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:-25-96

This SNB records the laboratory work carried out under the container life KTI (recorded in Black) & the Evolution of the near-field environment KTI (recorded in Blue). Research is concentrated on the microbial aspects of each & follows on from the experiment initiated in SNB 134.
Entries will be made by Peter Angell ph.D (Microbiology-Survey) & Alice Stone B.S. (Biology UTSA). 6-25-96

Please refer to Lab Notebook #134 page 123, 129 through 133 for procedures & identifications of Yucca Mountain samples

12/24/97

Results obtained from plate counts of samples incubated at 100°C for 48 hrs:

Ym-100-5-24-100°C #11	4.01×10^8 CFU/ml
Ym-100-5-24 100°C #12	2.83×10^8 CFU/ml
Ym-100-5-24 100°C #13	2.20×10^8 CFU/ml
control 100°C #11	1.15×10^4 CFU/ml
control 100°C #12	8.50×10^7 CFU/ml

Results obtained from plate counts of bacteria present on rocks; samples incubated at 100°C for 48 hrs.

Ym-100-5-24 100°C #11	3.75×10^5 CFU/ml
Ym-100-5-24 100°C #12	no counts
Ym-100-5-24 100°C #13	no counts

13:15 (846320) T=235 ≡ 454 hrs

T15 Using MEP2 (9:00) sampled red and blue faces as follows: Rinsed in 10ml 0.2um filtered RO H_2O & swabbed coupons. Placed swab tip in 1ml sterile PBS. (T15-454B3 & T1B-454B4)

T16 Using MEP2 (9:00) sampled red and blue faces as follows: Rinsed in 10 ml 0.2um filtered RO H_2O & swabbed coupons. Placed swab tip in 1ml sterile PBS. (T16-454B3 & T16-454B4)

Vortexed each tube for 1min and performed a 10-fold serial dilution and plated on LB4.

T15-425	1.33×10^7 CFU/ml	T16-425	2.83×10^6 CFU/ml
T15-432	2.58×10^7 CFU/ml	T16-432	2.67×10^6 CFU/ml

15:40
T15 29°C, plt 7.951, pt -190mV, b -275mV, r -231mV, w -255mV, y -177mV
T16 29°C, pH 8.141, pt -246mV, b -551mV, r -453mV, w -570mV, y -370mV
15:45 (855500) T= 238 ≡ 457 hrs
1ml samples taken from T15 & T16 for bacterial counts. Residual frozen (T15-457 & T16-457).
Alice Stone 6/25/96

6/26/96

8:10
T15 27°C, plt 7.981, pt -229mV, b -317mV, r -268mV, w -282mV, y -248mV
T16 27°C, pH 8.173, pt -306mV, b -596mV, r -578mV, w -609mV, y -558mV
8:15 (914575) T= 254 ≡ 473 hrs
1ml samples taken from T15 & T16 for bacterial counts. Residual labeled as (T15-473 & T16-473) and frozen.

Results obtained from plate counts of samples incubated at 100°C for 120 hrs.

Ym-100-5-24 100°C #14	2.25×10^8 CFU/ml
Ym-100-5-24 100°C #15	7.50×10^2 CFU/ml
Ym-100-5-24 100°C #16	no counts
control 100°C #13	no counts
control 100°C #14	no counts

Results obtained from bacteria on rocks, samples incubated at 100°C for 120 hrs.

Ym-100-5-24 #14	4.75×10^5 CFU/ml
Ym-100-5-24 #15	no counts
Ym-100-5-24 #16	no counts

Removed flasks Ym-100-5-24 80°C (21 thru 23) and controls 80°C (21&22) from shaking water bath and 100ul samples were taken from each. 10 fold serial dilutions were performed.

Weighed out 1g of Ym-100-5-24 80°c (21 thru 23) and placed in 10 ml of sterile PBS. Rinsed each sample three times with 10 ml of sterile PBS and then sonicated for 1 min. Did 10 fold serial dilutions.

3:00

T15 28°C, pH 7.934, pt -282 mV
b -345 mV, r -310 mV, w -309 mV, y -285 mV

T16 28°C, pH 8.165, pt -306 mV
b -592 mV, r -579 mV, w -606 mV, y -559 mV

3:05 (939318) T = 261 ≡ 480 hrs

1 ml samples taken from T15 & T16 for bacterial counts. Residual frozen (T15-480 & T16-480).

Results from bacterial counts on T15 & T16:

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
6/25/96	449	1.42×10^7	1.66×10^6
6/25/96	457	1.75×10^7	5.33×10^5

Results from biofilm counts on T15 & T16

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
6/25/96	454-B3	3.83×10^4	2.42×10^3
6/25/96	454-B4	6.67×10^3	6.67×10^3

Alice Stone

6/27/96

8:20

T15 27°C, pH 8.099, pt ~~-1,524~~ -279 mV
b -391 mV, r -352 mV, w -349 mV, y -339 mV

T16 27°C, pH 8.153, pt -287 mV
b -592 mV, r -581 mV, w -605 mV, y -562 mV

1 ml samples taken from T15 & T16 for bacterial counts at 8:30 (100184) T = 278 ≡ 497 hrs.
Residual frozen (T15-497 & T16-497)

Ran EIS using Solartron 1260 and EG&G 273 serial no. 41108 potentiostat. Setup as follows:
10 KHz to 5mHz, ±5mV frequency, 10 steps per decade, 100uA, 0.01 attenuator

T15 red =	T15r497.Z	RP = 794 KΩ
T15 white =	T15w497.Z	RP = 995 KΩ
T16 red =	T16r497.Z	RP = 428 KΩ
T16 white =	T16w497.Z	RP = 224 KΩ

Made 10 ml of MnO_2 media as follows:
0.17383 g MnO_2 lot # 960730
342 ul Na lactate lot # 943605
added to 10 ml 18.1 MΩ H_2O. Autoclaved for 30 min at 14 psi and 121°C.

plates made on 6/14/96 of M4, M4A + SP200 on M4AI plate. M4 media + agar + Bromthyol blue (pSI SWB 138) Shows growth has produced yellow colouration indicating the production of metabolic acids.

Results obtained from plate counts of samples incubated at 80°C for 48 hrs.

Ym-100-5-24 80°C #21	3.17×10^8 CFU/ml
Ym-100-5-24 80°C #22	3.70×10^8 CFU/ml
Ym-100-5-24 80°C #23	2.42×10^8 CFU/ml
control 80°C #21	no counts
control 80°C #22	no. counts

Results obtained from plate counts of bacteria present on rocks; samples incubated at 80°C for 48 hrs.

Ym-100-5-24	80°C #21	3.75×10^6 CFU/ml
Ym-100-5-24	80°C #22	3.33×10^5 CFU/ml
Ym-100-5-24	80°C #23	1.50×10^5 CFU/ml

Results from bacterial counts on T15 & T16

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
6/26/96	473	1.92×10^7	1.75×10^6
6/26/96	480	2.25×10^7	2.17×10^6

T15 & T16 runs restarted at 15:30 6/27/96 need to add 504 hrs to new values

15:30

T15 28°C, pH 7.980, pt -289 mV,
b -383 mV, r -354 mV, w -349 mV, y -330mV

T16 28°C, pH 8.126, pt -264 mV,
b -585mV, r -568 mV, w -597 mV, y -560mV

15:35 (600) T = 504

1ml samples taken from T15 & T16 for bacterial counts. Residual labeled as (T15-504 & T16-504) and frozen.

15:45 10 ml 2mM MnO_2 added to T16

Potentials carbon steel p.157 from Div 06

10% Thio medium	-631 mV
SRB	-711 mV
Lactate/acetate (abiotic)	-718 mV
Thio medium (abiotic)	-674 mV

Alice Stone

6/28/96 8:20

T15 27°C, pH 8.004, pt -308 mV
b -425 mV, r -388 mV, w -365 mV, y -364 mV

T16 27°C, pH 8.136, pt -243 mV
b -569 mV, r -547 mV, w -544 mV, y -340 mV

8:23 (61459) T = 17 ≡ 521 hrs

1 ml samples taken from T15 & T16 for bacterial counts. Residual labeled as T15-521 & T16-521 and frozen.

T15 -429	9.58×10^6 cfu ml^{-1}	T16-497	1.83×10^6 cfu ml^{-1}
T15 504	1.17×10^7 cfu ml^{-1}	T16-506	2.62×10^6 cfu ml^{-1}

Made biofilm counts on T15 + T16 10:55 (70576) (19.66v) using 16P#3 (3' o'clock) blue & red.

T15 blue -386 mV red. -360 mV

T16 blue -357 mV red. -463 mV

electrodes removed & rinsed in 10 ml of 18.1 mΩ H_2O (0.2 μm filt) stainless (red & blue) then swabbed & swirl plated = 1 ml of PBS vortexed on high for 1.5 min & plate counted (milk & 45 sec).

T15-B5 - 523 mV$_{SCE}$ T15-B6 -523 mV$_{SCE}$

T16-B5 - 523 mV$_{SCE}$ T16-B6 -523 mV$_{SCE}$

Removed flasks Ym-100-5-24 120°C (11 thru 13) and controls 120°C (11 & 12) from shaking water bath. 100 ul samples were taken from each flask and 10 fold serial dilutions performed.

Weighed out 1 g of Ym-100-5-24 120°C (11 thru 13) and placed in 10 ml of sterile PBS. Rinsed each sample three times with 10 ml of sterile PBS and then sonicated for 1 min. Did 10 fold serial dilutions

3:55

T15 30°C, pH 8.004, pt -197 mV
b -254 mV, r -202 mV, w -245 mV, y -177 mV

T16 30°C, pH 8.108, pt -293 mV
b -461 mV, r -431 mV, w -459 mV, y -325 mV

3:58 (88634) T = 25 ≡ 529 hrs.

1 ml samples taken from T15 & T16 for bacterial counts. Residual labeled as T15-529 & T16-529 and frozen

Performed Aw counts on sample Ym-100-5-24 120°C #13 using Aqua lab CX-2 SN 0493785 calibrated with

MgCl	0.330 ± 0.000	(Greenspan 0.331 ± 0.002)
NaCl	0.771 ± 0.000	(Greenspan 0.755 ± 0.001)
NaOH	0.068 ± 0.000	(Greenspan 0.089 ± 0.024)
Ym-100-5-24 120°C #13	0.051 ± 0.000	

Removed flasks Ym-100-5-24 120°C (14 thru 16) and controls 120°C (13 & 14) from oven and placed in RH chamber at 16:30.

Alice Stone

/29/96 15:50 complete crosbel yet again restarted as previous notes but D ie T16MNO2.DAT & T15TM100.DAT (add 553 hr to dwnl.)

