

# Lignocellulosic Characterization of Sulfate-Reducing Bioreactors (SRBRs) Treating Mining-Influenced Water (MIW)

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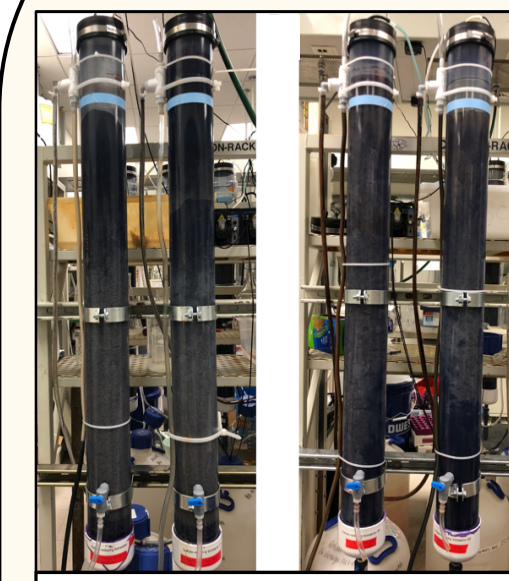
NSF Engineering Research Center for Bio-mediated and Bio-inspired Geotechnics (CBBG)

## What happens to the lignocellulosic composition of the substrate inside an SRBR treating MIW?

### Introduction

SRBRs are a passive treatment method for acidic MIW from active and legacy mine sites. In an SRBR, microbial communities degrade lignocellulosic substrates, producing electron donors for sulfate-reducing bacteria (SRB). Sulfate reduction produces free sulfide and alkalinity, which converts toxic and dissolved heavy metals from MIW into sulfide precipitates and neutralizes acidity. The pathways through which lignocellulose is made available as an organic carbon source for SRB are poorly understood. In this project, we aim to characterize lignocellulosic degradation throughout SRBRs packed with spent brewing grains (SBG) and sugarcane bagasse (SCB) to gain valuable knowledge of microbial processes for optimization of MIW treatment.

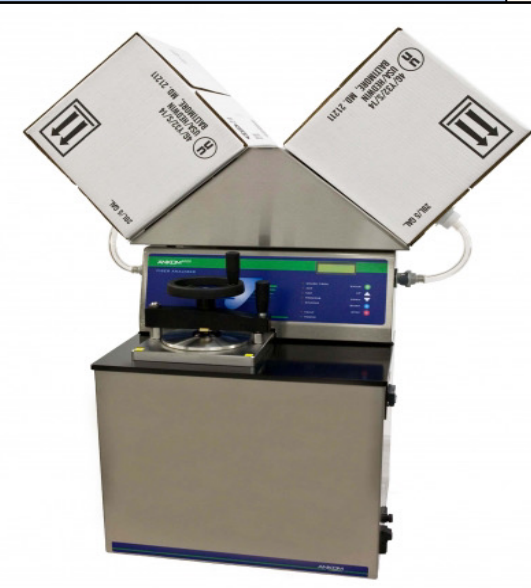
### Methodology



SRBRs - SBG & SCB

SRBRs were packed with 30% limestone and 70% SBG or SCB, treating MIW for 135 days. To quantify how lignocellulose (hemicellulose, cellulose, & lignin) changed throughout the SRBRs, an Ankom fiber analyzer was used:

- 1) *Neutral Detergent Fiber (NDF)* → cellulose, hemicellulose, and lignin remain (washes away proteins, fats, and starches)
- 2) *Acid Detergent Fiber (ADF)* → cellulose and lignin remain (washes away hemicellulose)  
**% Hemicellulose = (NDF - ADF)**
- 3) *Acid Detergent Lignin (ADL)* → Lignin remains (washes away cellulose)  
**% Cellulose = (ADF - ADL)**  
**% Lignin = (ADL)**



Ankom 2000 Fiber Analyzer

By weighing the dry weight before and after each wash, the lignocellulose content is characterized.

