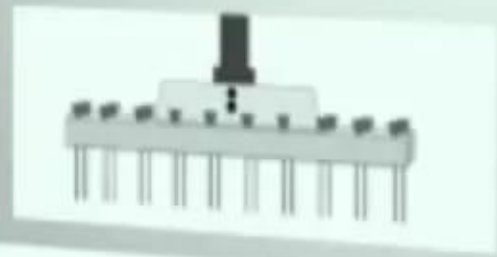
Methods (cont'd)

- Pegasus Planetary Simulation Chamber
- Evacuate to 1 mbar, fill with 80:20 H_2:CO_2 gas to 50 mbar, evacuate to 1 mbar (cycle 3x)
- Tubes punctured after 1 day
- CO_2 added on second-to-last day, tubes unpunctured
- 0.5 mL transferred to new media
- Expt. 6: 5 g sterilized JSC Mars-1 added atop cotton ball as diffusion barrier



1
00:00:12,470 --> 00:00:09,770
thank you so I am starting off the

2
00:00:14,390 --> 00:00:12,480
isotope section but I want to let you

3
00:00:17,150 --> 00:00:14,400
know that i am not talking at all about

4
00:00:19,939 --> 00:00:17,160
isotopes i actually asked the organizers

5
00:00:22,939 --> 00:00:19,949
but to put me on wednesday in case of

6
00:00:24,859 --> 00:00:22,949
travel problems on monday so anyway no

7
00:00:28,759 --> 00:00:24,869
isotopes sorry feel free to leave if you

8
00:00:31,130 --> 00:00:28,769
want so background why are we looking at

9
00:00:34,880 --> 00:00:31,140
low pressure well we try to mimic

10
00:00:37,280 --> 00:00:34,890
Martian conditions and Mars pressure is

11
00:00:39,470 --> 00:00:37,290
much much lower than that on earth it's

12
00:00:41,299 --> 00:00:39,480
about seven millibar well as Earth

13
00:00:43,490 --> 00:00:41,309

surface pressures about a thousand

14
00:00:46,069 --> 00:00:43,500
millibar and nowhere on earth can we

15
00:00:48,110 --> 00:00:46,079
actually get to seven millibar even at

16
00:00:51,590 --> 00:00:48,120
the top of Mount Everest and so we're

17
00:00:53,150 --> 00:00:51,600
looking to see you know there's no life

18
00:00:55,040 --> 00:00:53,160
on Earth here that evolved at low

19
00:00:57,350 --> 00:00:55,050
pressure so we're actually very

20
00:00:59,180 --> 00:00:57,360
interested in this topic and what's

21
00:01:01,250 --> 00:00:59,190
interesting is that few experiments

22
00:01:05,200 --> 00:01:01,260
actually test the effect of low pressure

23
00:01:07,700 --> 00:01:05,210
on organism metabolism and growth

24
00:01:10,490 --> 00:01:07,710
however if you were here Monday you

25
00:01:14,780 --> 00:01:10,500
heard Sam waters talk about low pressure

26
00:01:16,940 --> 00:01:14,790
and so she gave a great introduction to

27
00:01:18,950 --> 00:01:16,950
why we look at low pressure and her lap

28
00:01:21,530 --> 00:01:18,960
she worked in the Nicholson lab and

29
00:01:24,020 --> 00:01:21,540
Andrew sugar who is also at the

30
00:01:25,880 --> 00:01:24,030
University of Florida has looked at 22

31
00:01:28,039 --> 00:01:25,890
different bacterial species that are

32
00:01:30,140 --> 00:01:28,049
commonly found on earth some are found

33
00:01:33,170 --> 00:01:30,150
in spacecraft clean rooms and he's

34
00:01:36,620 --> 00:01:33,180
looked at survival and growth under low

35
00:01:39,890 --> 00:01:36,630
pressure and so sugar and Nicholson

36
00:01:43,399 --> 00:01:39,900
found that there's this 25 millibar

37
00:01:46,219 --> 00:01:43,409
limit to growth where most species are

38
00:01:48,410 --> 00:01:46,229

able to grow and metabolize down to 25

39
00:01:51,620 --> 00:01:48,420
millibar but then they reach this limit

40
00:01:54,080 --> 00:01:51,630
where many species either died off or

41
00:01:57,050 --> 00:01:54,090
they simply can't grow anymore and so

42
