Stable carbon isotope fractionation values (in per mil, ‰) of methane produced by *M. wolfeii* exposed to different pressure and temperature

Pressure	*45°C*	*55°C*	*65°C*
1atm	-69.12	-73.04	-69.77
400 atm	-72.54	-71.20	-69.69
800 atm	-72.51	-71.37	-69.38
1200 atm	-71.87	-70.14	-68.56

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} - 1 \right) \times 1000\ ‰$$

- Stable carbon isotope fractionation of methane:
  - differentiate between biotic and abiotic methane (Sinha and Kral, 2014)

1
00:00:14,420 --> 00:00:10,299
during temperatures and pressures and pH

2
00:00:16,640 --> 00:00:14,430
good afternoon everyone as we know

3
00:00:19,340 --> 00:00:16,650
methanogens our camera Auto tropicana

4
00:00:21,620 --> 00:00:19,350
robic archaea that mostly consume carbon

5
00:00:24,110 --> 00:00:21,630
dioxide for their carbon source and

6
00:00:26,450 --> 00:00:24,120
hydrogen for their energy source and

7
00:00:30,140 --> 00:00:26,460
produce methane as an end product of

8
00:00:33,260 --> 00:00:30,150
metabolism and we have been studying

9
00:00:35,479 --> 00:00:33,270
methanogens as ideal life form for on

10
00:00:37,819 --> 00:00:35,489
Mars for a long time because they are

11
00:00:41,000 --> 00:00:37,829
anaerobe they are not photosynthetic

12
00:00:43,849 --> 00:00:41,010
they do not require organics also they

13
00:00:46,009 --> 00:00:43,859

need carbon dioxide and hydrogen and we

14
00:00:49,189 --> 00:00:46,019
know that carbon dioxide is abundant in

15
00:00:51,049 --> 00:00:49,199
Martian atmosphere also hydrogen could

16
00:00:54,200 --> 00:00:51,059
be formed by serpentine ization

17
00:00:57,049 --> 00:00:54,210
reactions interestingly we have found

18
00:00:59,180 --> 00:00:57,059
methane on Mars on earth we know that

19
00:01:01,969 --> 00:00:59,190
most of methane has a biological in

20
00:01:05,509 --> 00:01:01,979
origin therefore mechanisms are ideal

21
00:01:07,760 --> 00:01:05,519
candidates for life on Mars also we know

22
00:01:11,480 --> 00:01:07,770
that the surface condition of Mars is

23
00:01:14,630 --> 00:01:11,490
extremely hostile to life because of low

24
00:01:17,270 --> 00:01:14,640
atmospheric air surface pressure low my

25
00:01:20,870 --> 00:01:17,280
surface temperature lack of liquid water

26
00:01:23,899 --> 00:01:20,880
presence of oxidizing compound and DNA

27
00:01:26,450 --> 00:01:23,909
damaging ultraviolet radiation but the

28
00:01:28,789 --> 00:01:26,460
subsurface conditions are different they

29
00:01:31,240 --> 00:01:28,799
are protected from radiation which will

30
00:01:35,149 --> 00:01:31,250
have higher pressure was really high

31
00:01:38,149 --> 00:01:35,159
temperature and water might be present

32
00:01:40,969 --> 00:01:38,159
in liquid form on earth we know that

33
00:01:43,310 --> 00:01:40,979
where there is water there is life even

34
00:01:45,830 --> 00:01:43,320
we have found life several kilometres

35
00:01:48,770 --> 00:01:45,840
below the surface of Earth and we are

36
00:01:50,539 --> 00:01:48,780
familiar with extreme of files based on

37
00:01:52,880 --> 00:01:50,549
their extreme conditions they are

38
00:01:55,719 --> 00:01:52,890

classified into different groups such as

39
00:01:59,270 --> 00:01:55,729
thermofoil Sparrow files as ido files

40
00:02:03,080 --> 00:01:59,280
sacrifice and hallow files so similar to

41
00:02:06,700 --> 00:02:03,090
earth a subsurface biosphere could exist