### Results

#### SRBR Performance

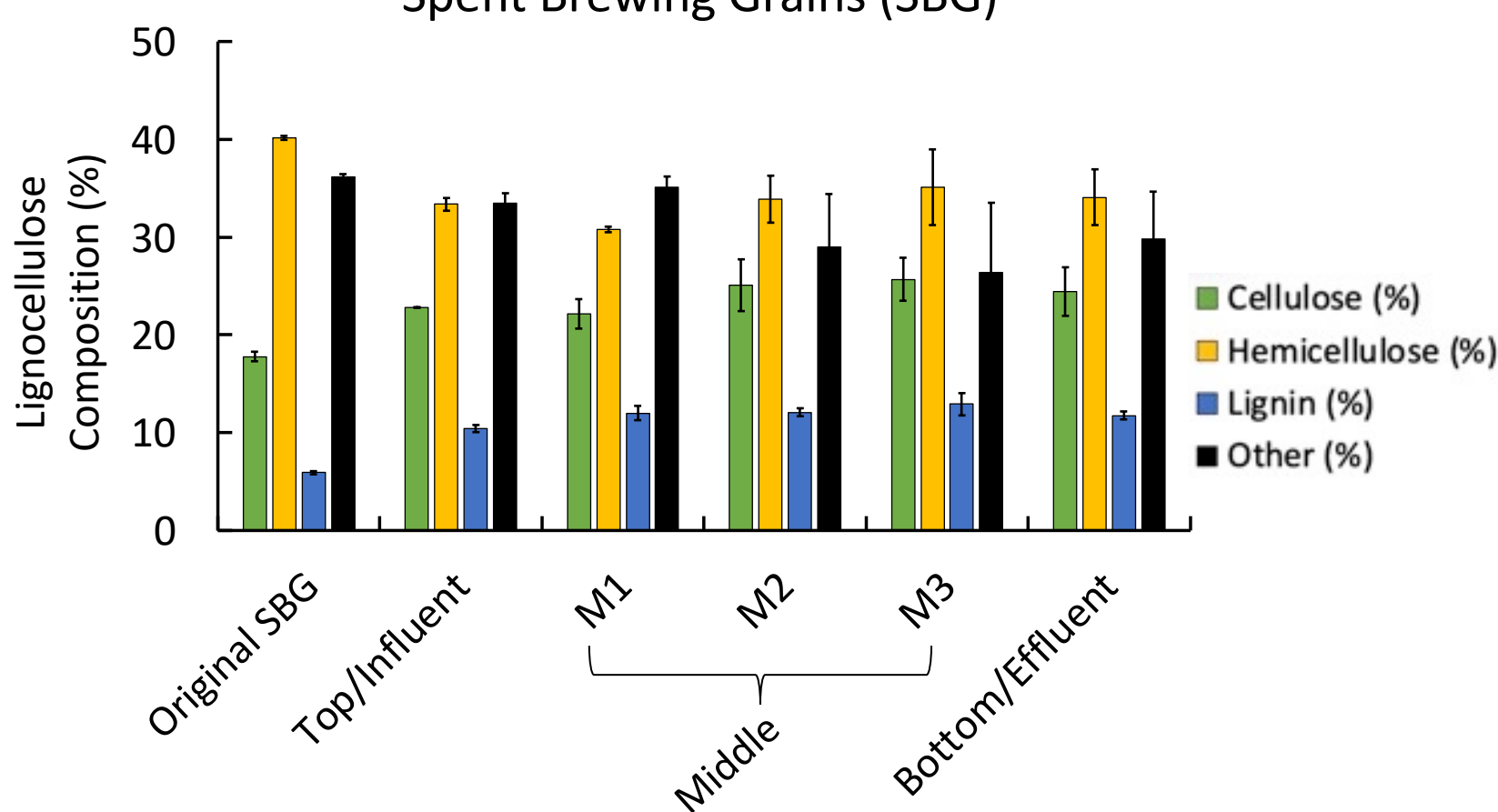
	pH (s.u.)	ORP (mV)	DO (mg/L)	Sulfate (mg/L)	Total VFAs (mM)	Total Metal(loid)s* (mg/L)	
Average MIW	2.6	520	5	500	N/A	120	--
SBG Effluent	5.9	260	1.3	41	20	1.9	Removal ~98%
SCB Effluent	6.3	100	2.5	414	BDL	5.4	Removal ~96%

- SBG → high-performing MIW treatment
- SCB → high-performing MIW treatment, except for low sulfate reduction and low VFA production

BDL = below detectable limit / VFA = volatile fatty acids / ORP = oxidation-reduction potential / DO = dissolved oxygen / \*Total metal(loid)s include Cd, Co, Cu, Fe, Ni, Pb, and Zn