00:01:58,819 --> 00:01:57,060
we're interested in figuring out maybe

43
00:02:03,200 --> 00:01:58,829
why this limit occurs and looking more

44
00:02:05,120 --> 00:02:03,210
into this limit so in my lab we use

45
00:02:07,520 --> 00:02:05,130
methanogens and they're members of the

46
00:02:10,070 --> 00:02:07,530
domain Archaea they're anaerobic and

47
00:02:11,860 --> 00:02:10,080
they use molecular hydrogen as an energy

48
00:02:13,660 --> 00:02:11,870
source in carbon

49
00:02:17,170 --> 00:02:13,670
side as a carbon source which is found

50
00:02:19,300 --> 00:02:17,180
on Mars and we use for species with a no

51
00:02:21,490 --> 00:02:19,310
thermo vector wolfy I nathanael star

52
00:02:25,020 --> 00:02:21,500
Cena Barker I Nathanael bacterium for me

53
00:02:27,820 --> 00:02:25,030
sikkim methanococcus Meeropol udhas so

54
00:02:30,339 --> 00:02:27,830
in the parenthesis you'll see their

55
00:02:32,259 --> 00:02:30,349
optimum growth temperature which it's

56
00:02:35,470 --> 00:02:32,269
obviously very high and not very Mars

57
00:02:38,020 --> 00:02:35,480
like but the reason that we don't use a

58
00:02:41,020 --> 00:02:38,030
cycra file or a cold loving with antigen

59
00:02:44,619 --> 00:02:41,030
is because they just grow so slowly that

60
00:02:47,490 --> 00:02:44,629
I would be here for another 20 years and

61
00:02:52,990 --> 00:02:47,500
my advisor would be like just just leave

62
00:02:57,009 --> 00:02:53,000
so we do use a thermophilic methanogens

63
00:02:58,539 --> 00:02:57,019

but they can survive low pressure and i

64
00:03:02,830 --> 00:02:58,549
have other experiments that show that

65
00:03:06,309 --> 00:03:02,840
but these four species are they

66
00:03:08,140 --> 00:03:06,319
basically show the breadth of the

67
00:03:12,130 --> 00:03:08,150
methanogenic archaea and that's why we

68
00:03:14,530 --> 00:03:12,140
use these for so in this talk i will

69
00:03:16,960 --> 00:03:14,540
talk to you about six experiments i ran

70
00:03:18,940 --> 00:03:16,970
from at varying pressure and the

71
00:03:22,030 --> 00:03:18,950
pressures are listed here and you can

72
00:03:25,150 --> 00:03:22,040
see that the first experiment was up to

73
00:03:27,699 --> 00:03:25,160
143 millibar and experiment 5 & 6

74
00:03:30,039 --> 00:03:27,709
arranged between six and twenty mellow

75
00:03:32,020 --> 00:03:30,049
bar and the reason we have arranged is

76
00:03:36,520 --> 00:03:32,030
because we are still developing our

77
00:03:40,930 --> 00:03:36,530
vacuum chamber and right now our Val are

78
00:03:42,250 --> 00:03:40,940
open and closed valve isn't as precise

79
00:03:45,039 --> 00:03:42,260
as we would like it to be and that's why

80
00:03:48,550 --> 00:03:45,049
we have that range there but we're going

81
00:03:50,710 --> 00:03:48,560
to fix that in the future but in a in

82
00:03:54,009 --> 00:03:50,720
each experiment I had 20 test tubes

83
00:03:55,479 --> 00:03:54,019
total which included five test tubes for

84
00:03:57,520 --> 00:03:55,489
each of the four species so there are

85
00:04:01,300 --> 00:03:57,530
five replicates and then these

86
00:04:04,990 --> 00:04:01,310
methanogens survive in liquid medium and

87
00:04:07,569 --> 00:04:05,000
so that is a problem at low pressure

88
00:04:09,699 --> 00:04:07,579