T15 28°c pH 7.99 pt -299mV b -311mV r -293mV w -319mV y -257
T16 25°c pH 8.1 pt -284mV b -543mV r -497mV w -521mV y -305mV

1ml Sampled for plate count & residual frozen T15-553 & T16-553

Removed flasks Ym-100-5-24 120°C (14 thru 16) & control 120°C (13 & 14) from ~~22~~ Refrigerator & placed in shaking H2O bath at 30°c after addition of SOW of R_1 & R_2

6/24/96

/30/96 16:10 94787
T15 28°c pH 7.9 pt -308 b -413, r -379, w -366 y -357mV
T16 28°c pH 8.0 pt -290mV b -554mV, r -524mV, w -542mV y -341mV
1ml Sampled for plate count on bluff stone residual frozen T15-579 T16-579

C/30/96

7/1/96 8:15 (145000) T = 40 ≡ 593 hrs
T15 27°C, pH 7.975, pt -302 mV
b -428 mV, r -389 mV, w -370 mV, y -370 mV
T16 27°C, pH 8.059, pt -289 mV
b -558 mV, r -531 mV, w -544 mV, y -343 mV
1 ml samples taken for plate counts & residual frozen (T15-593 & T16-593)

Results from biofilm counts on T15 & T16

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
B5-523	523	4.08×10^4	1.42×10^4
B6-523	523	2.92×10^4	2.08×10^4

Results from plate counts on T15 & T16:

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
6/28/96	521	2.17×10^6	1.25×10^7
6/28/96	529	2.17×10^7	1.80×10^6
6/29/96	553	2.92×10^6	2.58×10^7

Results obtained from plate counts incubated at 120°C for 48 hrs:

Ym-100-5-24 120°C #11	2.17×10^8 CFU/ml
Ym-100-5-24 120°C #12	2.17×10^8 CFU/ml
Ym-100-5-24 120°C #13	2.33×10^8 CFU/ml
control 120°C #11	no counts
control 120°C #12	no counts

Results obtained from plate counts of bacteria present on rocks; samples incubated at 120°C for 48 hrs.

Ym-100-5-24 120°C #11	2.33×10^5 CFU/ml
Ym-100-5-24 120°C #12	2.50×10^5 CFU/ml
Ym-100-5-24 120°C #13	2.83×10^5 CFU/ml

Made 10 ml 2mM $S_2O_3^-$ media as follows:
0.31642 g $Na_2S_2O_3$ lot # 923931A
342 ul Na lactate lot # 943605
added to 10 ml 18.1 MΩ H_2O. Autoclaved for 30 min at 14 psi and 121°C.

Run EIS parameters as before

T15 red	T15r594.2	Rp:	1.92 MΩ
T15 white	T15w594.2	Rp:	2.21 MΩ
T16 red	T16r594.2	Rp:	899 kΩ
T16 white	T16w594.2	Rp:	280 kΩ

Weighed out 3 x 4g of Ym-100-5-24 and placed in sterilized conical flasks labeled Ym-100-5-24 150°C (1 thru 3) and placed in oven at 150°C. (15:00) control flasks labeled control 150°C (1 & 2) were also placed in the oven.

Isolated two colony types from plates incubated at 120°c for 48 hrs.
YmI-9 irregular, white, flat
YmI-10 circular, cloudy white, very small, raised

3:50 (172806) T=48 ≡ 601 hrs
T15 29°C, pH 7.996, pt -309 mV
b -435 mV, r -384 mV, w -375 mV, y -375 mV
T16 26°C, pH 8.051, pt -283 mV
b -556 mV, r -529 mV, w -547 mV, y -349 mV
1 ml samples taken from T15 & T16 for plate counts & residual frozen. (T15-601 & T16-601)

Removed flasks Ym-100-5-24 100°C (21 thru 23) and controls 100°C (21 & 22) from shaking water bath 100ul samples were taken from each flask and 10 fold serial dilutions were performed

Weighed out 1g of Ym-100-5-24 100°C (21 thru 23) and placed in 10 ml of sterile PBS. Rinsed each sample three times with 10 ml of sterile PBS and sonicated for 1 min. Did 10 fold serial dilutions.

Added 10 ml of 2mM $S_2O_3^-$ media to T15 at 16:30

Alice Stone

7/2/96

8:05 (231255) T=64 ≡ 617 hrs
T15 29°C, pH 7.997, pt -308 mV
b -442 mV, r -390 mV, w -376 mV, y -373 mV
T16 29°C, pH 7.993, pt -285 mV
b -553 mV, r -530 mV, w -545 mV, y -344 mV
1 ml samples taken from T15 & T16 for plate counts & residual frozen. (T15-617 & T16-617)

Results from plate counts on T15 & T16:

Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
579	2.42×10^7	4.75×10^6
593	1.12×10^7	3.83×10^5

Determination of repassivation potential on carbon steel A516 grade 60 exposed to Desulfovibrio vulgaris in 10% lactate/acetate media (DIV06) and Thiobacillus ferrooxidans in 10% thio media (DIV06) p50/138.
Final potentials: SRB -726 mV SCE & thio -621 mV SCE
Put specimens in fresh media, degased 1hr with 99.99% N_2 at RT. Run Parameters:

initial delay	20 s	initial pot	-0.200 V
scan rate	0.1670 mV/s	vertex 1 pot	0.100 V
scan incr.	5.00 mV	i threshhold	1.00×10^{-3} A/cm²
sample area	8.00 cm²	Final pot	-0.300 V
density	7.90 g/ml		

(setup saved as: COPSETUP)

+Thiobacilli pt -286 mV SCE A516 -629 mV pH 7.25
+SRB pt -176 mV SCE A516 -225 mV

2:50 (255543) T = 71 ≡ 624 hrs
T15 31°C, pH 7.992, pt -310 mV
b -447 mV, r -395 mV, w -381 mV, y -378 mV
T16 30°C, pH 7.980, pt -180 mV
b -524 mV, r -494 mV, w -527 mV, y -306 mV
1 ml samples taken from T15 & T16 for plate counts & residual frozen. (T15-624 & T16-624)

Alice Stone 7/2/96

7/3/96

8:18 (318366) T = 88 ≡ 641 hrs
T15 29°C, pH 8.051, pt -304 mV
b -461 mV, r -403 mV, w -386 mV, y -390 mV
T16 29°C, pH 7.969, pt -295 mV
b -551 mV, r -529 mV, w -548 mV, y -346 mV

1 ml samples taken from T15 & T16 for plate counts.
Residual labeled as T15-641 & T16-641 and frozen.

Results from plate counts on T15 & T16:

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
7/1/96	601	1.83×10^7	1.83×10^6
7/2/96	617	9.33×10^6	7.17×10^6
7/2/96	624	3.25×10^6	1.50×10^7

Results obtained from samples incubated at 100°C for 96 hrs:

Ym-100-5-24 100°C #21	1.50×10^8 CFU/ml
Ym-100-5-24 100°C #22	1.25×10^8 CFU/ml
Ym-100-5-24 100°C #23	1.92×10^8 CFU/ml
control 100°C #21	4.50×10^7 CFU/ml
control 100°C #22	2.50×10^7 CFU/ml

Results obtained from plate counts of bacteria present on rocks; samples incubated at 100°C for 96 hrs:

Ym-100-5-24 100°C #21	1.66×10^5 CFU/ml
Ym-100-5-24 100°C #22	1.66×10^5 CFU/ml
Ym-100-5-24 100°C #23	2.08×10^5 CFU/ml

Made biofilm counts on T15 & T16 13:52 (338471)
T=94 ≡ 647 hrs using MEP #1 (12:00) white & yellow
T15 white -365 mV yellow -326 mV
T16 white -392 mV yellow -412 mV
Electrodes removed and rinsed in 10 ml of 18.1 mΩ H_2O 0.2 um filter sterilized. Swabbed and then placed swab in 1 ml of sterile PBS. Vortexed on high for 1.5 min and plate counted
T15-W1-647 T15-W2-647
T16-W1-647 T16-W2-647

14:24 (341493) T=95 ≡ 648 hrs
T15 30°C, pH 8.029, pt -188 mV
b -380 mV, r -320 mV, w -313 mV, y -287 mV

T16 30°C, pH 7.896, pt -181 mV
b -337 mV, r -330 mV, w -353 mV, y -248 mV
1 ml samples taken from T15 & T16 for plate counts & residual frozen. (T15-648 & T16-648).

Performed Aw counts on sample Ym-100-5-24 150°C #1 using Aqua lab CX-2 SN 0493785 calibrated with

MgCl	0.341 ± 0.000	(Greenspan 0.331 ± 0.002)
NaCl	0.773 ± 0.001	(Greenspan 0.755 ± 0.001)
NaOH	0.068 ± 0.000	(Greenspan 0.089 ± 0.024)
Ym-100-5-24 150°C #1	0.079 ± 0.000	

Removed flasks Ym-100-5-24 150°C (1 thru 3) and controls 150°C (1 & 2) from oven and placed in RH chamber at 16:30.
Alice Stone

7/5/96

8:33 (492094) T=137 ≡ 690 hrs
T15 25°C, pH 7.211, pt -82 mV
b -152 mV, r -99 mV, w -145 mV, y -77 mV
T16 27°C, pH 7.200, pt -11 mV
b -122 mV, r -130 mV, w -128 mV, y -156 mV
1 ml samples taken from T15 & T16 for plate counts & residuals frozen. (T15-690 & T16-690).

Results from plate counts on T15 & T16:

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
7/3/96	641	8.08×10^6	4.00×10^8
7/3/96	648	6.42×10^6	6.83×10^6

Results from biofilm counts on T15 & T16:

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
W1	647	2.08×10^4	2.58×10^5
W2	647	1.50×10^6	3.00×10^5

On 7/2/96 ~2.0 g of Ym-100-5-24 was placed in ~6 ml of sterile R2B and incubated. On 7/3/96 100 ul of sample was taken and a 10 fold serial dilution was performed.

Results from above sampling: 2.00×10^4 CFU/ml
4 different colony types visible and similar to:
YmI-1, YmI-2, YmI-3, & YmI-9.

Removed flasks Ym-100-5-24 150°C (1 thru 3) and controls 150°C (1 & 2) from RH chamber and added 50 ml of sterile R2B to each. Placed flasks in shaking water bath @ 14:00.

Removed flasks Ym-100-5-24 (14-16) 120°C and controls 120°C (13 & 14) from shaking water bath. 100 ul samples were taken and serial dilutions were performed.

7/8/96

120°C
Weighed out 1g of Ym-100-5-24^ (14 thru 16) and placed in 10 ml of sterile PBS. Rinsed each sample three times with 10 ml of sterile PBS. and then sonicated them for 1 min. Did 10-fold serial dilutions.

