Isolation and Characterization of Oak Ridge (FRC) Ground Water

Husna Yasini[1], Angelica Pattenato[2], Romy Chakarborty[2]

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1. Chabot Community College 2. Lawrence Berkeley National Laboratory

Abstract

In the 1940's Oak Ridge, Tennessee transformed from farmland to atomic testing ground. Currently, field research takes place at a series of contaminated and uncontaminated sites at the US Department of Energy's Oak Ridge Field Research Center. This study investigates microbes from groundwater that contains high plumes of uranium, technetium, nitrate, volatile compounds and has a pH ranging from 3-10. By isolating bacteria and using High-Throughput Isolation (HTI) techniques clonal isolates can efficiently and rapidly be characterized. Furthermore, select strains will be characterized to better understand how the groundwater microbes adapt to such contrasting geochemical gradients. A range of carbons are tested to see whether they can oxidize to CO_2 or ferment. Several groundwater samples including FW602, FW215, FW012, FW106, and DP16D were isolated and identified with the 16rRNA found to be composed of several species of Pseudomonas and Bascillus. Research is ongoing, and multiple isolation techniques are used in hopes of obtaining a diverse collection of isolates as a result.

Groundwater samples were tested for the ability to oxidize Humic. We are looking to find microbes than can use Humic substances as an electron donor for the anaerobic oxidation of organic compounds, by doing an iron assay. The finding that microorganisms can oxidize Humics has important implications for the mechanisms by which the microorganism oxidize organics in contaminated groundwater, and suggests that microbes have adapted to organics-contaminated groundwater.

Oak Ridge Field Research Center

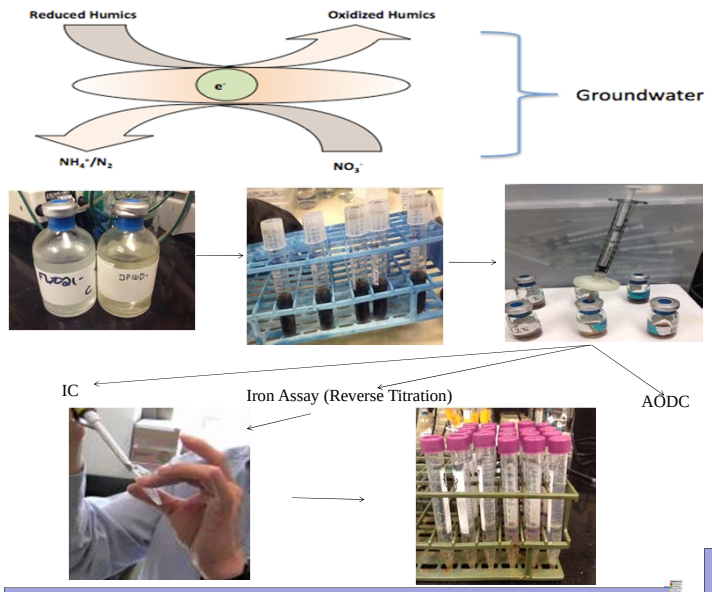
ORFC includes contaminated and uncontaminated area from which research can be conducted.

Contaminants include uranium, technetium-99, nitrate, thorium, and volatile organic compounds (ex=acetone, methylene chloride), and extreme pH.

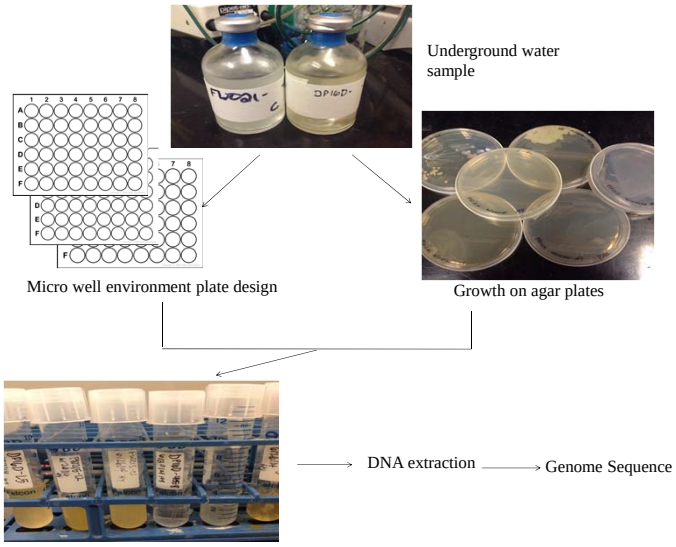
Groundwater samples from Oak Ridge (FRC)