which I'll get to but each tube is ten

89
00:04:11,589 --> 00:04:09,709
milliliters of liquid medium prepared

90
00:04:14,020 --> 00:04:11,599
anaerobically and so it contains carbon

91
00:04:15,970 --> 00:04:14,030
dioxide its pressurized with hydrogen

92
00:04:18,430 --> 00:04:15,980
gas which is their energy source and

93
00:04:24,580 --> 00:04:18,440
growth is monitored by methane

94
00:04:26,890 --> 00:04:24,590
action so once I make these test tubes

95
00:04:29,110 --> 00:04:26,900
with the myth antigens in them I put

96
00:04:30,610 --> 00:04:29,120
them in the Pegasus planetary simulation

97
00:04:35,440 --> 00:04:30,620
chamber at the University of Arkansas

98
00:04:37,330 --> 00:04:35,450
and before I sew these these tubes are

99
00:04:39,880 --> 00:04:37,340
sealed and before I puncture them i

100
00:04:42,040 --> 00:04:39,890
evacuate the chamber and then fill it

101
00:04:44,920 --> 00:04:42,050
with hydrogen and carbon dioxide gas

102
00:04:46,720 --> 00:04:44,930
then evacuated again and I cycle this

103
00:04:49,060 --> 00:04:46,730
three times in order to get rid of all

104
00:04:51,210 --> 00:04:49,070
that residual oxygen and atmosphere in

105
00:04:53,830 --> 00:04:51,220
there and then I let the chamber

106
00:04:56,160 --> 00:04:53,840
stabilize for about a day due to

107
00:05:00,130 --> 00:04:56,170
outgassing from the walls and whatnot

108
00:05:02,110 --> 00:05:00,140
then with this specialized device you

109
00:05:04,990 --> 00:05:02,120
see here which I have nicknamed the

110
00:05:08,230 --> 00:05:05,000
pokey device I'm able to turn a crank

111
00:05:10,240 --> 00:05:08,240
and it will either lower or raise and

112
00:05:12,640 --> 00:05:10,250
I'm able to puncture the tubes within

113
00:05:16,900 --> 00:05:12,650

the chamber at the pressure that I

114
00:05:19,090 --> 00:05:16,910
desire and so then the tubes remain

115
00:05:21,580 --> 00:05:19,100
punctured and they can équilibre twith

116
00:05:23,320 --> 00:05:21,590
the atmosphere and then on the second to

117
00:05:25,480 --> 00:05:23,330
last day I fill up the chamber with

118
00:05:28,390 --> 00:05:25,490
carbon dioxide otherwise there'd be a

119
00:05:30,460 --> 00:05:28,400
suction effect in the tubes and if I

120
00:05:33,700 --> 00:05:30,470
open the chamber it would allow oxygen

121
00:05:35,409 --> 00:05:33,710
in which would kill them and then on I

122
00:05:37,659 --> 00:05:35,419
unplug sure the tube so that they're

123
00:05:41,320 --> 00:05:37,669
full of carbon dioxide and then I take

124
00:05:43,270 --> 00:05:41,330
them out the methods were the same for

125
00:05:46,840 --> 00:05:43,280
all six experiments except for

126
00:05:49,900 --> 00:05:46,850
experiment six where I had a cotton ball

127
00:05:52,690 --> 00:05:49,910
right above the liquid and six or five

128
00:05:54,790 --> 00:05:52,700
grams of JSC Mars one to act as a

129
00:05:58,240 --> 00:05:54,800
diffusion barrier to slow the

130
00:06:01,420 --> 00:05:58,250
evaporation of water so here are my

131
00:06:03,820 --> 00:06:01,430
results for one of our organisms and

132
00:06:05,620 --> 00:06:03,830
it's very there's a lot of information

133
00:06:08,320 --> 00:06:05,630
here so I'm going to go over it slowly

134
00:06:10,060 --> 00:06:08,330