14:45 (514581) T = 143 ≡ 696 hrs
T15 27°C, pH 8.241, pt -463 mV
b -207 mV, r -187 mV, w -204 mV, y -182 mV
T16 29°C, pH 8.325, pt -249 mV
b -364 mV, r -341 mV, w -368 mV, y -321 mV
1 ml samples taken from T15 & T16 for plate counts & residual frozen. (T15-696 & T16-696)

Alice Stone
7/5/96

7/6/96

16:00 (605377) T = 168 ≡ 721 hrs
T15 29°C, pH 8.192, pt -392 mV
b -282 mV, r -239 mV, w -267 mV, y -235 mV
T16 31°C, pH 8.387, pt -343 mV
b -442 mV, r -406 mV, w -442 mV, y -359 mV
1 ml samples taken from T15 & T16 for plate counts & residual frozen. (T15-721 & T16-721).

Alice Stone 7/6/96

7/7/96

11:40 (676087) T = 188 ≡ 741 hrs
T15 26°C, pH 8.266, pt -460 mV
b -351 mV, r -326 mV, w -330 mV, y -314 mV
T16 29°C, pH 8.313, pt -293 mV
b -459 mV, r -421 mV, w -460 mV, y -356 mV
1 ml samples taken from T15 & T16 for plate counts & residuals frozen. (T15-741 & T16-741).

Alice Stone 7/7/96

8:20 (750500) T = 208 ≡ 761 hrs
T15 26°C, pH 8.426, pt -446 mV
b -377 mV, r -341 mV, w -343 mV, y -329 mV
T16 29°C, pH 8.210, pt -343 mV
b -483 mV, r -439 mV, w -478 mV, y -354 mV
1 ml samples taken from T15 & T16 for plate counts & residuals frozen. (T15-761 & T16-751)

Results from plate counts on T15 & T16:

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
7/5/96	690	4.75×10^7	3.08×10^7
7/5/96	696	4.58×10^7	4.16×10^6
7/6/96	724	5.25×10^6	2.25×10^7
7/7/96	741	4.42×10^7	3.37×10^7

Results obtained from samples incubated at 120°C for 120 hrs:

Ym-100-5-24 120°C #14	1.17×10^8 CFU/ml
Ym-100-5-24 120°C #15	no counts
Ym-100-5-24 120°C #16	no counts
control 120°C #13	no counts
control 120°C #14	no counts

Results obtained from plate counts of bacteria present on rocks; samples incubated at 120°C for 120 hrs:

Ym-100-5-24 120°C #14	1.42×10^5 CFU/ml
Ym-100-5-24 120°C #15	no counts
Ym-100-5-24 120°C #16	no counts

Made biofilm counts on T15 & T16 @ 13:54 (770544)
T= 214 ≡ 767 hrs using MEP#2 (9:00) white & yellow

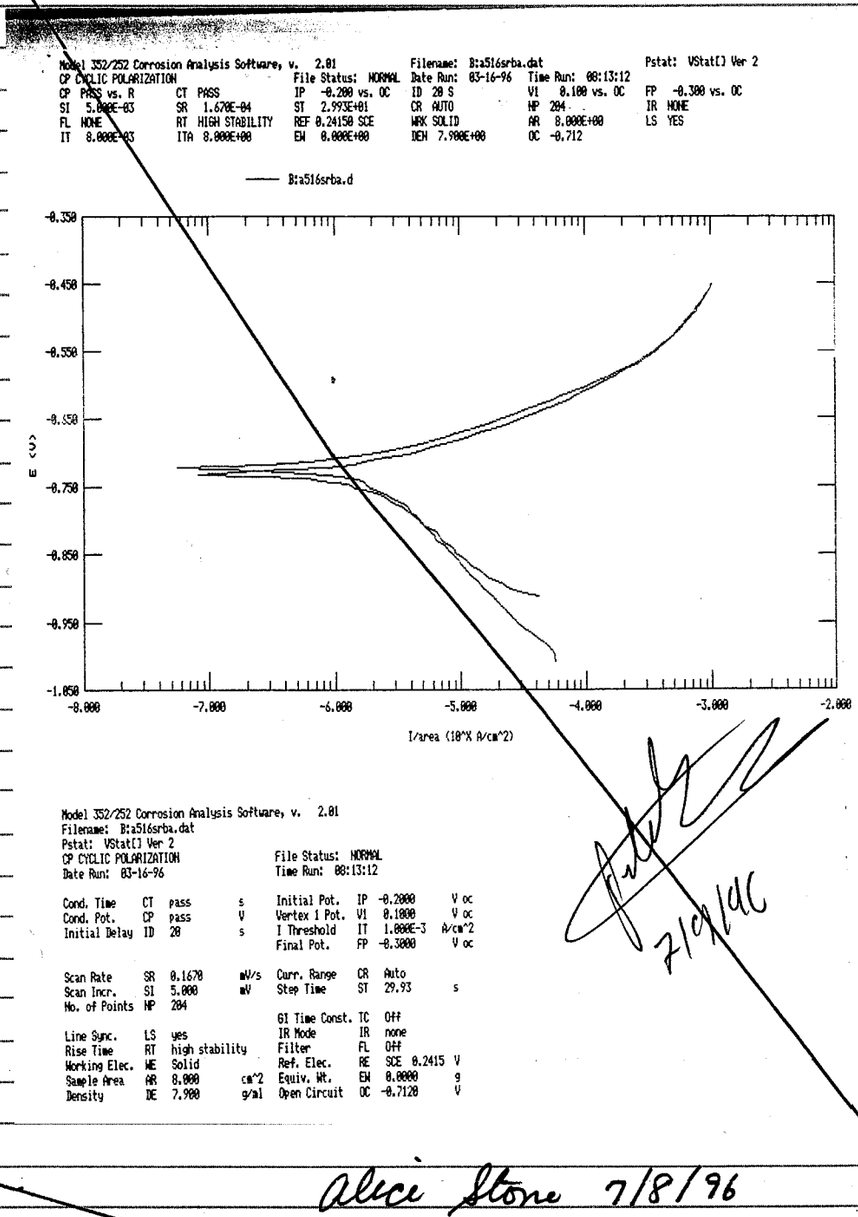
T15	white -142 mV	yellow -157 mV
T16	white -257 mV	yellow -378 mV

Electrodes removed and rinsed in 10ml of 18.1 MΩ H_2O 0.2μm filter sterilized. Swabbed and then placed in 1.0 ml of sterile PBS. Vortexed on high for 1.5 min and performed plate counts

T15-767-W3	T15-767-W4
T16-767-W3	T16-767-W4

#15 (771888) T: 214 ≡ 767 hrs
T15 27°C, pH 8.328, pt -102mV
b -264 mV, r -228 mV, w -248mV, y -225mV
T16 27°C, pH 8.174, pt -118 mV
b -373 mV, r -338mV, w -470 mV, y -298mV
1 ml samples taken from each vessel for plate counts & residual frozen

Weighed out 3X 4g of Ym-100-5-24 and placed in sterilized conical flasks labeled Ym-100-5-24 100°C (31-33). Placed flasks along with controls 100°C (31 & 32) in oven at 100°C (15:30).

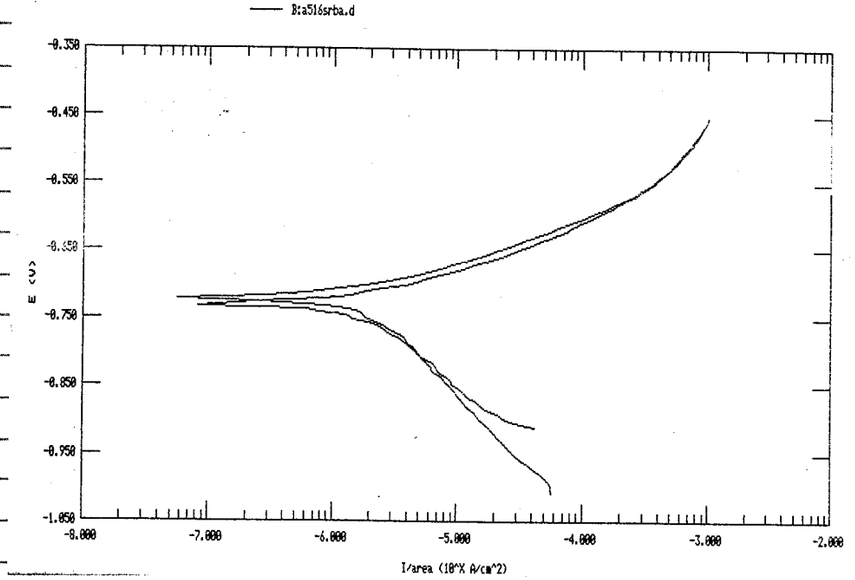


7/9/96

Alice Stone 7/8/96

10% lactate/acetate Medium abiotic (2 wk)

Model 352/252 Corrosion Analysis Software, v. 2.01
CP CYCLIC POLARIZATION
CP PASS vs. R | CT PASS | File Status: NORMAL | Filename: B:a516srba.dat | Pstat: VStat[] Ver 2
SI 5.000E-03 | SR 1.670E-04 | IP -0.200 vs. OC | Date Run: 03-16-96 | Time Run: 08:13:12
FL NONE | RT HIGH STABILITY | ST 2.993E+01 | ID 20 S | V1 0.100 vs. OC | FP -0.300 vs. OC
IT 8.000E-03 | ITA 8.000E+00 | REF 0.24150 SCE | CR AUTO | HP 204 | IR NONE
EW 0.000E+00 | WRK SOLID | AR 8.000E+00 | LS YES
DEN 7.900E+00 | OC -0.712



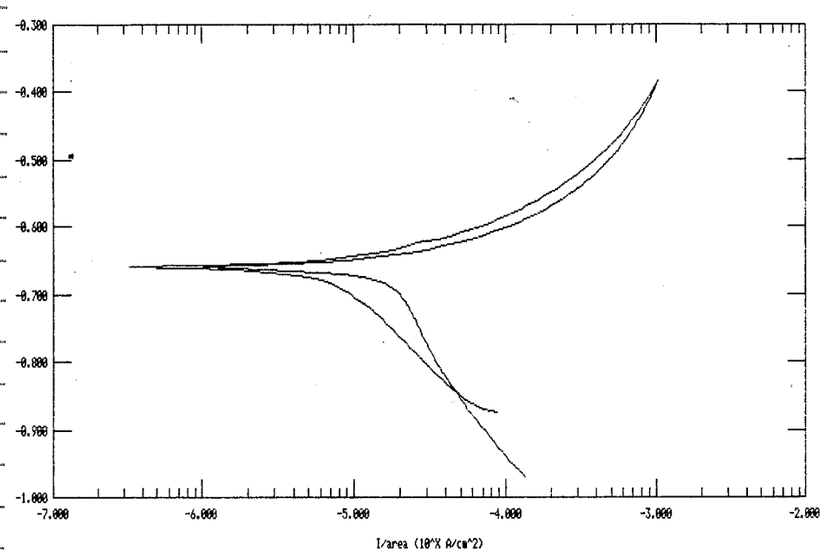
Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: B:a516srba.dat
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION | File Status: NORMAL
Date Run: 03-16-96 | Time Run: 08:13:12

