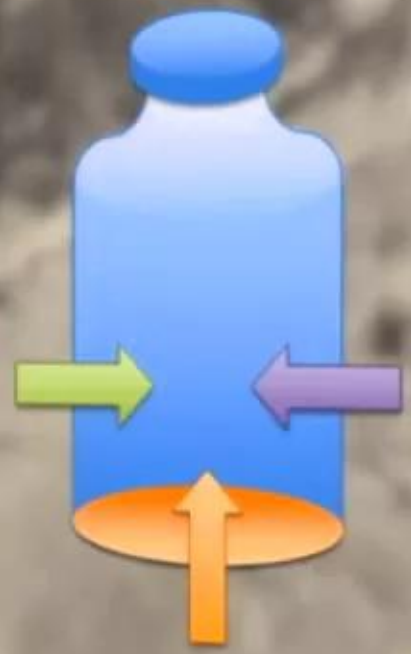
In vitro Fe(III) reduction assays

Electron donor limitation?

Potential sulfide-driven Fe(III) reduction?



+/- e^- donor (0.5 mM final concentration of acetate + lactate)

+/- molybdate (inhibitor of bacterial sulfate reduction)

Natural CP oxides and native microbial community

1
00:00:13,029 --> 00:00:10,480
okay cool so yeah today I will be

2
00:00:15,520 --> 00:00:13,039
telling you about in vitro and in situ

3
00:00:20,230 --> 00:00:15,530
iron geochemistry and microbial activity

4
00:00:22,750 --> 00:00:20,240
in chocolate pots hot spring to start

5
00:00:25,509 --> 00:00:22,760
with I'll show you all where my field

6
00:00:26,980 --> 00:00:25,519
site is a yellow stone if you have not

7
00:00:30,429 --> 00:00:26,990
been there first of all I recommend you

8
00:00:32,530 --> 00:00:30,439
go there but Yellowstone is in the

9
00:00:35,500 --> 00:00:32,540
northwest corner of the state of Wyoming

10
00:00:37,750 --> 00:00:35,510
and my field site is just below the

11
00:00:40,420 --> 00:00:37,760
norris geyser basin about five

12
00:00:42,580 --> 00:00:40,430
kilometers south along the given river

13
00:00:46,600 --> 00:00:42,590

and on the northern edge of Yellowstone

14
00:00:50,380 --> 00:00:46,610
Caldera here we have a face on view of

15
00:00:53,139 --> 00:00:50,390
my hot spring indicated in blue is the

16
00:00:54,729 --> 00:00:53,149
flow path of the main hot spring there

17
00:00:59,020 --> 00:00:54,739
are two satellite vents but I did not

18
00:01:00,849 --> 00:00:59,030
study those in this line of research and

19
00:01:03,639 --> 00:01:00,859
these two arrows indicate the sample

20
00:01:05,590 --> 00:01:03,649
sites from my Master's research one at

21
00:01:09,219 --> 00:01:05,600
the hot spring vent one about midway

22
00:01:10,809 --> 00:01:09,229
down the flow path and this is a little

23
00:01:12,460 --> 00:01:10,819
different from some of the other hot

24
00:01:14,620 --> 00:01:12,470
springs in Yellowstone perhaps some of

25
00:01:17,559 --> 00:01:14,630
the more famous ones this is a circum-

26
00:01:19,210 --> 00:01:17,569
tral ph hot spring and i like to say

27
00:01:22,080 --> 00:01:19,220
it's more of a warm spring because the

28
00:01:24,520 --> 00:01:22,090
event is about 50 degrees Celsius and

29
00:01:27,279 --> 00:01:24,530
cools off a little bit midway down the

30
00:01:30,639 --> 00:01:27,289
flow path and like I said circum neutral

31
00:01:34,990 --> 00:01:30,649
very slightly acidic at the event this

32
00:01:36,729 --> 00:01:35,000
is a result of hydrothermal acid

33
00:01:38,740 --> 00:01:36,739
sulphate groundwater is mixing with

34
00:01:43,150 --> 00:01:38,750
Lucille ground waters and you get the

35
00:01:44,800 --> 00:01:43,160
slightly acidic vent water and up in the

36
00:01:46,870 --> 00:01:44,810
corner just some of the properties of

37
00:01:49,749 --> 00:01:46,880
the water there's a lot of ferrous iron

38
00:01:53,080 --> 00:01:49,759

in the swatter near saturation with

39
00:01:55,990 --> 00:01:53,090
silica and there's also an appreciable

40
00:01:59,410 --> 00:01:56,000
amount of sulphate and this will come up

41
00:02:01,570 --> 00:01:59,420
a little bit later so just go over

42
00:02:04,869 --> 00:02:01,580
briefly the initial study that I did for

43
00:02:08,589 --> 00:02:04,879
my masters so this was more of a culture

44
00:02:10,719 --> 00:02:08,599
based study on the main goal was to

45
00:02:14,080 --> 00:02:10,729
determine the potential for iron

46
00:02:16,900 --> 00:02:14,090
reducing organisms in materials taken

47
00:02:19,210 --> 00:02:16,910
from these two sample sites the first

48
00:02:22,240 --> 00:02:19,220
