and VFAs produced within both types of SRBRs.

Table 6. Average concentration and removal of metals in duplicate SRBR effluents during the 3-d HRT. BDL = Below detection limit

	Spent brewing grains		Sugarcane bagasse	
Metal	Concentration (mg/L)	Removal	Concentration (mg/L)	Removal
Cd	BDL*	>99.5%	BDL*	>99.5%
Co	BDL*	>94.8%	0.004	75.2%
Cu	BDL*	>99.3%	0.006	99%
Fe	0.03	99.9%	1.4	97.6%
Ni	0.002	80%	0.004	56%
Pb	0.03	25%	0.008	78%
Zn	0.01	99.9%	2	96.5%

*Detection limits for Cd, Co, and Cu in mg/L were 0.0005, 0.001, and 0.005, respectively

Analysis of the lignocellulose composition and lignocellulolytic microbial genera present in the SRBRs gave insights into why there was higher availability of VFAs in the spent brewing grains SRBRs versus the sugarcane bagasse SRBRs. The lignocellulosic analysis of the organic material before and after operation and documented microbial genera are presented in Figure 2. The main difference between each organic substrate was in its composition. Spent brewing grains consisted primarily of hemicellulose (40%) and 'other' (34%). Whereas sugarcane bagasse consisted primarily of cellulose (47%) with a higher lignin (17%) percentage compared to the spent brewing grains. In general, of the three types of fiber in lignocellulosic material, hemicellulose is more readily microbially-degraded than both cellulose and lignin, followed by cellulose (Shrestha et al. 2017). Lignin is the most difficult of the three for microbial degradation and it is more readily degraded in an aerobic environment than in an anaerobic environment, although this is still a topic that is not well understood (Shrestha et al. 2017, Li et al. 2009). The 'other' category is even more readily available for microbial fermentation, consisting of sugars, starches, other carbohydrates, proteins, and amino acids. At the conclusion of the 135-d trial, we observed a decrease in the spent brewing grains SRBRs in the amount of hemicellulose and 'other' category (7% and 4 %, respectively), suggesting that these two types of carbon sources were utilized by the microbial community. In the sugarcane bagasse SRBRs, we observed a decrease in the hemicellulose and cellulose category (3% and 2%, respectively).

Panels B and D of Figure 2 show the lignocellulosic degrading microbial genera identified in each set of SRBRs. In the spent brewing grains SRBRs, *Bacteroides, Butyrivibrio, Clostridium, and Ruminococcus* containing species capable of degrading hemicellulose and cellulose (Brunecky et al. 2012, Shrestha et al. 2017, Tsavkelova and Netrusov 2011) comprised about half of the total sequences of microbes available for lignocellulose degradation. *Acetivibrio, Fibrobacter* and *Eubacterium*, comprising the other half, are genera known to degrade cellulose (Shrestha et al. 2017). In the sugarcane bagasse SRBRs, *Hydrogenispora, Ruminiclostridium, Bacteroides,* and *Clostridium*, which are all capable of degrading hemicellulose or fermentation (Wu and Cheng 2018, Xie et al. 2021, Shrestha et al. 2017, Tsavkelova and Netrusov 2011), made up about a third of sequences of microbes with putative capabilities for lignocellulosic degradation, while *Cellulomonas* and *Geobacillus*, made up the remainder. Interestingly, *Geobacillus* has been shown to degrade lignin under oxic conditions but can also degrade cellulose (Meslé et al. 2022). The difference in performance of the spent brewing grains and sugarcane bagasse can be attributed to the lignocellulose composition and the microbial community's ability to degrade the lignocellulose. In each set of SRBR before and after operation, the spent brewing grains showed larger decreases in hemicellulose while sequences belonging to genera capable of hemicellulose degradation were also more abundant in the spent brewing grains SRBRs than in the sugarcane bagasse

SRBRs. The higher percentage of hemicellulose present in the spent brewing grains could also explain the higher availability of VFAs promoting microbial growth and hence higher rates of sulfate reduction.