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	V1	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	204					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7120	V

Alice Stone
7/8/96

10% Thio medium abiotic (2 wk)

Model 352/252 Corrosion Analysis Software, v. 2.01
CP CYCLIC POLARIZATION
CP PASS vs. R | CT PASS | File Status: NORMAL | Filename: B:a516thia.dat | Pstat: VStat[] Ver 2
SI 5.000E-03 | SR 1.670E-04 | IP -0.200 vs. OC | Date Run: 03-16-96 | Time Run: 13:44:51
FL NONE | RT HIGH STABILITY | ST 2.993E+01 | ID 20 S | V1 0.100 vs. OC | FP -0.300 vs. OC
IT 8.000E-03 | ITA 8.000E+00 | REF 0.24150 SCE | CR AUTO | HP 216 | IR NONE
EW 0.000E+00 | WRK SOLID | AR 8.000E+00 | LS YES
DEN 7.900E+00 | OC -0.674



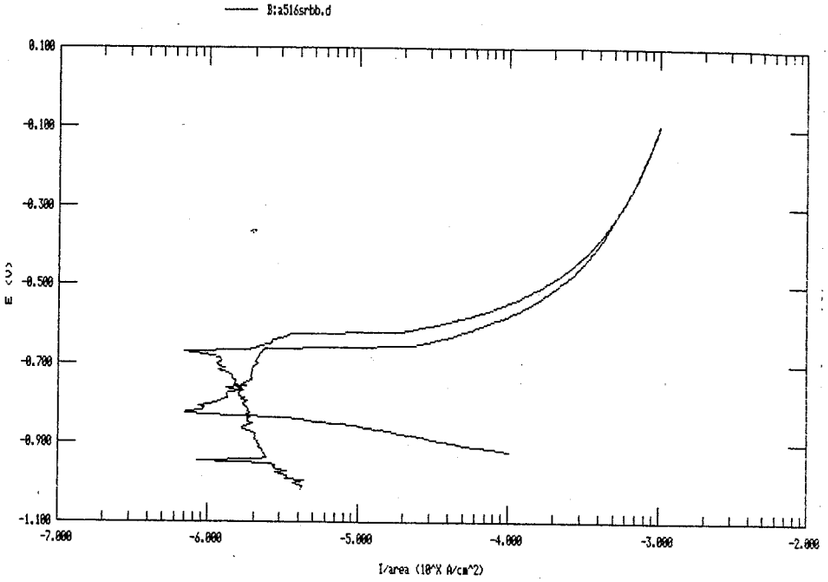
Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: B:a516thia.dat
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION | File Status: NORMAL
Date Run: 03-16-96 | Time Run: 13:44:51

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	V1	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	216					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.6740	V

Alice Stone
7/8/96

Desulfovibrio vulgaris 2 wk exposure

Model 352/252 Corrosion Analysis Software, v. 2.01
CP CYCLIC POLARIZATION
CP PASS vs. R CT PASS
SI 5.000E-03 SR 1.670E-04
FL NONE RT HIGH STABILITY
IT 8.000E-03 ITA 8.000E+00
File Status: NORMAL
IP -0.200 vs. OC
ST 2.993E+01
REF 0.24150 SCE
EW 0.000E+00
Filename: B:a516srbb.dat
Date Run: 03-15-96 Time Run: 13:23:04
ID 20 S VI 0.100 vs. OC
CR AUTO NP 350
WRK SOLID AR 8.000E+00
DEN 7.900E+00 OC -0.721
Pstat: VStat[] Ver 2
FP -0.300 vs. OC
IR NONE
LS YES



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: B:a516srbb.dat
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION
Date Run: 03-15-96
File Status: NORMAL
Time Run: 13:23:04

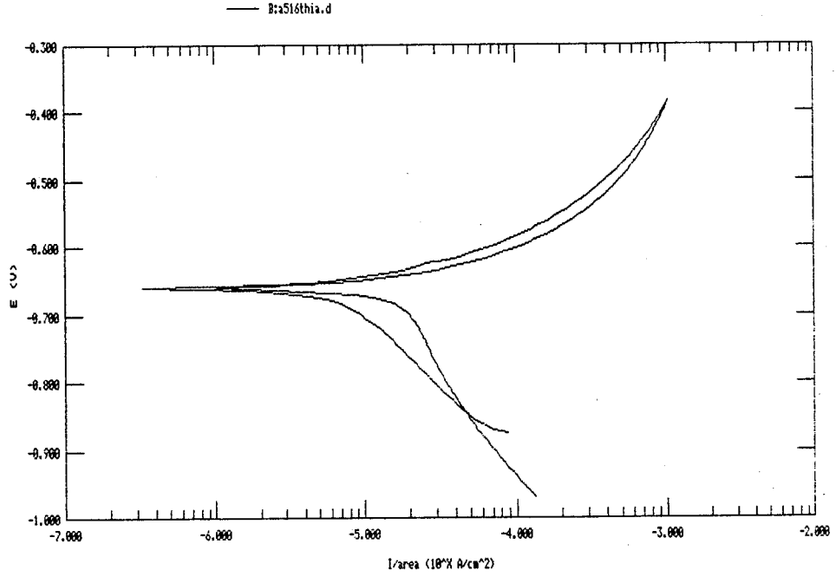
Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	VI	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	350					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7210	V

Alice Stone
7/8/96

7/9/96

8:30 (837420) T = 236 ≡ 789 hrs
T15 26°C, pH 8.008, pt -9 mV
b -183 mV, r -148 mV, w -177 mV, y -141 mV
T16 24°C, pH 8.129, pt -324 mV
b -468 mV, r -408 mV, w -462 mV, y -346 mV
1 ml samples taken from T15 & T16 for plate counts & residuals frozen: (T15-789 & T16-789).

Model 352/252 Corrosion Analysis Software, v. 2.01
CP CYCLIC POLARIZATION
CP PASS vs. R CT PASS
SI 5.000E-03 SR 1.670E-04
FL NONE RT HIGH STABILITY
IT 8.000E-03 ITA 8.000E+00
File Status: NORMAL
IP -0.200 vs. OC
ST 2.993E+01
REF 0.24150 SCE
EW 0.000E+00
Filename: B:a516thia.dat
Date Run: 03-16-96 Time Run: 13:44:51
ID 20 S VI 0.100 vs. OC
CR AUTO NP 216
WRK SOLID AR 8.000E+00
DEN 7.900E+00 OC -0.674
Pstat: VStat[] Ver 2
FP -0.300 vs. OC
IR NONE
LS YES



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: B:a516thia.dat
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION
Date Run: 03-16-96
File Status: NORMAL
Time Run: 13:44:51

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	VI	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	216					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.6740	V

7/9/96

16:18 (865594) T = 240 ≡ 793 hrs
T15 30°C, pH 8.319, pt -395 mV
b -273 mV, r -231 mV, w -256 mV, y -224 mV
T16 28°C, pH 8.095, pt -320 mV
b -477 mV, r -417 mV, w -471 mV, y -346 mV
1 ml samples taken from T15 & T16 for plate counts and residual frozen. (T15-793 & T16-793)

Alice Stone 7/9/96

7/10/96 8:20 (923323) T = 256 ≡ 809 hrs
T15 25°C, pH 8.469, pt -395 mV
b -318 mV, r -262 mV, w -304 mV, y -283 mV
T16 24°C, pH 8.086, pt -315 mV
b -494 mV, r -433 mV, w -485 mV, y -351 mV
1 ml samples taken from T15 & T16 for plate counts. Residual labeled as T15-809 & T16-809 and frozen.

Results from plate counts on T15 & T16:

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
7/8/96	761	5.08×10^7	4.25×10^7
7/8/96	767	2.00×10^7	3.08×10^7
7/9/96	789	4.25×10^7	3.08×10^7
7/9/96	793	7.08×10^7	3.67×10^7

Results from biofilm counts on T15 & T16:

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
W3	767	1.33×10^5	3.75×10^4
W4	767	1.00×10^5	2.33×10^4

Removed flasks ym-100-5-24 100°C (31 thru 33) and controls 100°C (31 & 32) from oven and placed in RH chamber @ 14:30.

Weighed out 3x 4 g of ym-100-5-24 and placed in sterilized conical flasks labeled ym-100-5-24

120°C (21 thru 23). Placed flasks along with controls 120°C (21 & 22) in oven set at 120°C @ 14:30.

15:00 (947493) T = 263 ≡ 816 hrs
T15 26°C, pH 8.529, pt -417 mV
b -330 mV, r -280 mV, w -314 mV, y -294 mV
T16 25°C, pH 8.069, pt -314 mV
b -499 mV, r -439 mV, w -490 mV, y -353 mV
1 ml samples taken from T15 & T16 for plate counts and residuals frozen. (T15-816 & T16-816)

Alice Stone 7/10/96

7/11/96 8:30 (1010158) T = 281 ≡ 834 hrs
T15 26°C, pH 8.559, pt -454 mV
b -362 mV, r -321 mV, w -333 mV, y -344 mV
T16 24°C, pH 8.059, pt -311 mV
b -509 mV, r -449 mV, w -500 mV, y -354 mV
1 ml samples taken from T15 & T16 for plate counts and residuals frozen (T15-834 & T16-834).

Results from plate counts on T15 & T16:

7/10/96	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
	809	8.33×10^7	2.83×10^7

Ran EIS using Solartron 1260 and EG&G 273 serial no. 41108 potentiostat. Set up as before
T15 red = T15r835.z
T15 white = T15w835.z
T16 red = T16r835.z
T16 white = T16w835.z

Made biofilm counts on T15 & T16 @ 10:40 (1017899)
T = 282 ≡ 835 hrs using MEP4 (center)
T15 white = -314 mV T16 white = -466 mV
T15 red = -293 mV T16 red = -400 mV

T15 blue = -352 mV T16 blue = -496 mV
T15 yellow = -327 mV T16 yellow = -310 mV
Electrodes removed and rinsed in 18.1 mΩ H_2O 0.2um filter sterilized. Swabbed and then placed in 1.0 ml of sterile PBS. Vortexed on high for 1.5min and performed plate counts.

T15b-835	T16b-835
T15r-835	T16r-835
T15w-835	T16w-835
T15y-835	T16y-835

Removed flasks Ym-100-5-24 100°c (31 thru 33) and Controls 100°c (31 & 32) from Rit chamber and added 50 ml of sterile R2B to each. Placed flasks in shaking water bath @ 16:30.
Alice Stone 7/11/96

7/12/96 Results from plate counts on T15 & T16

	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
7/10/96	816	5.42×10^7	3.42×10^7
7/11/96	834	6.67×10^7	3.50×10^7

Removed flasks Ym-100-5-24 150°c (1 thru 3) and Controls 150°C (1 & 2) were removed from the shaking water bath and 100 ul samples were taken. Serial dilutions were performed.