42
00:02:09,770 --> 00:02:06,710
on Mars this type of information

43
00:02:12,670 --> 00:02:09,780
inspired us to know the growth and

44
00:02:14,960 --> 00:02:12,680
survivability of methanogenic archaea

45
00:02:17,830 --> 00:02:14,970
after exposing them to different

46
00:02:20,059 --> 00:02:17,840
pressure temperature and low pH

47
00:02:23,300 --> 00:02:20,069
simulating subsurface in

48
00:02:26,509 --> 00:02:23,310
or mint and we also wanted to see their

49
00:02:28,429 --> 00:02:26,519
morphology due to the exposure to

50
00:02:31,129 --> 00:02:28,439
different pressure and temperature and

51
00:02:33,860 --> 00:02:31,139
we also wanted to see the effect of

52
00:02:37,819 --> 00:02:33,870
temperature and pressure on their stable

53
00:02:40,280 --> 00:02:37,829
carbon isotope fractionation so we use

54
00:02:42,920 --> 00:02:40,290
the meta no thermal back thermo

55
00:02:46,039 --> 00:02:42,930
bacterial feel that grows optimally at

56
00:02:48,500 --> 00:02:46,049
55 degrees centigrade they are obligate

57
00:02:51,770 --> 00:02:48,510
anaerobes and they use hydrogen and

58
00:02:54,949 --> 00:02:51,780
carbon dioxide and produce methane we

59
00:02:57,709 --> 00:02:54,959
prepare three different sets set one was

60
00:03:00,649 --> 00:02:57,719
varying temperature and we made growth

61
00:03:03,280 --> 00:03:00,659
media in bicarbonate buffer methanogens

62
00:03:06,250 --> 00:03:03,290
were inoculated in these media and

63
00:03:10,729 --> 00:03:06,260

incubated at four different temperatures

64
00:03:14,059 --> 00:03:10,739
45 55 65 and 75 degree centigrade at

65
00:03:17,110 --> 00:03:14,069
atmospheric pressure and we analyzed

66
00:03:20,209 --> 00:03:17,120
headspace gaseous sample Rio de clé

67
00:03:23,270 --> 00:03:20,219
second set was varying pH in which we

68
00:03:28,839 --> 00:03:23,280
made media in bicarbonate buffer of

69
00:03:31,819 --> 00:03:28,849
different ph level 4 5 6 and 7 and again

70
00:03:34,069 --> 00:03:31,829
they were pressurized with hydrogen and

71
00:03:36,679 --> 00:03:34,079
incubated at their optimum growth

72
00:03:39,439 --> 00:03:36,689
temperature which is 55 degree

73
00:03:43,159 --> 00:03:39,449
centigrade and haida space gas sample

74
00:03:45,800 --> 00:03:43,169
were analyzed periodically third set was

75
00:03:49,580 --> 00:03:45,810
combined pressure and temperature and we

76
00:03:53,149 --> 00:03:49,590
use four different pressures one a 400

77
00:03:57,229 --> 00:03:53,159
800 and 1200 atmospheric pressure for

78
00:04:00,740 --> 00:03:57,239
temperature we used 45 55 and 65 degree

79
00:04:03,110 --> 00:04:00,750
centigrade this is the diagram of high

80
00:04:06,559 --> 00:04:03,120
hydrostatic pressure temperature chamber

81
00:04:09,469 --> 00:04:06,569
in which we used a small high-pressure

82
00:04:12,800 --> 00:04:09,479
cubed and was placed from the top of the

83
00:04:14,990 --> 00:04:12,810
chamber and the a piston was used to

84
00:04:18,439 --> 00:04:15,000
pressurize the water and the pressure

85
00:04:20,749 --> 00:04:18,449
was measured with pressure gas and to

86
00:04:24,379 --> 00:04:20,759
maintain the temperature of the system

87
00:04:26,959 --> 00:04:24,389
we use circulating water bath so this

88
00:04:30,350 --> 00:04:26,969

small high-pressure pivot was filled

89
00:04:33,230 --> 00:04:30,360
with methanogens in our anaerobic