### Lignocellulose Characterization

#### Spent Brewing Grains (SBG)

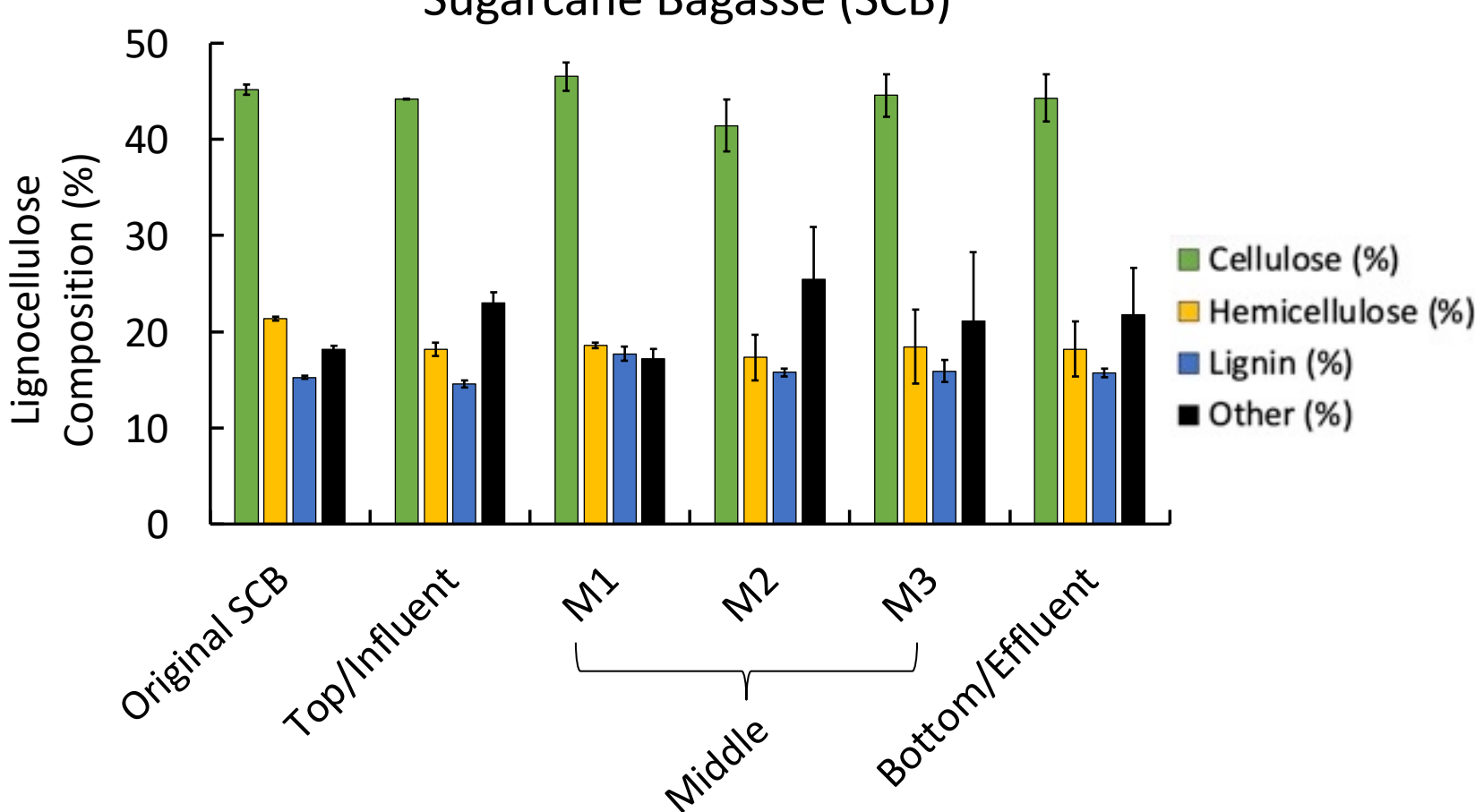


Average % Removal Compared to Original				
	Cellulose	Hemicellulose	Lignin	Other
Original SBG	17.8	40.2	5.91	36.1
Top/Influent	--	17	--	7.40
M1	--	23.5	--	2.70
M2	--	15.7	--	19.7
M3	--	12.7	--	27.0
Bottom/Effluent	--	15.3	--	17.4