Weighed out 1g of Ym-100-5-24 150°c (1 thru 3) and placed in sterile PBS. Rinsed each sample three times with 10 ml sterile PBS and then sonicated for 1 min. 10-fold serial dilutions were performed

Alice Stone
7/12/96

7/15/96 Results obtained from samples incubated at 150°c for 48 hrs:

Ym-100-5-24	150°c #1	6.67×10^7 CFU/ml
Ym-100-5-24	150°c #2	3.33×10^8 CFU/ml
Ym-100-5-24	150°c #3	no counts
control	150°c #1	no counts
control	150°c #2	no counts

Results obtained from samples incubated at 150°c for 48 hrs; Plate counts for bacteria present on rocks:

Ym-100-5-24	150°c #1	1.33×10^5 CFU/ml
Ym-100-5-24	150°c #2	3.92×10^5 CFU/ml
Ym-100-5-24	150°c #3	no counts

Results from biofilm counts on T15 & T16:

Coupon	Time (hrs)	T15 (CFU/ml)	T16 (CFU/ml)
red	835	6.42×10^6	1.08×10^4
white	835	5.33×10^6	2.00×10^5
blue	835	9.00×10^5	3.83×10^4
yellow	835	2.42×10^6	3.92×10^4

Made 10% TSB in 500ml and 250ml flask as follows:
TSB lot#58855JE 3.0g per 500ml 18.1 MΩ H_2O
TSB " 1.5g per 250ml 18.1 MΩ H_2O
autoclaved for 45min at 121°c and 14psi.

pH calibration using Orion EA920 meter serial no. 5001A. Electrode in size 7 bung Orion EA940 & Electrode in size 6 bung Orion EA920 serial no 5001A. pH buffer solutions used: buffer solution pH 10.00 lot # 960984-24, buffer solution pH 7.00 lot # 961196-24, & buffer solution pH 4.00 lot # 960230-24.
pH 7.00 readings:
size 6 bung 10.5 mV
size 7 bung 15.8 mV

pH 4.0 readings:

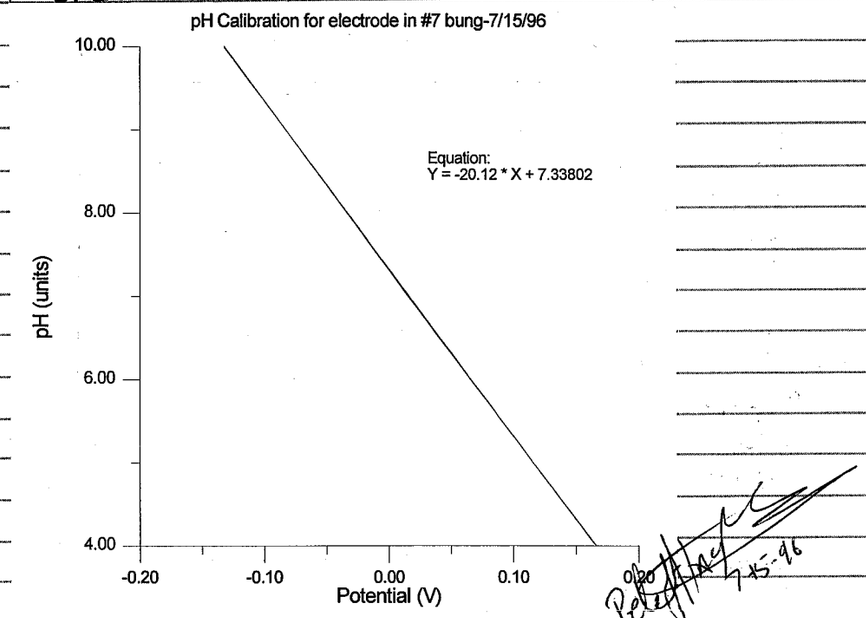
size 6 bung 151.1 mV

Size 7 bung 166.4 mV

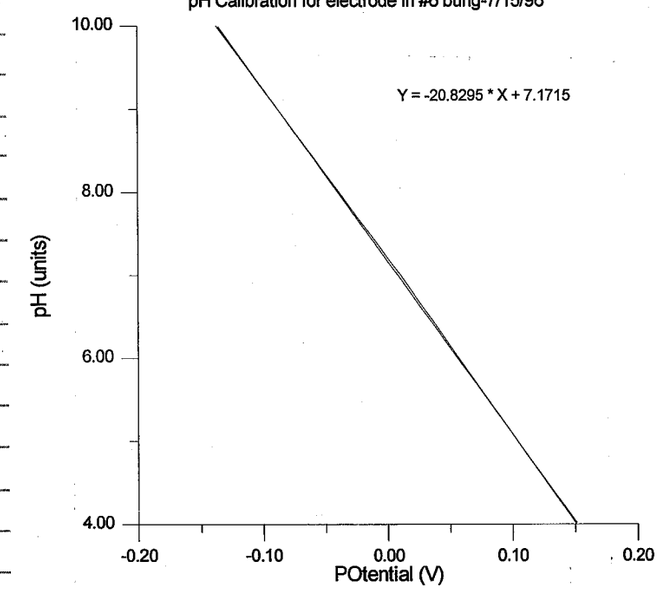
pH 10.00 readings:

size 6 bung -136.9 mV

Size 7 buna -131.8 mV



Peter Lacy 7-15-96



Made 500 ml of 0.525% sodium hypochlorite solution. pH electrodes where sterilized overnight.

Sample Ym-100-5-16 b was crushed and fractionated to 2-4mm. Now called Ym-100-6-24. sieve (CASTE-11).

Removed flasks Ym-100-5-24 120°C (21-23) and controls 120°C (21 & 22) from oven and placed in RH chamber @ 15:30.

Weighed out 3 x 4 g of Ym-100-6-24 and placed in sterilized conical flasks labeled Ym-100-6-24 150°C (4 thru 6); ~~also~~ placed flasks along with controls 150°C (3 & 4) in oven set at 150°c @ 16:30.

Alice Stone 7/15/96

7/16/96 Removed pH electrodes from 0.525% sodium hypochlorite solution and placed in 10% TSB (p.27/182).

Made 1000ml of media based on J13 well water at Yucca Mtn. site as follows:

NaCl	lot # 947723	1.6485g ≈ 1000 ppm Cl^-	1.64852 g
$NaHCO_3$	897789	0.1170 g ≈ 85 ppm HCO_3^-	0.11704 g
Na_2SO_4	901213	0.0296 g ≈ 25 ppm SO_4^-	0.02954 g
$NaNO_3$	897183	0.0137 g ≈ 10 ppm NO_3^-	0.13699 g
NaF	950992	0.0044 g ≈ 2 ppm F^-	0.00446 g

into 1000 ml 18.1 MΩ H_2O.

Removed flasks Ym-100-5-24 120°c (21 thru 23) and controls 120°c (21 & 22) from RH chamber and adde 50ml of sterile R2B to each. Placed in shaking water bath at 10:30

Vessels ethylene sterilized at CBS.

Made 1L of media to be used in anaerobic T17 vessel as follows:

0.31642 g $Na_2S_2O_3$ (2mM)	lot #	923931A
1.64859 g NaCl		947723
1ml trace minerals		
100ml 10x Mn media (p. 121/134)		
900ml 18.1MΩ H_2O		

Autoclaved for 1hr at 121°C & 14psi

Made 1L of media to be used in aerobic T18 vessel as follows:

0.16998 g $NaNO_3$ (2mM)	lot #	~~923931A~~ 897183
1.64859 g NaCl		947723
1 ml trace minerals		
100ml 10x Mn media (p. 121/134)		
900ml 18.1MΩ H_2O		

Autoclaved for 1hr at 121°C & 14psi.

Alice Stone 7/16/96

7/17/96 Made 1L of modified J13 media as follows:

Basal -

NaCl	lot # 947723	1.64850 g
$NaHCO_3$	897789	0.11700 g
$MgSO_4 \cdot 7H_2O$	947409	0.05647 g
$FeSO_4 \cdot 7H_2O$	946178	0.01000 g
NaF	950992	0.00440 g
$NaNO_3$	897183	0.01370 g
Lactate 60%	943605	341 ul

into 1000ml 18.1MΩ H_2O. 6 x 100ml of basal was placed in 250ml flasks labeled 1-6. Flask 1 contained 100ml of basal.

Flask 2 - 100ml basal + 0.001g yeast extract (lot#59695JB) 10~~0~~ppm yeast extract

Flask 3 - 100ml basal + 0.0001g ascorbic acid (lot # 947449) 1ppm ascorbic acid

Flask 4 - 100ml basal + 0.001g ascorbic acid (lot # 947449) 10ppm ascorbic acid

Flask 5 - 100ml basal + 0.001g Yeast extract + 0.001g ascorbic acid. 10ppm yeast extract & 10ppm ascorbic acid

Flask 6 - 100ml basal + 0.001g yeast extract + 0.0001g ascorbic acid. 10ppm yeast extract & 1ppm ascorbic acid.

Flasks were degased with 95% N_2 & 5% H_2. Dispensed into 9ml aliquots and autoclaved for 45min at 121°C and 14psi.

Removed flasks Ym-100-5-24 $^{100°C}$ (31 thru 33) and controls 100°C (31 & 32) from the shaking water bath 100ul samples were taken and 10 fold serial dilutions were performed.

Weighed out 1g of Ym-100-5-24 (31 thru 33) 100°C and placed in sterile PBS. Rinsed each sample three times with 10ml sterile PBS and then sonicated for 1min. Performed 10 fold serial dilutions.

On 7/15/96 reaction vessels set up with PPE, drip tubes & medium outlet, gas sparge stones, and other holes filled with bungs, except for one covered with autoclave paper. Autoclaved at 121°C for 1hr @ 14psi. As cooling MEP's were sonicated for 10min in absolute acetone and air dried. MEP's were placed in vessels immediately upon removal from autoclave. Thermometers sterilized with isopropanal and placed in vessels. (SN 0323007 & SN 0323005). Vessels were then placed in incubator at 70°C.

7/17/96 Alice Stone

7/18/96

Set up vessels T17 & T18. Round top vessel (T17) labeled as T17 Thio and filled with media prepared on p. 30. Other vessel designated T18 was filled with media prepared on p. 30. pH electrodes removed from 10% TSB and placed in appropriate vessels. Began abiotic run.