90
00:04:35,170 --> 00:04:33,240
chamber and was placed

91
00:04:38,839 --> 00:04:35,180
hydrostatic temperature chamber

92
00:04:42,439 --> 00:04:38,849
temperature pressure chamber for 15

93
00:04:45,110 --> 00:04:42,449
hours after exposure to desired

94
00:04:47,540 --> 00:04:45,120
temperature and pressure we took images

95
00:04:50,779 --> 00:04:47,550
of some cells and some cells were

96
00:04:53,510 --> 00:04:50,789
inoculated into phrase growth media and

97
00:04:55,640 --> 00:04:53,520
they again pressurized with a hydrogen

98
00:04:58,279 --> 00:04:55,650
and incubated at optimum growth

99
00:05:01,339 --> 00:04:58,289
temperature which is 55 at one

100
00:05:05,210 --> 00:05:01,349
atmospheric pressure and headspace gas

101
00:05:07,670 --> 00:05:05,220
sample was analyzed periodical you to

102
00:05:09,920 --> 00:05:07,680
measure methane concentration we used

103
00:05:12,860 --> 00:05:09,930
gas chromatograph and to measure a

104
00:05:16,749 --> 00:05:12,870
stable carbon isotope fractionation we

105
00:05:20,570 --> 00:05:16,759
used Picaro cavity lingdon spectrometer

106
00:05:23,629 --> 00:05:20,580
now results so at all different

107
00:05:27,200 --> 00:05:23,639
temperatures even at 75 degree

108
00:05:30,170 --> 00:05:27,210
centigrade they produced methane and the

109
00:05:32,809 --> 00:05:30,180
second set was varying pH and they

110
00:05:36,499 --> 00:05:32,819
produce methane at all different ph

111
00:05:41,719 --> 00:05:36,509
level however their lag phase increases

112
00:05:43,939 --> 00:05:41,729
with the decrease in the ph level this

113
00:05:46,520 --> 00:05:43,949

is the graph of a combined pressure and

114
00:05:49,520 --> 00:05:46,530
temperature experiment the top graph

115
00:05:52,850 --> 00:05:49,530
represents when they were at 45 degree

116
00:05:55,129 --> 00:05:52,860
centigrade at different places and the

117
00:05:57,589 --> 00:05:55,139
left side of the graph represents when

118
00:06:00,499 --> 00:05:57,599
they were at 55 degree centigrade at

119
00:06:02,959 --> 00:06:00,509
different places and the graph

120
00:06:06,020 --> 00:06:02,969
represents well they were at 65 degree

121
00:06:08,480 --> 00:06:06,030
centigrade at different places so in

122
00:06:11,300 --> 00:06:08,490
this graph we see that they produce very

123
00:06:14,390 --> 00:06:11,310
high concentration of methane after they

124
00:06:17,809 --> 00:06:14,400
were brought back to normal pressure and

125
00:06:22,159 --> 00:06:17,819
their optimum growth temperature which

126
00:06:24,620 --> 00:06:22,169
is 55 then we calculated their growth

127
00:06:26,959 --> 00:06:24,630
rate and we found that their growth rate

128
00:06:29,390 --> 00:06:26,969
did not change until four hundred

129
00:06:31,820 --> 00:06:29,400
atmospheric pressure but their growth

130
00:06:38,149 --> 00:06:31,830
rate increases with the increase in the

131
00:06:40,430 --> 00:06:38,159
pressure at 55 degree centigrade this is

132
00:06:43,279 --> 00:06:40,440
the table of a stable carbon isotope

133
00:06:45,560 --> 00:06:43,289
fractionation and in this table we do

134
00:06:45,970 --> 00:06:45,570
not see any significant difference in

135
00:06:50,800 --> 00:06:45,980
the

136
00:06:53,860 --> 00:06:50,810
effect of different pressure or

137
00:06:56,170 --> 00:06:53,870
different temperatures we use this

138
00:06:59,530 --> 00:06:56,180