thing I did was a most probable number

49
00:02:26,410 --> 00:02:22,250
enumeration and this is a technique to

50
00:02:31,630 --> 00:02:26,420
get an estimated number of the organisms

51
00:02:33,100 --> 00:02:31,640
in in these communities and following

52
00:02:37,660 --> 00:02:33,110
that I did a series of enrichment

53
00:02:39,940 --> 00:02:37,670
cultures and in these these cultures

54
00:02:43,330 --> 00:02:39,950
they were able to sustain their level of

55
00:02:48,520 --> 00:02:43,340
activity for more than 30 transfers well

56
00:02:52,030 --> 00:02:48,530
over 400 days and I also did some 16s

57
00:02:55,150 --> 00:02:52,040
and metagenomic microbial community

58
00:02:57,699 --> 00:02:55,160
analyses and this identified well known

59
00:03:01,980 --> 00:02:57,709
and potentially novel dissimilatory iron

60
00:03:06,280 --> 00:03:01,990
reducing organisms so here we have the

61
00:03:08,190 --> 00:03:06,290
activity for the cultures for like I

62
00:03:10,720 --> 00:03:08,200
said well over 3d transfers well over

63
00:03:14,110 --> 00:03:10,730

400 days they just passed their

64
00:03:15,730 --> 00:03:14,120
thousandth day of existence little while

65
00:03:18,370 --> 00:03:15,740
ago so I'm really excited about that

66
00:03:20,380 --> 00:03:18,380
that I've kept them alive and happy for

67
00:03:22,330 --> 00:03:20,390
this long and like I said there's this

68
00:03:25,990 --> 00:03:22,340
sustained level of activity call it

69
00:03:30,400 --> 00:03:26,000
about 30 millimoles per liter of ferrous

70
00:03:31,930 --> 00:03:30,410
iron being produced and if you're not

71
00:03:36,960 --> 00:03:31,940
familiar with metagenomics don't be

72
00:03:40,300 --> 00:03:36,970
scared by this this is the one of the

73
00:03:43,060 --> 00:03:40,310
meta-genome accrue salts from my vent

74
00:03:45,340 --> 00:03:43,070
community this picture here each one of

75
00:03:47,560 --> 00:03:45,350
the colors represents a different

76
00:03:49,360 --> 00:03:47,570
organism and the shapes don't really

77
00:03:51,430 --> 00:03:49,370
mean anything but they all clustered

78
00:03:54,670 --> 00:03:51,440
together each of those dots clustering

79
00:03:59,440 --> 00:03:54,680
together those are all part of all the

80
00:04:01,509 --> 00:03:59,450
DNA coming from one organism so this is

81
00:04:08,289 --> 00:04:01,519
perhaps more intuitive way of looking at

82
00:04:11,199 --> 00:04:08,299
it these correspond it's a way of

83
00:04:14,580 --> 00:04:11,209
teasing apart the number of organisms

84
00:04:18,699 --> 00:04:14,590
that you have in your meta-genome and

85
00:04:21,099 --> 00:04:18,709
from the meta-genome identified eight

86
00:04:24,130 --> 00:04:21,109
taxonomic groups call it eight different

87
00:04:27,760 --> 00:04:24,140
organisms that are dominating this

88
00:04:30,100 --> 00:04:27,770

community four of these I was able to

89
00:04:31,779 --> 00:04:30,110
recover nearly complete reconstruct

90
00:04:32,559 --> 00:04:31,789
genome so I have almost the entire

91
00:04:35,409 --> 00:04:32,569
complement

92
00:04:38,589 --> 00:04:35,419
DNA from those organisms and for four of

93
00:04:41,260 --> 00:04:38,599
those I had hits for the PCC porin what

94
00:04:43,689 --> 00:04:41,270
is this PCC porin PCC stands for poor

95
00:04:46,299 --> 00:04:43,699
and cytochrome complex and this has

96
00:04:51,309 --> 00:04:46,309
recently been identified by liang sheet

97
00:04:54,489 --> 00:04:51,319
from pnnl as a gene complex that has

98
00:04:56,290 --> 00:04:54,499
been found in all geobacter species

99
00:04:58,059 --> 00:04:56,300
which are well-known iron reducers and

100
00:05:01,629 --> 00:04:58,069
