Characterization of Microbial Life in Different Stages of Water Treatment at Sanford Underground Research Facility

Molly Devlin[1] and Dave Bergman[2]

1.SUNY College of Environmental Science and Forestry, Syracuse NY 2. Black Hills State University, Spearfish SD

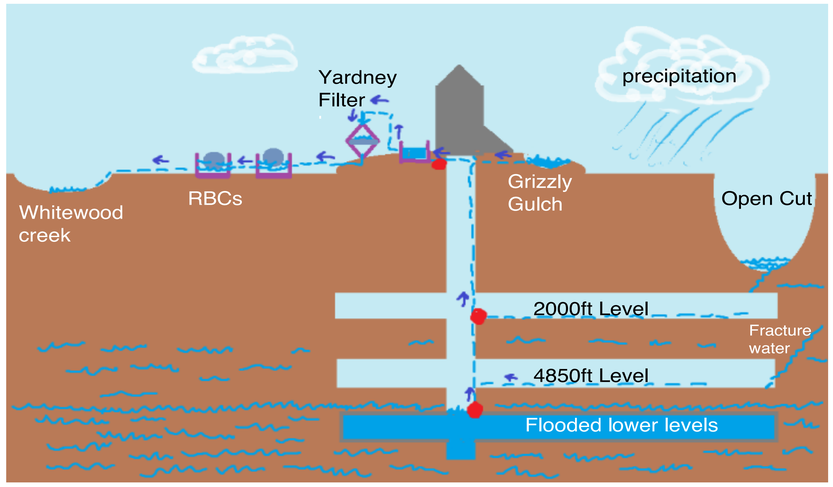

Figure 1. Water flow in SURF Water Treatment Facility

Introduction:

The Sanford Underground Research Facility (SURF) is located in the former Homestake gold mine. Water must be constantly pumped out of SURF to keep from flooding labs and equipment built below the natural water table. This water is eventually discharged into Gold Run Creek, which joins Whitewood Creek. During the early days of the Homestake gold mine, this water, toxic due to the high levels of, ammonium cyanide, and precipitated iron, was released untreated, resulting in severe pollution of the Whitewood Creek drainage. A water treatment plant using Yardney filters and Rotating Biological Contactors (RBCs) was constructed to remove these contaminants from the water before being it is released into the Gold Run Creek. The Yardney filters[1] have anthracite coal and sand to remove the precipitated iron. The RBCs have rotating disks that expose SURF water to ammonia-oxidizing biofilms and air. During the last years of gold mining at Homestake, cyanide was also removed by the bacterium *Pseudomonas paucimobilis* on the RBCs. Gold Run and Whitewood Creeks now have excellent water quality and is now a state record winning trout fishery.[2] In this study, we examine how the microbial flora of SURF wastewater changes as it moves through the different stages of wastewater collection and treatment.

Figure 2. Gallionella taken from a biofilm in the 2000ft level of SURF

References:

1. "Sand Media Filters Automatic Backwash System." *Https://Www.yardneyfilters.com*, Yardley Water Filtration Systems, Inc, 2018, www.yardneyfilters.com/sand_media_filters.aspx.

2. Whitlock, James. (1990). Biological detoxification of precious metal processing wastewaters. Geomicrobiology Journal - GEOMICROBIOL J. 8. 241-249. 10.1080/01490459009377896.

3. Parada, A., Needham, D.M., and Furman, J.A.2015. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series, and global field samples. Environ. Microbiol. doi:10.1111/1462-2920.13023

Materials and methods:

Sampling:

About 20 L of sump water from the 2000 foot level of SURF was filtered using a MasterFlex E/S portable sampler peristaltic pump with sterile silicon tubing and a 0.2μm filter. 9.0 L of water from Grizzly Gulch tailings pond was filtered using the same equipment. In the lab the filters were placed in the -80˚C freezer. Water from the Yardney filter backwash, which contained biofilms from the surface of the Yardney filters, was collected in two 500 mL bottles. The Yardney filter back wash was centrifuged at 10,000 rpm for 30 minutes and supernatant was decanted. The resulting pellet was stored at -20˚C. Biofilms were collected with sterile spatulas from two rotating biological contactors (RBCs) in the water treatment facility for SURF, and briefly stored in 15 mL Falcon tubes on ice. The first RBC was located near the outflow of the Yardney filters, while the second was near the discharge of RBC-treated wastewater into Gold Run creek. The RBC biofilms and filter were placed in the -80˚C freezer.