9:30 T = 0 hrs

T18 24°C, pt 30 mV, pH 7.978
b -112 mV, r -106 mV, w -114 mV, y -123 mV

T17 24°C, pt -4 mV, pH 7.814
b -122 mV, r -120 mV, w -128 mV, y -120 mV

Determination of repassivation potential on carbon steel A516 grade 60 in J13 media (p. 29/182). Put specimen in fresh media and degased for 1 hr with 99.99% N_2 at RT.
Run parameters:

initial delay	20 s	initial potential	-0.200 V
scan rate	0.1670 mV/s	vertex I pot.	0.100 V
scan incr.	5.00 mV	I threshhold	1.00×10^{-3} A/cm²
sample area	8.00 cm²	final potential	-0.300 V
density	7.90 g/ml	(setup saved as: CPPSETUP)	

Results obtained from samples incubated at 100°C for 48 hrs:

Ym-100-5-24 100°C #31	5.00×10^{8} CFU/ml
Ym-100-5-24 100°C #32	$5.50 \times 10^{+8}$ CFU/ml
Ym-100-5-24 100°C #33	$5.17 \times 10^{+8}$ CFU/ml
control 100°C #31	no counts
control 100°C #32	no counts

Results obtained from plate counts of bacteria present on rocks; samples incubated at 100°C for 48 hrs:

Ym-100-5-24 100°C #31	3.30×10^{5} CFU/ml
Ym-100-5-24 100°C #32	2.75×10^{5} CFU/ml
Ym-100-5-24 100°C #33	3.50×10^{5} CFU/ml

Model 352/252 Corrosion Analysis Software, v. 2.01 Filename: B:A516j13.dat Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION File Status: NORMAL Date Run: 03-17-96 Time Run: 10:27:25

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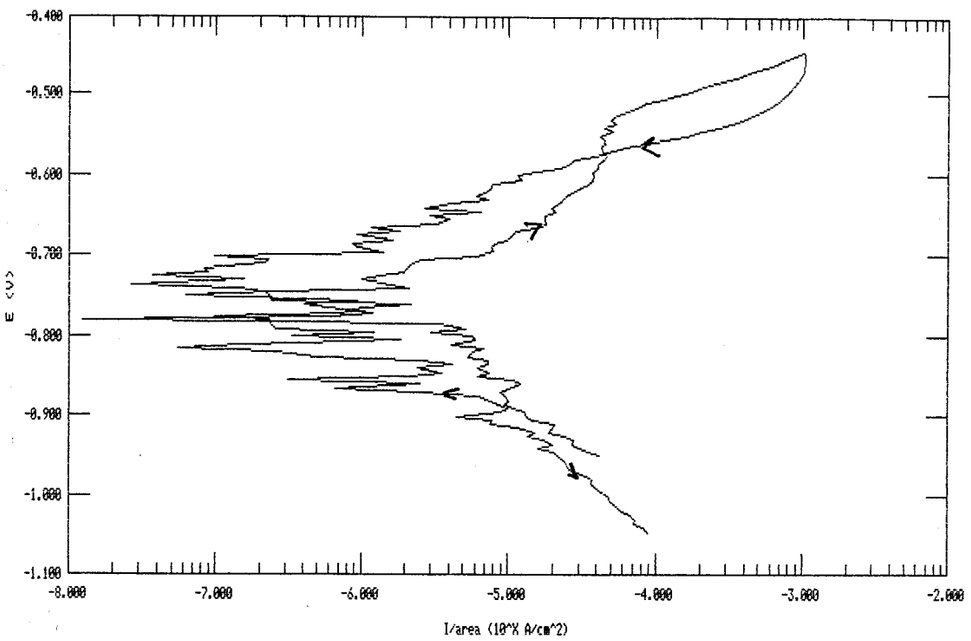
FL	NONE	RT	HIGH STABILITY	REF	0.2415S SCE	WRK	SOLID
IT	8.000E-03	ITA	8.000E+00	EW	0.000E+00	DEN	7.900E+00

VI	0.100 vs. OC	FP	-0.300 vs. OC		
NP	222	IR	NONE		
AR	8.000E+00	LS	YES		
OC	-0.751				



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: B:A516j13.dat
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION File Status: NORMAL
Date Run: 03-17-96 Time Run: 10:27:25

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	VI	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	222					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7510	V

Alice Stone 7/18/96

7/19/96

8:20 (85206) T = 24 hrs
T17 26°C, pH 8.111, pt -138mV, b -139mV, r -137mV, w -139mV, y -134 mV
T18 25°C, pH 7.795, pt 23mV, b -103mV, r -137mV, w -115mV, y -177mV
Checked above values using Kiethly 614 serial no. 467-374 calibrated Feb. 26, 1996. Results are as follows:
T17 pt -136mV, b -139mV, r -137mV, w -139mV, y -135mV
T18 pt 23mV, b -102mV, r -139mV, w -115mV, y -177mV

Removed flasks Ym-100-6-24 150°C (4 thru 6) and controls 150°C (3 & 4) from oven and placed in RH chamber @ 10:00.

Weighed out 3X4g of Ym-100-6-24 and placed in sterilized conical flasks labeled Ym-100-6-24 180°C (1 thru 3). Placed flasks along with controls 180°C (1 & 2) in oven at 180°C. (10:30).

Took 1ml samples from T17 & T18 for plate counts to check for contamination. (T17-24 & T18-24)

15:20 (110477) T = 31 hrs
T17 32°C, pH 8.110, pt -116mV, b -139mV, r -137mV, w -138mV, y -133mV
T18 30°C, pH 7.770, pt 22mV, b -105mV, r -141mV, w -118mV, y -182mV

Ran EIS using Solartron 1260 and EG&G 273 serial no. 41108 potentiostat. Setup as follows: 10KHz to 5mHz, ±5mV frequency, 10 steps per decade, 100uA, 0.01 attenuator.
T17 red = T17r31.Z Rp 2.918 mΩ * * SA 10cm2
T17 white = T17w31.Z Rp 1.829 mΩ* of 2cm²
T18 red = T18r31.Z Rp 2.154 mΩ*
T18 white = T18w31.Z Rp 2.132 mΩ* 7/21/96

Alice Stone
7/19/96

7/20/96

12:00 (185234) T = 51 hrs
T17 26°C, pH 8.180, pt -120mV
b -130mV, r -127mV, w -126mV, y -123mV
T18 25°C, pH 7.837, pt 19mV
b -109mV, r -150mV, w -115mV, y -190mV
1 ml samples taken from T17 & T18 for plate counts to check for contamination. (T17-51 & T18-51)

Alice Stone 7/20/96

7/21/96

18:30 (295161) T = 82 hrs
T17 30°C, pH 8.100, pt -129mV
b -134mV, r -130mV, w -126mV, y -121mV
T18 30°C, pH 7.731, pt 18mV
b -106mV, r -160mV, w -108mV, y -188mV

Removed flasks Ym-100-6-24 150°C (4 thru 6) and controls 150°C (3 & 4) from RH chamber. Added 50ml of sterile RZB to each and placed in shaking water bath.

Removed flasks Ym-100-6-24 180°C (1 thru 3) and controls 180°C (1 & 2) from oven and placed in RH chamber @ 18:30. Aluminum foil caps were replaced with sterilized bungs and paper caps.

Alice Stone 7/21/96

7/22/96

8:30 (345176) T = 96 hrs
T17 25°C, pH 8.106, pt -138mV
b -140mV, r -123mV, w -120mV, y -117mV
T18 25°C, pH 8.033, pt 13mV
b -116mV, r -167mV, w -118mV, y -188mV

Checked above values using Kiethly 614 serial no. 467-374 calibration Feb 26, 1996. Results are as follows:

T17 pt -138 mV, b -138 mV, r -122 mV, w -120 mV, y -117 mV
T18 pt 19 mV, b -116 mV, r -166 mV, w -118 mV, y -182 mV

1 ml samples taken from T17 & T18 for plate counts and residual frozen (T17-96 & T18-96).

Results from previous contamination checks:

hrs	counts
T17-24	no counts
T18-24	no counts
T17-51	no counts
T18-51	no counts

Preparation of samples to be innoculated into T17 & T18 as follows:
1 ml of sterile media removed from T17 and placed in sterile mc tube. Aseptically transfered 1 loop of SP200 from plate seeded on 7/20/96 (p. 65/138) to mctube. Vortexed for 30 seconds and innoculated into T17 @ 9:30.
1 ml of sterile media removed from T18 and placed in sterile mc tube. Aseptically transfered 1 small loop of Ym-10-A-04, Ym-10-H-12, Ym-10-H-05 & Ym-10-M-02 from plates seeded on 7/20/96 (p. 65/138) to mc tube. Vortexed for 30 seconds and innoculated into T18 @ 9:30.

10:40 (352877) T = 98 hrs
T17 28°C, pH 8.086, pt -121 mV
b -128 mV, r -121 mV, w -117 mV, y -115 mV
T18 26°C, pH 7.994, pt 22 mV
b -116 mV, r -113 mV, w -121 mV, y -110 mV
1 ml samples taken from T17 & T18 for plate counts and residual frozen (T17-98 & T18-98)

Ran EIS using Solartron 1260 and EG&G 273 serial no. 41108 potentiostat. Setup as follows: 10KHz to 5mHz, ± 5mV frequency, 10 steps per decade, 100uA, 0.01 attenuator

T17 red = T17r 98.2 3.861 mΩ* cm² SA set at 1 cm²
T17 white = T17w 98.2 4.262 mΩ* cm²
T18 red = T18r 98.2 4.720 mΩ* of 2 cm²
T18 White = T18w 98.2 2.551 mΩ* pebbt 6-26-96

Removed flasks Ym-100-5-24 (31 thru 33) 120°C and controls 120°C (31 & 32) from shaking water bath 100 ul samples were taken and serial dilutions were performed.

Weighed out 1g of Ym-100-5-24 120°C (31 thru 33) and placed in sterile PBS. Rinsed each sample three times with 10 ml sterile PBS and then sonicated for 1 min. 10-fold serial dilutions were performed.
Alice Stone 7/22/96

7/23/96 8:25 (431073) T = 120 hrs
T17 30°C, pH 8.064, pt -149 mV
b -138 mV, r -131 mV, w -129 mV, y -125 mV
T18 26°C, pH 8.011, pt 20 mV
b -107 mV, r -106 mV, w -135 mV, y -115 mV
1 ml samples taken from T17 & T18 for plate counts and residual frozen (T17-120 & T18-120)

Results obtained from samples incubated at 120°C for 120 hrs:

Ym-100-5-24 120°C # 31	1.67×10^7 CFU/ml
Ym-100-5-24 120°C # 32	2.08×10^7 CFU/ml
Ym-100-5-24 120°C # 33	1.92×10^7 CFU/ml
control 120°C #31	no counts
control 120°C #32	no counts

Results obtained from plate counts of bacteria present on rocks; samples incubated at 120°C for 120hrs:

Ym-100-5-24 120°C #31 2.50×10^5 CFU/ml
Ym-100-5-24 120°C #32 2.67×10^5 CFU/ml
Ym-100-5-24 120°C #33 2.33×10^5 CFU/ml

14:30 (452498) T= 126 hrs
T17 32°C, pH 8.017, pt -140mV
b -133mV, r -127mV, w -124mV, y -114mV
T18 30°C, pH 7.902, pt 17mV
b -105mV, r -102mV, w -134mV, y -112mV
1ml samples taken from T17 & T18 for plate counts and residual frozen (T17-126 & T18-126)
Alice Stone 7/23/96

7/24/96 8:20 (517348) T= 144 hrs
T17 30°C, pH 8.075, pt -160mV
b -145mV, r -139mV, w -138mV, y -134mV
T18 26°C, pH 7.745, pt 29mV
b -100mV, r -109mV, w -138mV, y -117mV
1ml samples taken from T17 & T18 for plate counts and residual frozen (T17-144 & T18-144).