also in some other species one in

101
00:05:03,879 --> 00:05:01,639
particular ignazio a curium album if you

102
00:05:05,619 --> 00:05:03,889
haven't heard of it that's okay but what

103
00:05:07,799 --> 00:05:05,629
is cool about that organism while it has

104
00:05:10,929 --> 00:05:07,809
not been implicated as an iron reducer

105
00:05:13,989 --> 00:05:10,939
this guy here miele re back to roseus is

106
00:05:16,959 --> 00:05:13,999
a relative of it a cousin of this

107
00:05:19,689 --> 00:05:16,969
Ignacio acti reham and I identified this

108
00:05:22,629 --> 00:05:19,699
organism in my enrichment cultures and

109
00:05:24,339 --> 00:05:22,639
it also has this porn cytochrome complex

110
00:05:26,709 --> 00:05:24,349
which is exciting because it has some

111
00:05:30,159 --> 00:05:26,719
implications that it's involved in the

112
00:05:33,279 --> 00:05:30,169
iron reduction cycle moving on to the

113
00:05:36,790 --> 00:05:33,289

current study so the main goal here is

114
00:05:39,009 --> 00:05:36,800
to look at the Institute community my

115
00:05:40,570 --> 00:05:39,019
previous study was looking at cultures I

116
00:05:44,909 --> 00:05:40,580
want to know what's actually going on at

117
00:05:47,679 --> 00:05:44,919
the hot spring so this is looking at the

118
00:05:50,230 --> 00:05:47,689
using the endogenous electron donor pool

119
00:05:53,799 --> 00:05:50,240
and again determining that iron

120
00:05:56,769 --> 00:05:53,809
reduction potential and once again

121
00:06:01,420 --> 00:05:56,779
characterize the microbial community so

122
00:06:03,429 --> 00:06:01,430
far looking only at 16s sequencing again

123
00:06:06,219 --> 00:06:03,439
we have hot spring here is the flow path

124
00:06:09,159 --> 00:06:06,229
again and these symbols in red are spots

125
00:06:13,269 --> 00:06:09,169
that we took core samples this was from

126
00:06:17,290 --> 00:06:13,279
summer two summers ago six spots along

127
00:06:19,779 --> 00:06:17,300
the flow path here we have some pretty

128
00:06:22,989 --> 00:06:19,789
field shots here's the flow path from

129
00:06:24,999 --> 00:06:22,999
the top one of the core samples we

130
00:06:29,079 --> 00:06:25,009
collected and then this is the pool that

131
00:06:31,149 --> 00:06:29,089
forms at the top of hot spring so

132
00:06:41,219 --> 00:06:31,159
there's a couple different treatments

133
00:06:43,179 --> 00:06:41,229
for this experiment there were I added

134
00:06:45,639 --> 00:06:43,189
there were treatments with and without

135
00:06:45,940 --> 00:06:45,649
additional electron donor and treatments

136
00:06:50,170 --> 00:06:45,950
with

137
00:06:52,990 --> 00:06:50,180
molybdate edition was to knock out any

138
00:06:56,680 --> 00:06:53,000

sulfate-reducing bacteria which might be

139
00:06:58,660 --> 00:06:56,690
contributing to the amount of iron being

140
00:07:00,610 --> 00:06:58,670
reduced from sulfate producers

141
00:07:04,930 --> 00:07:00,620
by-product sulfide which can chemically

142
00:07:07,330 --> 00:07:04,940
react with iron here's the results from

143
00:07:10,810 --> 00:07:07,340
that set so this is site 1 that means

144
00:07:13,120 --> 00:07:10,820
the hot spring vent site 3 is the third

145
00:07:17,860 --> 00:07:13,130
core site which is about 2 meters away

146
00:07:20,920 --> 00:07:17,870
and the main takeaway from these these

147
00:07:22,300 --> 00:07:20,930
figures is within without additional

148
00:07:23,980 --> 00:07:22,310
electron donor and with and without

149
00:07:26,770 --> 00:07:23,990
molybdate there's not a whole lot of

150
00:07:29,770 --> 00:07:26,780
difference at site 1 however you move

151
00:07:31,870 --> 00:07:29,780