DNA extraction:

The DNA from the RBC biofilms and the Yardney filter backwash was extracted with the Qiagen DNeasy PowerLyzer Power Soil Kit. Filters containing microbes from the SURF 2000 foot level sump water and Grizzly Gulch water were opened with a sterilized pipe cutter and the filter membrane was removed. DNA from the filters was extracted with the Qiagen DNeasy Power Water Kit and quantified with Nanodrop Spectrophotometer. Purification was done using a Zymo Research Genomic Clean and Concentration kit.

16S rDNA Library Preparation:

DNA was diluted to 5ng/μL in PCR grade H_20. PCR was performed to amplify the V4 to V5 regions of the 16S ribosomal RNA genes using primers 515f and 926r (Parada et al. 2015), and indices attached to 16s rDNA amplicon library using a Illumina Nextera kit. Concentration of the 16S rDNA libraries were assessed with a Qubit fluorometer. DNA sequencing was performed using an Illumina MiSeq System. Sequencing output was analyzed with the CLC Bio Microbial Genomics software from Qiagen.

Results:

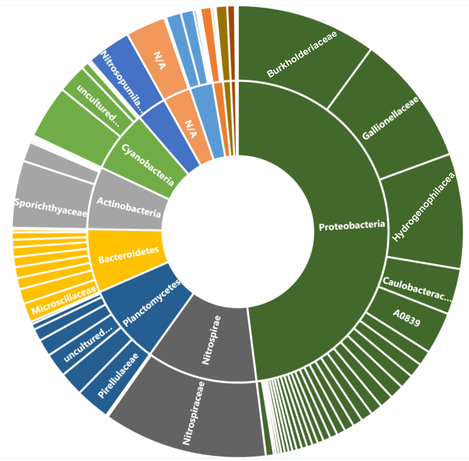

Figure 3. Overall Microbial diversity

Alpha and Beta Diversity of Samples

Chao-1 alpha diversity shows high levels of diversity in the First RBC, Filter Backwash and Last RBC (346, 394 and 374 OTUs respectively), and low level of diversity in the 2000 foot level and Grizzly Gulch (156 and 131 OTUs respectively).

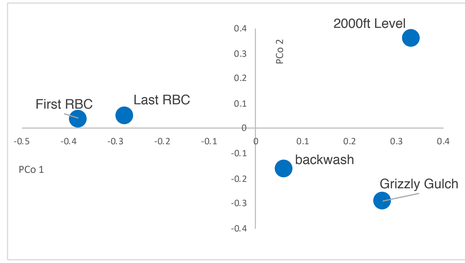

Figure 4. Relatedness of Samples

Principal Coordinate analysis (PCo) illustrates the relatedness of samples based on their abundance of different taxa (Figure 2). The PCo1 axis accounts for 56% variation and the PCo2 accounts for 34% variation.