formula to calculate their carbon

139
00:07:02,080 --> 00:06:59,540
isotopic fractionation values actually a

140
00:07:04,480 --> 00:07:02,090
stable carbon isotope fractionation is

141
00:07:08,080 --> 00:07:04,490
one of the method that can distinguish

142
00:07:10,900 --> 00:07:08,090
between biogenic and a biogenic methane

143
00:07:13,060 --> 00:07:10,910
and here we wanted to see effect of high

144
00:07:17,950 --> 00:07:13,070
pressure high temperature on their

145
00:07:20,650 --> 00:07:17,960
carbon isotopic values these are the

146
00:07:23,800 --> 00:07:20,660
images of wilfy I at 55 degree

147
00:07:25,930 --> 00:07:23,810
centigrade and this this image was taken

148
00:07:28,600 --> 00:07:25,940
when they were at one atmospheric

149
00:07:31,180 --> 00:07:28,610
pressure and this image was taken when

150
00:07:34,300 --> 00:07:31,190
they were at 1200 atmospheric pressure

151
00:07:36,970 --> 00:07:34,310
at higher pressure we found the

152
00:07:42,400 --> 00:07:36,980
increased number of elongated cells

153
00:07:44,850 --> 00:07:42,410
suggesting lack of cell division in

154
00:07:47,760 --> 00:07:44,860
general the growth and survivability of

155
00:07:50,800 --> 00:07:47,770
microorganisms depends on their ambient

156
00:07:55,030 --> 00:07:50,810
physical and chemical conditions such as

157
00:07:58,000 --> 00:07:55,040
pressure temperature and pH and we know

158
00:08:00,610 --> 00:07:58,010
that extremophiles mostly belong to the

159
00:08:03,670 --> 00:08:00,620
domain Archaea because of they have

160
00:08:06,310 --> 00:08:03,680
unique membrane lipid bonding their

161
00:08:09,000 --> 00:08:06,320
cytoplasmic membrane contains ether

162
00:08:12,100 --> 00:08:09,010
bonds which are more resistant to heat

163
00:08:15,940 --> 00:08:12,110

also some archaeal cytoplasmic membrane

164
00:08:19,270 --> 00:08:15,950
have a tetraethyl lipids which forms

165
00:08:22,600 --> 00:08:19,280
mono layer which are almost impermeable

166
00:08:25,900 --> 00:08:22,610
to ions and Samardzija can produce

167
00:08:28,840 --> 00:08:25,910
pressure induced proteins such as heat

168
00:08:32,440 --> 00:08:28,850
and cold shock protein which helped them

169
00:08:35,620 --> 00:08:32,450
to survive in extreme conditions in our

170
00:08:38,530 --> 00:08:35,630
experiment we have not investigated that

171
00:08:41,080 --> 00:08:38,540
which biomolecules are responsible for

172
00:08:44,470 --> 00:08:41,090
their survivability at high pressure

173
00:08:47,260 --> 00:08:44,480
high temperature and low pH so we will

174
00:08:50,140 --> 00:08:47,270
investigate them later but currently we

175
00:08:53,230 --> 00:08:50,150
are combining all three factors pressure

176
00:08:55,750 --> 00:08:53,240
temperature and pH we want to see their

177
00:08:59,380 --> 00:08:55,760
growth rate and survivability at these

178
00:09:01,740 --> 00:08:59,390
conditions so in conclusion at

179
00:09:06,040 --> 00:09:01,750
can say that goofy I can tolerate

180
00:09:08,620 --> 00:09:06,050
pressure for 1,200 atmospheric pressure

181
00:09:14,050 --> 00:09:08,630
temperature up to 75 degree centigrade

182
00:09:16,090 --> 00:09:14,060
and low pH up to 4 and their growth rate

183
00:09:18,820 --> 00:09:16,100
increases with the increase in the

184
00:09:21,940 --> 00:09:18,830
pressure and the temperature and

185
00:09:24,490 --> 00:09:21,950