just the short distance way the purple

152
00:07:33,580 --> 00:07:31,880
and green symbols are the ones that were

153
00:07:37,420 --> 00:07:33,590
amended with additional electron donor

154
00:07:41,020 --> 00:07:37,430
and there's a much increased level of

155
00:07:42,970 --> 00:07:41,030
activity but again there is not much

156
00:07:45,310 --> 00:07:42,980
difference but with with and without

157
00:07:48,340 --> 00:07:45,320
molybdate so the takeaway from that is

158
00:07:52,720 --> 00:07:48,350
there's not a significant influence from

159
00:07:54,730 --> 00:07:52,730
sulfate reducers this is another measure

160
00:07:58,570 --> 00:07:54,740
of microbial activity this is the

161
00:08:00,220 --> 00:07:58,580
measure of fe 2 over fe total the more

162
00:08:02,590 --> 00:08:00,230
fe 2 that's produced the more active

163
00:08:06,120 --> 00:08:02,600

they are so once again you have a high

164
00:08:08,170 --> 00:08:06,130
level have activity at the vent

165
00:08:12,400 --> 00:08:08,180
diminished you when you move a little

166
00:08:16,600 --> 00:08:12,410
bit away and at site five there's almost

167
00:08:19,420 --> 00:08:16,610
no activity so what does this mean we're

168
00:08:21,310 --> 00:08:19,430
still interested in where the electrons

169
00:08:23,470 --> 00:08:21,320
excuse me where the carbon source is

170
00:08:25,870 --> 00:08:23,480
coming from this is by no means a closed

171
00:08:28,420 --> 00:08:25,880
system there is plenty of influx of

172
00:08:30,760 --> 00:08:28,430
other plant and animal debris flowing

173
00:08:34,360 --> 00:08:30,770
into the system but you see this

174
00:08:37,660 --> 00:08:34,370
decrease in activity and my thought is

175
00:08:40,330 --> 00:08:37,670
if the only source of carbon is this

176
00:08:43,630 --> 00:08:40,340
exogenous material coming in you would

177
00:08:46,930 --> 00:08:43,640
see a more similar level of yes in

178
00:08:51,220 --> 00:08:46,940
similar level of activity throughout hot

179
00:08:53,760 --> 00:08:51,230
spring but you don't see that so to me

180
00:08:56,380 --> 00:08:53,770
this would suggest that there is a

181
00:08:58,860 --> 00:08:56,390
source of carbon coming from the hot

182
00:09:00,750 --> 00:08:58,870
spring vent and to support this

183
00:09:05,600 --> 00:09:00,760
from the core samples that were

184
00:09:10,640 --> 00:09:08,040
relatives of sitter or accidents which

185
00:09:14,670 --> 00:09:10,650
is an autotrophic iron oxidizer so

186
00:09:18,210 --> 00:09:14,680
there's good indication that this carbon

187
00:09:21,360 --> 00:09:18,220
might be being produced in situ so the

188
00:09:23,120 --> 00:09:21,370

vent is the hot spot of activity so

189
00:09:26,340 --> 00:09:23,130
comparing these different communities

190
00:09:28,470 --> 00:09:26,350
this is a principal components analysis

191
00:09:30,390 --> 00:09:28,480
plot each one of these symbols

192
00:09:32,940 --> 00:09:30,400
represents an entire microbial community

193
00:09:34,890 --> 00:09:32,950
and this is a way of very quickly

194
00:09:37,829 --> 00:09:34,900
looking at how related all of these

195
00:09:39,960 --> 00:09:37,839
communities are so in blue we have

196
00:09:45,240 --> 00:09:39,970
everything from site 1 again this is the

197
00:09:50,700 --> 00:09:45,250
hot spring vent this is the t0 of my in

198
00:09:53,430 --> 00:09:50,710
vitro reduction assay this is day 20 all

199
00:09:55,500 --> 00:09:53,440
of those treatments cluster very very

200
00:09:57,300 --> 00:09:55,510
closely together this would mean that

201
00:10:00,360 --> 00:09:57,310
there is not much difference between the

202
00:10:02,700 --> 00:10:00,370
microbial community and what it also

203