Functional Categorization of Microbes in SURF Samples

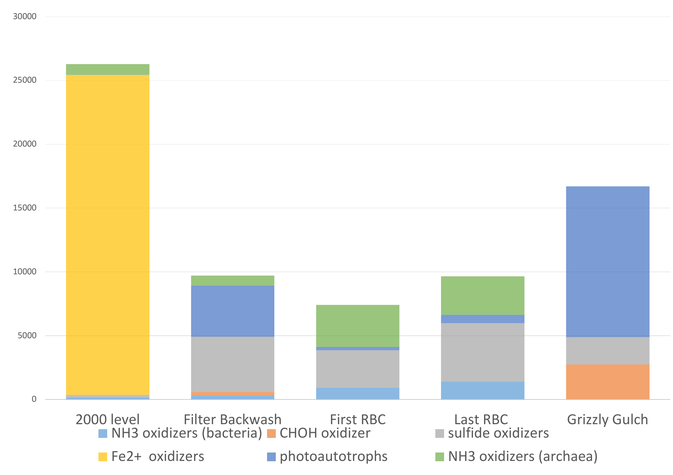

Figure 5. Functional Categorization of Microbes in SURF Samples

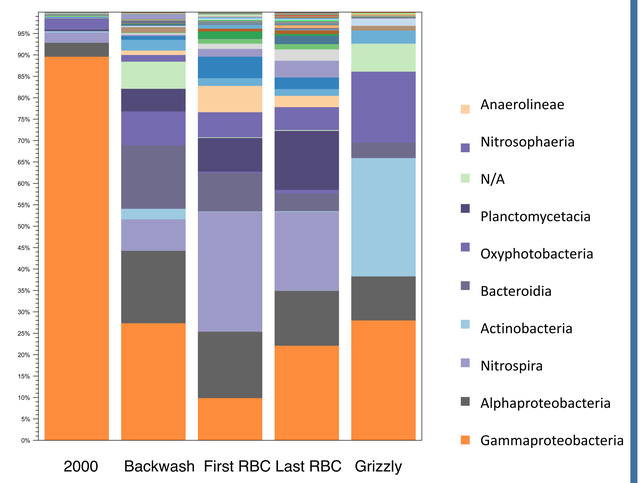

Figure 6. Microbial Diversity by Class

The majority of Fe^{2+} oxidizers are in the 2000 foot level, which correlates with the high levels of Ferrous Iron (Fe^{2+}) present (Figure 4). These are mostly Gammaproteobacteria, mostly in the genera *Sideroxydans* or *Gallionella*, which oxidize ferrous iron for energy ($Fe^{2+} + O_2 \rightarrow Fe^{3+}$).

Most of the photoautotrophs are in Grizzly Gulch and the Backwash. The photoautotroph sequences are from the chloroplasts of eukaryotic algae, which are common in bodies of water on the surface as they make energy from oxygenic photosynthesis.

Sulfide oxidizers are present in all samples, except for the 2000 level. The sulfide oxidizers are mostly Betaproteobacteria of the genus *Thiobcillus*.

There is a high level of ammonia (NH_3) oxidizing archaea and bacteria in both RBCs that will convert ammonia to nitrite ($NH_3 + O_2 \rightarrow NO_2^-$). The archaea are mostly *Nitrosospheria* and the bacteria are mostly Gamaproteobacteria from the family Nitrosomonadaceae. Other microbes, mainly Nitrospirae, oxidize nitrite to nitrate ($NO_2^- + O_2 \rightarrow NO_3^-$)

Conclusion:

- Chemoautrotrophic iron-oxidizing bacteria dominate the microbial flora of the sump water on the 2000 ft. level, but are not seen in wastewater from the surface in SURF. Perhaps this is due to their requirements for dissolved ferrous iron and low oxygen levels.
-There was a surprisingly high amount of Sporichthyaceae, a family of Actinobacteria, in Grizzly Gulch pond water. Sporichthyaceae are chemoheterotrophic and have been reported in soils.
- *Thiobacillus*, a sulfide oxidizer, was found in Grizzly Gulch water, both RBCs, and the Yardney filters. The water in the area has sulfate in it, which may be converted to sulfide by sulfate-respiring microbes in localized anaerobic environments in sediments or biofilms. The sulfide can then be oxidized by *Thiobacillus*.
-The amount of ammonia- and nitrite-oxidizing microbes was the same in the first and last RBCs. This was unexpected because, although some ammonia is present in SURF wastewater prior to RBC treatment, none is present in the final effluent discharged into Gold Run Creek. It is possible that some ammonia in the last RBC is produced within biofilms from deamination of organic matter, and it is all oxidized by microbes prior to being discharged from the wastewater plant.

Acknowledgments:

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