on the left is methane production which

135
00:06:13,090 --> 00:06:10,070
is how we measure the growth in our of

136
00:06:15,190 --> 00:06:13,100
our methanogens and on the bottom are

137
00:06:17,560 --> 00:06:15,200
the experiments so there's two columns

138
00:06:22,330 --> 00:06:17,570

for each of the six experiments and the

139
00:06:24,880 --> 00:06:22,340
pressures are also listed there and each

140
00:06:27,520 --> 00:06:24,890
column the light gray is the original

141
00:06:31,120 --> 00:06:27,530
tube and that corresponds to the tube

142
00:06:31,959 --> 00:06:31,130
that was actually in the chamber under

143
00:06:34,899 --> 00:06:31,969
low pressure

144
00:06:37,659 --> 00:06:34,909
and then the dark great column is the

145
00:06:40,059 --> 00:06:37,669
tube that I transferred point five

146
00:06:44,319 --> 00:06:40,069
milliliters from the original tube to

147
00:06:46,809 --> 00:06:44,329
the transfer tube okay so these all this

148
00:06:49,059 --> 00:06:46,819
methane production is actually following

149
00:06:51,549 --> 00:06:49,069
exposure and following an incubation

150
00:06:54,159 --> 00:06:51,559
period so i had the tubes in the chamber

151
00:06:57,159 --> 00:06:54,169
exposed to low pressure i took them out

152
00:06:59,319 --> 00:06:57,169
I'd performed a transfer and then I put

153
00:07:02,499 --> 00:06:59,329
all the tubes at their ideal incubation

154
00:07:06,429 --> 00:07:02,509
temperature and so what we can say is

155
00:07:07,989 --> 00:07:06,439
that initially when I fill the chamber

156
00:07:10,509 --> 00:07:07,999
up with carbon dioxide the methane

157
00:07:12,399 --> 00:07:10,519
content is about zero because the tubes

158
00:07:16,509 --> 00:07:12,409
are being filled with carbon dioxide and

159
00:07:21,339 --> 00:07:16,519
so we can compare these post-exposure

160
00:07:23,889 --> 00:07:21,349
methane values to the zero points of the

161
00:07:26,829 --> 00:07:23,899
actual experiment and so you'll see that

162
00:07:30,579 --> 00:07:26,839
there's growth or methane production in

163
00:07:33,009 --> 00:07:30,589

each of the six experiments even at even

164
00:07:35,979 --> 00:07:33,019
if it's just a few percent at six Mel

165
00:07:38,290 --> 00:07:35,989
bar and so the reason there are no

166
00:07:39,850 --> 00:07:38,300
original tubes for experiments five and

167
00:07:43,749 --> 00:07:39,860
six is because there was so much

168
00:07:45,609 --> 00:07:43,759
evaporation that there wasn't after

169
00:07:47,829 --> 00:07:45,619
performing the point five milliliter

170
00:07:51,579 --> 00:07:47,839
transfer there wasn't anything left in

171
00:07:54,850 --> 00:07:51,589
those tubes so I just discarded them so

172
00:07:56,589 --> 00:07:54,860
this is for Barker I wolfie I and

173
00:07:59,019 --> 00:07:56,599
Meeropol udhas you see the same thing

174
00:08:02,949 --> 00:07:59,029
there's methane production following

175
00:08:04,779 --> 00:08:02,959
exposure to low pressure and for me

176
00:08:07,019 --> 00:08:04,789
cecum the reason I show this one

177
00:08:09,759 --> 00:08:07,029
separately is because you can see that

178
00:08:11,979 --> 00:08:09,769
the methane production in both the

179
00:08:15,279 --> 00:08:11,989
original and the transfer tubes is very

180
00:08:17,499 --> 00:08:15,289