pressure did not affect on their carbon

186
00:09:26,590 --> 00:09:24,500
isotopic data and the high at high

187
00:09:29,950 --> 00:09:26,600
pressure we found increased number of

188
00:09:32,560 --> 00:09:29,960

elongated cells suggesting lack of cell

189
00:09:35,170 --> 00:09:32,570
division and this work was supported by

190
00:09:43,740 --> 00:09:35,180
nasa astrobiology exobiology program

191
00:10:02,920 --> 00:09:59,320
questions for nevada hi um great talk a

192
00:10:05,530 --> 00:10:02,930
question is do you have any plans to get

193
00:10:06,670 --> 00:10:05,540
a yes in your model a little bit earn

194
00:10:09,790 --> 00:10:06,680
your experimental work a little bit

195
00:10:11,830 --> 00:10:09,800
closer to like near surface conditions

196
00:10:14,140 --> 00:10:11,840
on Mars so like decreasing that pressure

197
00:10:15,700 --> 00:10:14,150
decreasing that temperature getting a

198
00:10:20,590 --> 00:10:15,710
little bit closer to what the surface

199
00:10:22,720 --> 00:10:20,600
conditions would be so near surface we

200
00:10:25,060 --> 00:10:22,730
are talking about Miss temperature will

201
00:10:26,980 --> 00:10:25,070
be what temperature are you expecting

202
00:10:30,610 --> 00:10:26,990
well I mean I'm asking if you have any

203
00:10:32,350 --> 00:10:30,620
plans to get any closer or no okay as my

204
00:10:34,990 --> 00:10:32,360
colleague see is working on low

205
00:10:38,260 --> 00:10:35,000
temperature and low pressure oh okay so

206
00:10:46,930 --> 00:10:38,270
please working on that okay I won't be

207
00:10:48,760 --> 00:10:46,940
working on okay hi um you might have

208
00:10:50,620 --> 00:10:48,770
gone over this too quickly and I missed

209
00:10:52,780 --> 00:10:50,630
it but were you calculating growth rate

210
00:10:54,640 --> 00:10:52,790
by methane production in the headspace

211
00:11:00,250 --> 00:10:54,650
or did you do something like optical

212
00:11:04,060 --> 00:11:00,260
density no hay de space nitin yes okay

213
00:11:07,900 --> 00:11:04,070

not optical density okay can you explain

214
00:11:09,840 --> 00:11:07,910
why oh why yeah like white why didn't

215
00:11:12,390 --> 00:11:09,850
you something like actual density or

216
00:11:15,420 --> 00:11:12,400
because our meta no gel

217
00:11:18,960 --> 00:11:15,430
it's very difficult to use optical

218
00:11:25,530 --> 00:11:18,970
density it precipitate quickly so we

219
00:11:29,400 --> 00:11:25,540
tried but we did not get good data of OD

220
00:11:31,980 --> 00:11:29,410
so we decided to use only method

221
00:11:37,190 --> 00:11:31,990
concentration okay the chaos media is

222
00:11:41,040 --> 00:11:37,200
also like very turbid and very initial

223
00:11:47,730 --> 00:11:41,050
OD is very high so we doesn't work in

224
00:11:50,100 --> 00:11:47,740
our experiment yeah wonderful talk I

225
00:11:55,020 --> 00:11:50,110
also work at intelligence so EA

226
00:11:57,860 --> 00:11:55,030
methanogens you changed many of the

227
00:12:00,180 --> 00:11:57,870
conditions are you also thinking about

228
00:12:03,660 --> 00:12:00,190
analyzing or actually what was the

229
00:12:09,090 --> 00:12:03,670
hydrogen concentration did you measure

230
00:12:12,630 --> 00:12:09,100
it or no no no right to me know we

231
00:12:15,510 --> 00:12:12,640
pressurize so my residual we did not

232
00:12:21,030 --> 00:12:15,520
measure hydrogen concentration just to