(--)= no removal

★ Original SBG → Relatively high in hemicellulose

#### Sugarcane Bagasse (SCB)

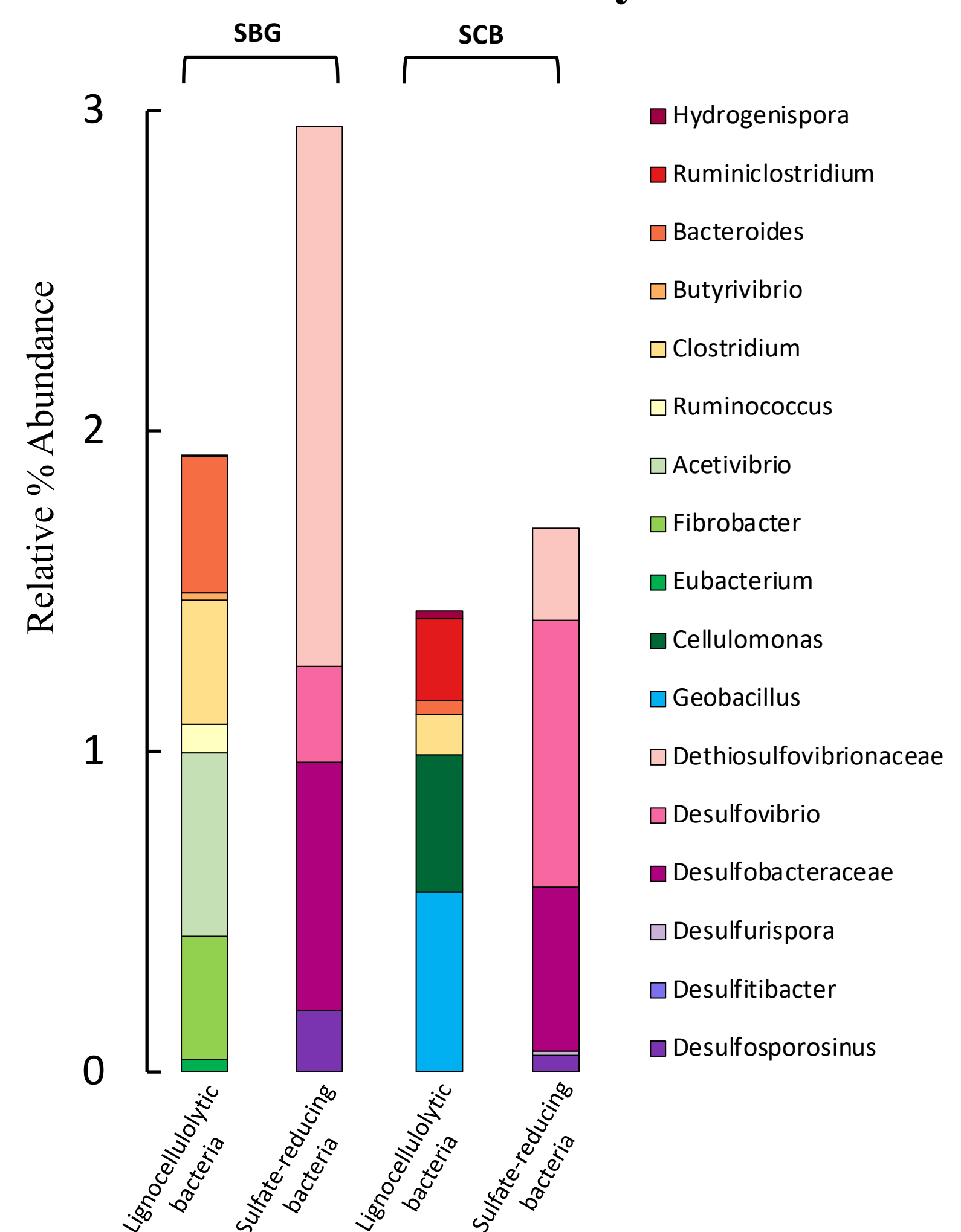


Average % Removal Compared to Original				
	Cellulose	Hemicellulose	Lignin	Other
Original SCB	45.2	21.3	15.3	18.2
Top/Influent	2.13	14.7	4.63	--
M1	--	12.9	--	5.73
M2	8.32	18.8	--	--
M3	1.38	13.6	--	--
Bottom/Effluent	1.96	14.7	--	--

(--)= no removal

★ Original SCB → Relatively high in cellulose and lignin

### Microbial Analysis



### Key Findings

- ❖ Metal(loid) removal was largely unaffected by lignocellulosic composition under project conditions.
- ❖ Fermentation products (e.g., VFAs) likely came from the biodegradation of hemicellulose and/or other category (i.e., starches, sugars, proteins, fats).
- ❖ Lignocellulose composition largely affects the composition of the lignocellulolytic bacteria.
- ❖ The substrate rich in hemicellulose (SBG) promoted a higher sulfate reduction in this project.

### Future Work

Analyze lignocellulose composition of SRBRs operating in the field at mining sites for many years to understand SRBR longevity and effective MIW treatment.

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