Results obtained from plate counts on T17 & T18:

Time (hrs)	T17 (CFU/ml)	T18 (CFU/ml)
96	no counts	no counts
98	7.75×10^4	2.17×10^6
120	2.75×10^5	3.50×10^6

Removed flasks Ym-100-6-24 180°C (1thru3) and controls 180°C (1&2) from RH chamber and added 50ml of sterile R2B to each. Placed in shaking water bath.

14:35 (539690) T= 150 hrs
T17 34°C, pH 8.025, pt -141mV
b -133mV, r -127mV, w -123mV, y -115mV
T18 29°C, pH 7.684, pt 24mV
b -102mV, r -111mV, w -140mV, y -117mV
1ml samples were taken from T17 & T18 for plate counts and residual frozen. (T17-150 & T18-150).
Alice Stone 7/24/96

7/25/96 8:25 (603970) T= 168 hrs
T17 30°C, pH 8.002, pt -143mV
b -139mV, r -133mV, w -132mV, y -131mV
T18 27°C, pH 7.541, pt 24mV
b -93mV, r -107mV, w -135mV, y -114mV
1ml samples were taken from T17 & T18 for plate counts and residual frozen (T17-168 & T18-168).

Results obtained from plate counts on T18 & T17:

Time (hrs)	T17 (CFU/ml)	T18 (CFU/ml)
126	2.67×10^5	2.83×10^6

14:25 (625475) T= 174 hrs
T17 33°C, pH 8.076, pt -125mV
b -125mV, r -120mV, w -119mV, y -117mV
T18 29°C, pH 7.569, pt 25mV
b -97mV, r -109mV, w -131mV, y -116mV
1ml samples were taken from T17 & T18 for plate counts and residual frozen. (T17-174 & T18-174).
Alice Stone 7/25/96

7/26/96 8:25 (690195) T= 192 hrs
T17 29°C, pH 8.091, pt -133mV
b -141mV, r -135mV, w -135mV, y -133mV

T18 26°C, pH 7.708, pt 33mV
b -101 mV, r -112 mV, w -138 mV, y -121 mV
1 ml samples were taken from T17 & T18 for plate counts. Residuals frozen (T17-192 & T18-192).

Ran EIS using Solartron 1260 and EG&G 273 serial no. 41108 potentiostat. Setup as before.
T17 red = T17r193.z Rp = 7.710 MΩ
T17 white = T17w193.z Rp = 8.491 MΩ
T18 red = T18r193.z Rp = 5.976 MΩ
T18 white = T18w193.z Rp = 11.671 MΩ

15:20 (715166) T = 199hrs
T17 29°C, pH = 8.067, pt = -119mV
b -122mV, r -117 mV, w -117mV, y -114 mV
T18 25°C, pH = 7.591, pt = 23mV
b -108mV, r -123mV, w -144m, y -134mV
1 ml samples were taken from T17 & T18 for plate counts. Residuals frozen (T17-199 & T18-199)

Removed flasks Ym-100-6-24 150°C (4 thru 6) and controls 150°C (3 & 4) from shaking water bath. 100 µl samples were taken from each and serial dilutions were performed.

Weighed out 1g of Ym-100-6-24 150°C (4 thru 6) and placed in sterile PBS. Rinsed each sample three times with 10 ml sterile PBS and then sonicated for 1 min. Performed 10-fold serial dilutions

Added 0.5 ml of Ym-FeOx-2 To vessel T18

Alice Stone 7/26/96

7/27/96 13:45 (795762) T = 221 hrs
T17 30°C, pH 8.032, pt -122mV
b -146 mV, r -141 mV, w -141 mV, y -140 mV
T18 26°C, pH 7.398, pt 39mV
b -76 mV, r -79mV, w -111 mV, y -95 mV
1 ml samples taken from T17 & T18 for plate counts. Residual frozen (T17-221 & T18-221)

Results obtained from samples incubated at 150°C for 120 hrs:

Ym-100-6-24 150°C # 4	0 CFU/ml
Ym-100-6-24 150°C # 5	0 CFU/ml
Ym-100-6-24 150°C # 6	0 CFU/ml
control 150°C #3	0 CFU/ml
control 150°C #4	0 CFU/ml

Plate counts for bacteria present on rocks:

Ym-100-6-24 150°C #4	0 CFU/ml
Ym-100-6-24 150°C #5	0 CFU/ml
Ym-100-6-24 150°C #6	0 CFU/ml

Results obtained from plate counts on T17 & T18:

Time (hrs)	T17 (CFU/ml)	T18 (CFU/ml)
144	1.58×10^5	2.50×10^7
150	1.67×10^5	2.33×10^7
168	1.92×10^5	3.33×10^7
174	1.50×10^5	3.20×10^7
192	2.08×10^5	2.92×10^7

Weighed out 3X 4 g Ym-100-6-24 and placed in sterilized conical flasks labeled Ym-100-6-24 130°C (1 thru 3). Placed flasks along with controls 130°C (1 & 2) in oven set at 130°C at 14:30.

Alice Stone
7/27/96

7/28/96

18:00 (897707) T= 249 hrs
T17 32°C, pH 7.968, pt -138mV
b -141 mV, r -136mV, w - 134 mV, y -134mV
T18 30°C, pH 7.307, pt 37mV
b -82mV, r -84 mV, w -120 mV, y -99mV
1 ml samples taken from T17 & T18 for plate counts & residuals frozen. (T17-249 & T18-249).

7/29/96

9:20 (952939) T=265 hrs
T17 30°C, pH 7.967, pt -149 mV
b -121mV, r -116 mV, w -116 mV, y -114mV
T18 27°C, pH 7.395, pt 40 mV
b -83mV, r -85mV, w -119mV, y -98mV
1 ml samples taken from T17 & T18 for plate counts & residuals frozen (T17-265 & T18-265).

Results obtained from plate counts on T17 & T18:

Time (hrs)	T17 (CFU/ml)	T18 (CFU/ml)
199	6.60×10^4	4.08×10^7
221	8.25×10^4	2.67×10^7

Removed flasks Ym-100-6-24 180°C (1 thru 3) and controls 180°C (1&2) from shaking water bath. 100 ul samples were taken from each and serial dilutions were performed.

Removed flasks Ym-100-6-24 130°C (1 thru 3) and controls 130°C (1&2) from oven and placed in RH chamber at 14:30 Aluminum foil caps were replaced with sterilized bungs and paper caps.

Weighed out 3x 4g of Ym-100-6-24 and placed in sterilized conical flasks

labeled Ym-100-6-24 140°C (1 thru 3). Placed flasks along with controls 140°C (1&2) in oven at 140°C @ 15:00.

Weighed out 1g of Ym-100-6-24 180° (1 thru 3) and placed in 10ml of sterile PBS. Rinsed each sample three times with 10 ml of sterile PBS and then sonicated for 1 min. Performed plate counts on each sample.

15:00 (972825) T= 270 hrs
T17 33°C, pH 7.934, pt -139mV
b -99mV, r -92mV, w -92mV, y -90mV
T18 30°C, pH 7.337, pt 39mV
b -86mV, r -87mV, w -122mV, y -100mV
1 ml samples taken from T17 & T18 for plate counts & residuals frozen. (T17-270 & T18-270).

Prepared 1 L of modified J13 media with 1 ppm Ascorbic Acid as follows:

NaCl	lot# 947723	1.64850 g
$NaHCO_3$	897789	0.11700 g
$MgSO_4 \cdot 7H_2O$	947409	0.06470 g
NaF	950992	0.00440 g
$NaNO_3$	897183	0.01370 g
Lactate	943605	341 ul

into 1000 ml sterilized 18.1 MΩ H_2O. Added 0.00100 g (≈ 1ppm) ascorbic acid lot # 947749.

Alicia Stone 7/29/96

7/30/96

8:30 (1036848) T= 288 hrs
T17 30°C, pH 7.991, pt -153 mV
b -110mV, r -106 mV, w -107mV, y -106mV
T18 27°C, pH 7.349, pt 46 mV
b -83mV, r -89mV, w -120mV, y -101 mV
1 ml samples taken from T17 & T18 for plate counts & residuals frozen (T17-288 & T18-288).

Ran EIS using Solartron 1260 and EG&G 273 serial no. 41108 potentiostat. Setup as before.

T17 red = T17r288.z
T17 white = T17w288.z
T18 red = T18r288.z
T18 white = T18w288.z

Determination of repassivation potential on A516 grade 60 carbon steel in modified J13 media with 1ppm ascorbic acid (p.43/182). Put specimen in fresh media and degased for 1hr with 99.99% NT at Rt. Ran EIS using Solartron 1260 and EG&G 273 serial no. 41108 potentiostat with the same setup as before.

Run parameters for CP:

initial delay	20s	initial pot.	-0.200V
scan rate	0.1670 mV/s	vertex I pot	0.100V
scan incr.	5.00 mV	I threshhold	1.00×10^{-3} A/cm²
sample area	8.00 cm²	final pot.	-0.300V
density	7.90 g/ml		

Results obtained from samples incubated at 180°C for 48 hrs:

Ym-100-6-24	180°C #1	0 CFU/ml
Ym-100-6-24	180°C #2	0 CFU/ml
Ym-100-6-24	180°C #3	0 CFU/ml
control	180°C #1	0 CFU/ml
control	180°C #2	0 CFU/ml

Plate counts for bacteria present on rocks:

Ym-100-6-24	180°C #1	0 CFU/ml
Ym-100-6-24	180°C #2	0 CFU/ml
Ym-100-6-24	180°C #3	0 CFU/ml

pH before: 7.073

pH After: 7.280

Results obtained from plate counts on T17 & T18:

Time (hrs)	T17 (CFU/ml)	T18 (CFU/ml)
249	2.50×10^5	2.92×10^7
265	2.58×10^5	3.00×10^7

14:20 (1057290) T = 294 hrs

T17 32°C, pH 7.942, pt -140mV
b -83 mV, r -76 mV, w -76 mV, y -72mV

T18 30°C, pH 7.435, pt 44 mV
b -90 mV, r -85mV, w -86 mV, y -104mV

1ml samples taken from T17 & T18 for plate counts. Residuals frozen (T17-294 & T18-294).