similar and so if you were considering

181
00:08:21,279 --> 00:08:17,509
that oh well you know you transfer them

182
00:08:22,959 --> 00:08:21,289
with antigens and they have new media

183
00:08:25,379 --> 00:08:22,969
and new growth so maybe they went

184
00:08:28,659 --> 00:08:25,389
dormant and then you know we're able to

185
00:08:31,089 --> 00:08:28,669
regrow again maybe the transfer media is

186
00:08:33,369 --> 00:08:31,099
different maybe the low pressure you

187
00:08:35,019 --> 00:08:33,379
know affected their media the growth

188
00:08:39,040 --> 00:08:35,029

between the original tubes and the

189
00:08:42,009 --> 00:08:39,050
transfer tubes are the same so in

190
00:08:44,820 --> 00:08:42,019
conclusion the low pressure exposure is

191
00:08:47,340 --> 00:08:44,830
not lethal to actively met

192
00:08:51,180 --> 00:08:47,350
rising cells so right now we can't

193
00:08:52,410 --> 00:08:51,190
measure in situ growth of of them

194
00:08:54,360 --> 00:08:52,420
advantage ins during the exposure

195
00:08:57,450 --> 00:08:54,370
because it's actually fairly difficult

196
00:09:00,390 --> 00:08:57,460
to take a sample out of a low pressure

197
00:09:02,430 --> 00:09:00,400
vacuum but so there's sort of a black

198
00:09:05,070 --> 00:09:02,440
box of during the exposure what is

199
00:09:06,870 --> 00:09:05,080
actually happening but we know that we

200
00:09:08,940 --> 00:09:06,880
had actively metabolizing methanogens

201
00:09:10,830 --> 00:09:08,950
before the experiment and then we had

202
00:09:14,010 --> 00:09:10,840
actively metabolising with antigens

203
00:09:15,840 --> 00:09:14,020
after the experiment the limiting factor

204
00:09:18,600 --> 00:09:15,850
is currently the evaporation of liquid

205
00:09:20,880 --> 00:09:18,610
media this is not something we can get

206
00:09:22,740 --> 00:09:20,890
rid of considering we're working at low

207
00:09:25,710 --> 00:09:22,750
pressure if we lowered the temperature

208
00:09:28,350 --> 00:09:25,720
20 degrees the methanogens wouldn't grow

209
00:09:29,820 --> 00:09:28,360
anyway even if we had a psycho Phillip

210
00:09:32,700 --> 00:09:29,830
meth antigen it would just grow so

211
00:09:36,600 --> 00:09:32,710
slowly that even the slowly rate of

212
00:09:38,610 --> 00:09:36,610
evaporation of water might be faster

213
00:09:42,480 --> 00:09:38,620

than the actual slow growth rate of a

214
00:09:44,640 --> 00:09:42,490
psycho Phillip organism and then the

215
00:09:46,680 --> 00:09:44,650
methane production and optical density

216
00:09:49,710 --> 00:09:46,690
measurements that I haven't shown do

217
00:09:52,740 --> 00:09:49,720
indicate growth at least following

218
00:09:54,810 --> 00:09:52,750
exposure but what we really want to know

219
00:09:57,630 --> 00:09:54,820
is are the cells capable of active

220
00:10:01,490 --> 00:09:57,640
growth at low pressure institute during

221
00:10:06,360 --> 00:10:01,500
the experiment and so this fall we've

222
00:10:08,850 --> 00:10:06,370
achieved or obtained a device that we're

223
00:10:11,390 --> 00:10:08,860
able to put an actual whole system

224
00:10:13,980 --> 00:10:11,400
within our chamber to measure real time

225
00:10:16,230 --> 00:10:13,990
methane production and hopefully we're

226