Well ID	Temp. (°C)	DO (mg/L)	Redox (mV)	pH	Ferrous Iron (mg/L)	Sulfide (ppm)
FW106	19.64	0.03	424.00	3.55	1.38	0.013
FW215	17.44	0.22	-6.00	6.59	1.1	0.8
FW602	17.09	1.28	130.00	6.29	NA	NA
FW126	22.35	0.08	234.00	2.87	2.18	0.583
FW126	19.45	0.02	197.00	2.97	0.89	0.291
FW021	20.09	0.24	261.00	3.64	0.78	0.009
DP16D	19.42	0.17	14.00	5.54	1.68	0

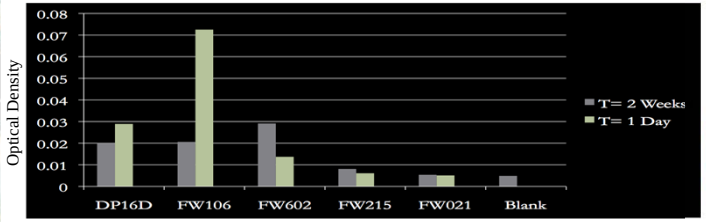
1. Investigating Humics oxidation



2. Isolation scheme for obtaining diverse strains



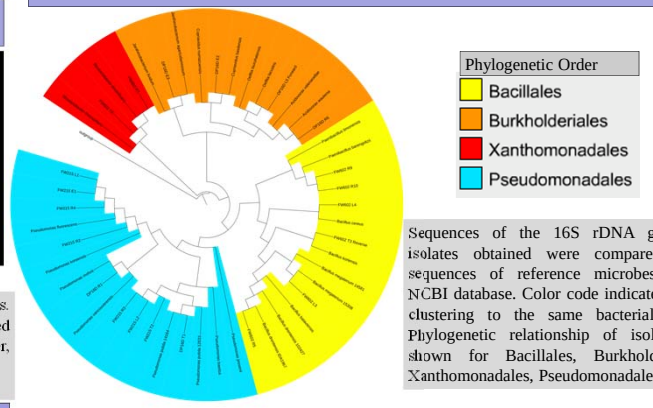
Humics Oxidation Results



Groundwater samples from Oak Ridge (FRC)

The figure above illustrates the trend of Humics oxidation in our underground water samples. Samples FW602, FW215, and FW021 show that the longer the inoculated Humic was incubated the more Humics is oxidized, therefore, more electrons are released to reduce Fe charge. However, samples DP16D and FW106 show otherwise, which could be as a result of systematical error.

Phylogenetic Tree



Sequences of the 16S rDNA gene of isolates obtained were compared with sequences of reference microbes using NCBI database. Color code indicate clades clustering to the same bacterial order. Phylogenetic relationship of isolates is shown for Bacillales, Burkholderiales, Xanthomonadales, Pseudomonadales.

Conclusion

Microbes were successfully isolated from the groundwater samples and found to be of the orders Bacillales, Burkholderiales, Xanthomondales, and Pseudomonadales. Groundwater samples were also tested for the ability to utilize Humic substances as electron donors for anaerobic respiration by doing an iron assay. The ability of the groundwater samples to oxidize Humic further validates that the microbes have adapted to organics- contaminated groundwater, or that they have learned to make use of what carbon sources are available to them in their environment.

Future Projects

- The microbial isolates and functional genes of metabolic pathways will be further investigated with an emphasis on Pseudomonas spp., to better understand denitrification in these isolates.
- The isolates will also be tested for the role of metals, including Fe, Co, and Mo, as limiting factors in nitrate reduction, in both monoculture and coculture with other isolates [1].
- A range of carbons will also be tested to see whether they can oxidize to CO_2 or ferment.

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Contact Information

Husna Yasini, Chabot Community College

Email: husna94_yasini@yahoo.com

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