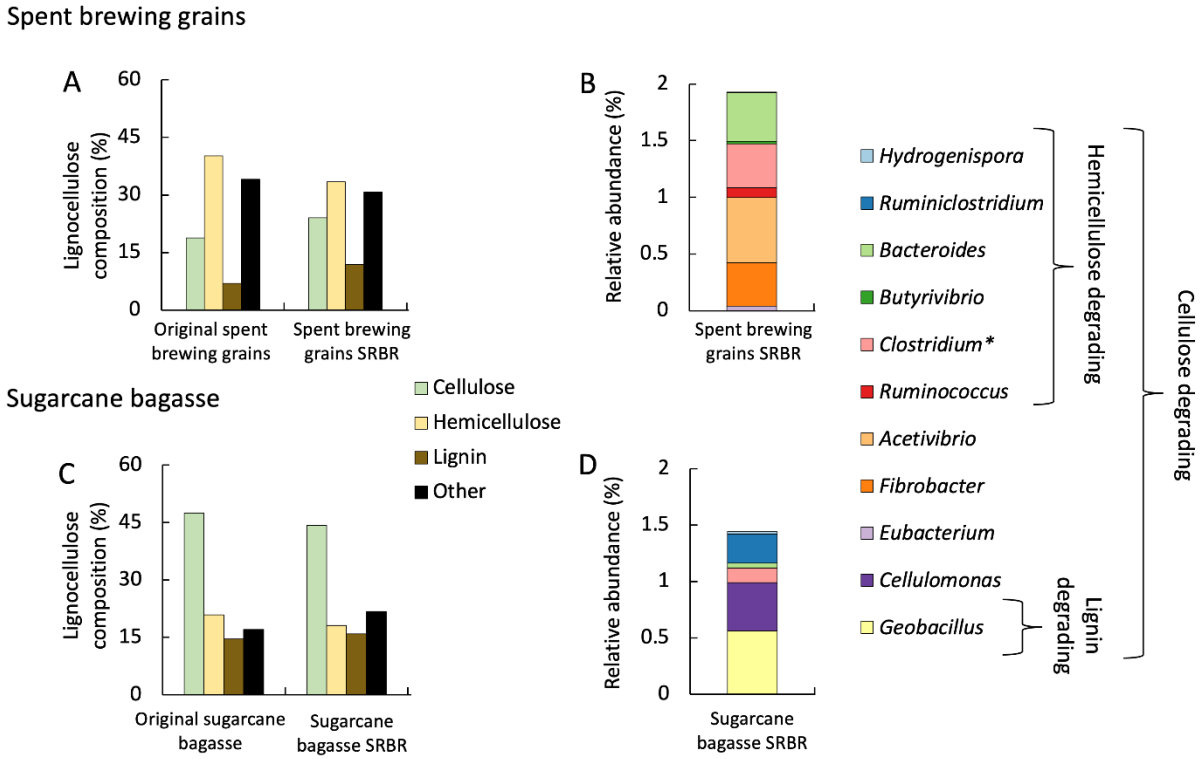

Figure 2. A-D Lignocellulose composition in SRBRs and selected genera containing lignocellulose - degrading species in SRBRs containing spent brewing grains (SBG) or sugarcane bagasse (SCB). The SRBR data are averages of duplicates.

Conclusions

While SRBR technology has been in use for several decades, strategies remain for refining their design and improving performance. For example, designing passive SRBRs for application at mines around the globe to help remediate mining-influenced waters benefits from a more expanded view of the organic materials that can potentially be utilized. A wide variety of cellulosic and organic wastes, such as agricultural by-products or locally-available organic materials, have potential for use in passive SRBRs. Some of these have already been evaluated by others (e.g., see review by Sheoran et al. 2010), but various cellulosic and organic wastes remain to be evaluated. It is critical that these materials be capable of supporting the diverse array of microbial communities needed to optimize sulfate reduction without hindering them (e.g., via anti-microbial compounds) or inadvertently leaching additional metals into waters being treated. Designing SRBRs containing the organic substrates evaluated in this paper for long-term success will also benefit from a more critical assessment of what characteristics of the cellulosic and organic wastes influence SRBR microbial communities, and ultimately sulfate-reduction and metal removal performance. It may be

beneficial to further test lignocellulosic organics high in hemicellulose such as the spent brewing grains for long-term microbial community support. Additionally, a combination of lignocellulosic materials high in hemicellulose and cellulose may be beneficial due to the differences in lignocellulosic microbial community composition supported by each to support long-term treatment system efficiency and performance. Additional analysis into these mechanisms and lingering questions would benefit the design, operation, and monitoring of full-scale SRBRs treating MIW.

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