00:10:20,850 --> 00:10:16,240
going to see if it works and it should

227
00:10:22,800 --> 00:10:20,860
but you know science so we'll see and

228
00:10:25,530 --> 00:10:22,810
then we're also going to attempt to

229
00:10:27,270 --> 00:10:25,540
lengthen our experiments using various

230
00:10:30,960 --> 00:10:27,280
diffusion barriers such as different

231
00:10:33,720 --> 00:10:30,970
soils or salts in the media or even just

232
00:10:35,640 --> 00:10:33,730
adding a hydrophobic filter on top of

233
00:10:39,690 --> 00:10:35,650
our syringe needles in the chamber just

234
00:10:43,500 --> 00:10:39,700
to slow that evaporation down and so I'm

235
00:10:45,870 --> 00:10:43,510
going to be that guy and I do have a

236
00:10:48,390 --> 00:10:45,880
call for help I've spoken to many of you

237
00:10:50,910 --> 00:10:48,400
on Monday and greatly appreciate your

238
00:10:53,130 --> 00:10:50,920

input and probably be emailing you in

239
00:10:54,930 --> 00:10:53,140
the future but you know we're trying to

240
00:10:56,879 --> 00:10:54,940
set up our low pressure vacuum chamber

241
00:10:58,859 --> 00:10:56,889
if anyone has

242
00:11:03,090 --> 00:10:58,869
the information about any of these

243
00:11:06,900 --> 00:11:03,100
topics it'd be greatly appreciated but

244
00:11:08,699 --> 00:11:06,910
you know competition as well so I'm

245
00:11:19,169 --> 00:11:08,709
working on it yeah we're all friends

246
00:11:21,869 --> 00:11:19,179
here but other than that thank you all

247
00:11:30,150 --> 00:11:21,879
right do we have any questions either

248
00:11:31,499 --> 00:11:30,160
online or in person so you're growing

249
00:11:34,409 --> 00:11:31,509
these cells on hydrogen and you're

250
00:11:39,119 --> 00:11:34,419
growing them at Steven millibars total

251
00:11:40,829 --> 00:11:39,129
preciate it was a t20 h202 so may it

252
00:11:42,809 --> 00:11:40,839
just be that that low-pressure there's

253
00:11:45,210 --> 00:11:42,819
just not enough hydrogen for them to

254
00:11:48,720 --> 00:11:45,220
grow yeah and that that's definitely a

255
00:11:51,059 --> 00:11:48,730
possibility other studies have shown

256
00:11:53,819 --> 00:11:51,069
that they can grow on very very limited

257
00:11:55,289 --> 00:11:53,829
amounts of hydrogen but that's something

258
00:11:57,749 --> 00:11:55,299
that we definitely need to take into

259
00:11:59,579 --> 00:11:57,759
account in our our false we should talk

260
00:12:09,470 --> 00:11:59,589
about Steven phallic hydrogen limitation

261
00:12:12,449 --> 00:12:09,480
okay so I know at least a seat of orange

262
00:12:13,619 --> 00:12:12,459
can use acetate as an electron donor can

263
00:12:16,199 --> 00:12:13,629

you use that instead of hydrogen a

264
00:12:21,359 --> 00:12:16,209
fascinating factory um yes that's

265
00:12:23,579 --> 00:12:21,369
possible where we use these species just

266
00:12:25,799 --> 00:12:23,589
because they don't require any organic

267
00:12:28,319 --> 00:12:25,809
compounds and hydrogen and carbon

268
00:12:30,569 --> 00:12:28,329
dioxide well carbon dioxide Minos on

269
00:12:32,549 --> 00:12:30,579
Mars but it's also postulated that

270
00:12:34,679 --> 00:12:32,559
hydrogen is available and they're the

271
00:12:37,079 --> 00:12:34,689
simplest compounds so that's why we use