00:10:06,030 --> 00:10:02,710
shows is that activity was stimulated by

204
00:10:07,740 --> 00:10:06,040
the addition of electron donor but that

205
00:10:13,260 --> 00:10:07,750
the microbial community was not changed

206
00:10:14,519 --> 00:10:13,270
as a result of it and I would also like

207
00:10:17,370 --> 00:10:14,529
to point out that within these

208
00:10:19,769 --> 00:10:17,380
communities there is also that organism

209
00:10:21,930 --> 00:10:19,779
I mentioned earlier eggnog bacterium i

210
00:10:25,890 --> 00:10:21,940
did get hits for this organism in here

211
00:10:32,300 --> 00:10:25,900
so again this is all leading up to what

212
00:10:35,519 --> 00:10:32,310
is potentially the in situ iron reducer

213
00:10:37,740 --> 00:10:35,529

so my my latest line of research is a

214
00:10:41,100 --> 00:10:37,750
stable isotope probing experiment this

215
00:10:43,650 --> 00:10:41,110
is using carbon 13 labeled acetate to

216
00:10:46,530 --> 00:10:43,660
probe the microbial community and again

217
00:10:50,280 --> 00:10:46,540
look for that active those active

218
00:10:53,370 --> 00:10:50,290
organisms so this looks fairly similar

219
00:10:56,360 --> 00:10:53,380
to the results from my insta to their

220
00:10:58,530 --> 00:10:56,370
excuse me in vitro reduction assay the

221
00:11:00,810 --> 00:10:58,540
activity is stimulated by the addition

222
00:11:02,790 --> 00:11:00,820
of electron donor but in green here

223
00:11:05,130 --> 00:11:02,800
there is still an appreciable amount of

224
00:11:09,810 --> 00:11:05,140
activity without additional electron

225
00:11:11,680 --> 00:11:09,820
donor and some conclusions there's an

226
00:11:15,130 --> 00:11:11,690
active community at the vent

227
00:11:16,990 --> 00:11:15,140
um the community appears to be electron

228
00:11:20,290 --> 00:11:17,000
donor limited within a short distance of

229
00:11:23,440 --> 00:11:20,300
the vent there are organisms related to

230
00:11:25,720 --> 00:11:23,450
a dissimilatory iron reducers that I

231
00:11:28,240 --> 00:11:25,730
identified in my enrichment culture and

232
00:11:30,580 --> 00:11:28,250
I did not have time to go into the iron

233
00:11:35,620 --> 00:11:30,590
isotope fractionation but we are also

234
00:11:39,730 --> 00:11:35,630
looking at that to see if there is a bio

235
00:11:43,600 --> 00:11:39,740
signature of the microbial activity with

236
00:11:46,450 --> 00:11:43,610
distance and depth from the vent and

237
00:11:48,610 --> 00:11:46,460
that thanks to everyone who funded me

238
00:11:56,140 --> 00:11:48,620

and organized this conference and with

239
00:12:09,460 --> 00:11:56,150
that we'll take questions okay we have

240
00:12:12,820 --> 00:12:09,470
time for a few questions em okay last

241
00:12:18,630 --> 00:12:12,830
month I was in Yellowstone and I saw

242
00:12:22,810 --> 00:12:18,640
warning signs all over Hot Springs and

243
00:12:25,180 --> 00:12:22,820
how acidic they could be and why why

244
00:12:29,200 --> 00:12:25,190
they are so a city that you cannot even

245
00:12:30,970 --> 00:12:29,210
touch them uh yeah so like I said with

246
00:12:33,940 --> 00:12:30,980
my hat spring it's a little anomalous

247
00:12:35,880 --> 00:12:33,950
from the rest in Yellowstone but yeah

248
00:12:41,050 --> 00:12:35,890
the extremely acidic ones that is

249
00:12:45,630 --> 00:12:41,060
there's a lot of sulfide in the ground

250
00:12:52,470 --> 00:12:45,640
waters so I think that's probably the

251
00:12:56,230 --> 00:12:52,480
yeah yeah sulfuric acid is probably the

252
00:12:59,920 --> 00:12:56,240
most of the source of acidity and hot

253
00:13:09,370 --> 00:13:06,460
and you want else okay um I guess we