Biofilm counts on T17 & T18 using MEP #1 (12:00) blue & red

T17 blue -100 mV T17 red -92 mV
T18 blue -106 mV T18 red -90 mV

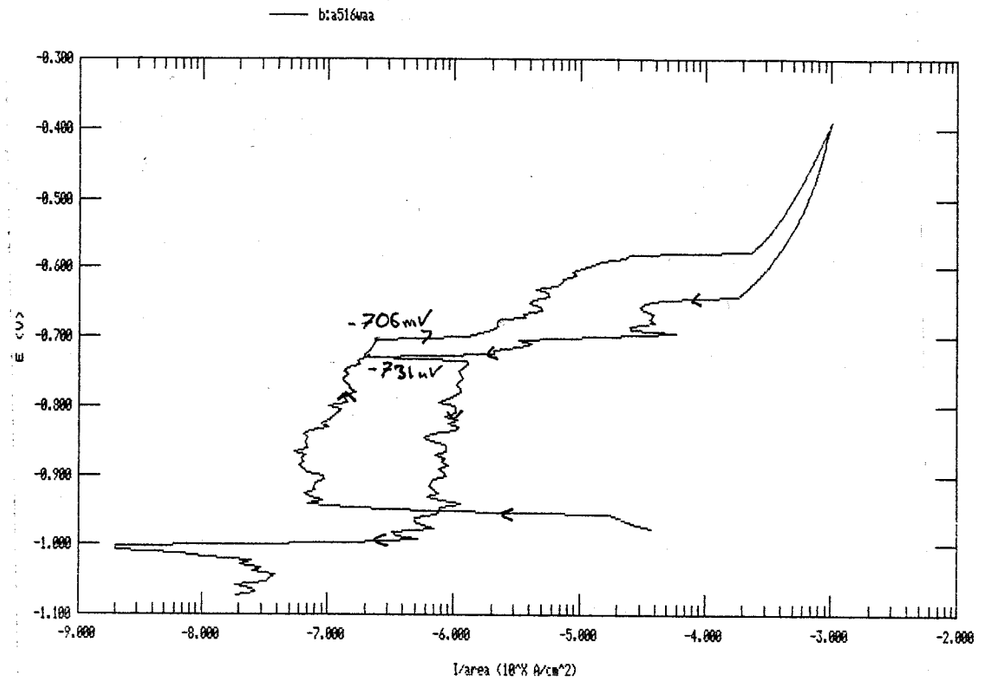
electrodes removed and rinsed in 10ml of 18.1MΩ H2O 0.2 um filter sterilized. Swabbed and then placed in 1ml of sterile PBS. Vortexed on high for 1.5min and performed serial dilutions.

T17-294-B1 & T17-294-B2
T18-294-B1 & T18-294-B2

Placed 200ml of modified J13 media with 1ppm ascorbic acid into two 250ml sterilized conical flasks. A516 Grade 60 carbon steel specimens were placed in each flask. Flasks were then degased with 95% N_2 & 5% H_2 and allowed to cool. 0.5ml of D. vulgaris 25779 was injected into each flask. Flasked were then placed in the anaerobic glove box @ 15:30

Model 352/252 Corrosion Analysis Software, v. 2.01 Filename: b:a516waa Pstat: VStat[] Ver 2 CP CYCLIC POLARIZATION

File Status:	NORMAL	Date Run:	03-18-96	Time Run:	10:50:56						
CP	PASS vs. R	CT	PASS	IP	-0.200 vs. OC	ID	20 S	VI	0.100 vs. OC	FP	-0.300 vs. OC
SI	5.000E-03	SR	1.670E-04	ST	2.993E+01	CR	AUTO	NP	254	IR	NONE
FL	NONE	RT	HIGH STABILITY	REF	0.24150 SCE	WRK	SOLID	AR	8.000E+00	LS	YES
IT	8.000E-03	ITA	8.000E+00	EW	0.000E+00	DEN	7.900E+00	OC	-0.776		



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: b:a516waa
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION File Status: NORMAL
Date Run: 03-18-96 Time Run: 10:50:56

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	VI	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	254					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7760	V

Alice Stone 7/30/96

7/31/96

8:20 (1121910) T = 312 hrs
T17 29°C, pH 7.845, pt -156mV
b -108mV, r -104mV, w -104mV, y -103 mV
T18 26°C, pH 7.768, pt -12mV
b -103 mV, r -97mV, w -128mV, y -117mV
1 ml sample taken from each vessel for plate counts. Residuals frozen. (T17-312 & T18-312).

Removed flasks Ym-100-6-24 130°C (1thru3) and controls 130°C (1 & 2) from RH chamber and added 50ml of sterile R2B to each. Placed in shaking water bath at 14:30.

Removed flasks Ym-100-6-24 140°C (1thru 3) and controls 140°C (1&2) from oven. Aluminum foil caps were replaced by sterilized bungs and paper caps. Placed flasks in RH chamber at 14:40.

15:10 (1146545) T= 318 hrs
T17 32°C, pH 7.805, pt -151mV
b -94mV, r -89mV, w -88mV, y -86mV
T18 30°C, pH 7.797, pt -20mV
b -112mV, r -102mV, w -135 mV, y -123mV
1 ml samples taken from T17 & T18 for plate counts. Residuals frozen. (T17-318 & T18-318).

Weighed out 3x 4g of Ym-100-6-24 and placed in sterilized conical flasks labeled: Ym-100-6-24 120°C (1thru3). Placed flasks along with controls 120°C (1&2) in oven set at 120°C @ 16:00.

Alice Stone
7/31/96

8/1/96

8:30 (1208967) T = 336 hrs
T17 30°C, pH 7.572, pt 82 mV
b -53 mV, r -42 mV, w -40 mV, y -43 mV
T18 26°C, pH 7.891, pt -24 mV
b -117 mV, r -110 mV, w -137 mV, y -127 mV
1 ml samples taken from T17 & T18 for plate counts. Residuals frozen. (T17-336 & T18-336).

Biofilm counts on T17 & T18 using MEP#2 (9:00) blue & red faces
T17 blue -53mV T17 red -45 mV
T18 blue -111 mV T18 red -95 mV
Electrodes removed and rinsed in 10ml 18.1 mΩ 0.2um filtered H_2O. Swabbed and placed in 1 ml of PBS. Vortexed on high for 1.5 min and performed serial dilutions.
T17-336 B_3 T17-336 B4
T18-336 B_3 T18-336 B4

Ran EIS using Solartron 1260 and EG&G 273 serial no. 41108 potentiostat. Setup as before.
T17 red = T17r 337.Z
T17 white = T17w 337.Z
T18 red = T18r 337.Z
T18 white = T18w 337.Z

Results obtained from plate counts on T17 & T18:

Time (hrs)	T17 (CFU/ml)	T18 (CFU/ml)
270	5.00×10^4	2.75×10^7
288	5.17×10^4	3.08×10^7
294	5.00×10^5	2.58×10^7
312	3.58×10^4	2.75×10^7

Switched from 95% N_2 & 5% H_2 to 99.99% N_2 for T17 @ 12:00.

8/2/96

8/6/96

Results obtained from biofilm counts:

coupon	T17 (CFU/ml)	T18 (CFU/ml)
294B1	5.58×10^3	6.42×10^5
294B2	5.91×10^3	6.75×10^5

14:00 (1229065) T = 341 hrs
T17 32°C, pH 7.770, pt -112 mV
b -118 mV, r -112 mV, w -113 mV, y -111 mV
T18 30°C, pH 8.040, pt -27 mV
b -140 mV, r -117 mV, w -147 mV, y -144 mV
1 ml samples taken from T17 & T18 for plate counts. Residuals frozen (T17-341 & T18-341).

Alice Stone 8/1/96

12:10 flesh 4M-100-6-24 140°C (1 thru 3) 0 controls 140°C 1&2 removed from RM chamber 50 ml of R_2B aseptically added (pH 7.1/135) & flasks placed in 30°C water bath

8/3/96

T time (hr)	T17 (cfu/ml)	T18 (cfu/ml)
318	1.08×10^5	2.66×10^7
336	2.08×10^7	1.25×10^7
341	2.91×10^7	1.41×10^7

T17-336-B3 = 1.25×10^6 cfu/ml. T17-336-B4 = 1.50×10^6 cfu/ml.
T18-336-B3 = 1.16×10^6 cfu/ml. T18-336-B4 = 1.00×10^6 cfu/ml

14:15 1661820
T17 31°C pH 7.9 pt -120 mV b -79 r -72 w -71 y -67
T18 27°C pH 8.1 pt -27 mV b -157 r -140 w -172 y -152 mV
1 ml Samples taken for plate counts from T17 & T18 residuals frozen T17-461.5 T18-461.5

8/7/96

8:22 (1726929) T = 480 hrs
T17 30°C, pH 7.842, pt 43 mV
b -51 mV, r -36 mV, w -32 mV, y -26 mV
T18 26°C, pH 8.187, pt -17 mV
b -140 mV, r -122 mV, w -150 mV, y -136 mV
1 ml samples taken from T17 & T18 for plate counts. Residuals frozen (T17-480 & T18-480).

Ran EIS using Solartron 1260 and EG&G 273 serial no. 41108 potentiostat. Setup as before.
T17red = T17r481.Z
T17white = T17w481.Z
T18red = T18r481.Z
T18white = T18w481.Z

Removed flasks Ym-100-6-24 130°C (1thru3) and controls 130°C (1&2) from shaking water bath 100ul samples were taken from each and serial dilutions were performed.

Weighed out 1g of Ym-100-6-24 130°C (1thru3) and placed in 10 ml of sterile PBS. Rensed each sample three times with 10 ml sterile PBS and then sonicated for 1 min. 100ul samples were taken from each and serial dilutions were performed.

UPS crashed and system restarted. Add 487 hrs to new time. Replace pt electrode in vessel T17 with a new pp pt electrode.

16:15 (2303) T = 1 ≡ 488 hrs
T17 32°C, pH 8.410, pt -414 mV
b -90 mV, r -92 mV, w -92 mV, y -90 mV
T18 29°C, pH 8.128, pt -21 mV
b -151 mV, r -128 mV, w -158 mV, y -145 mV
1 ml samples taken from T17 & T18 for plate

counts. Residuals frozen (T17-488 & T18-488).
Alice Stone 8/7/96

8/8/96

8:30 (61171) T = 17 ≡ 504 hrs
T17 29°C, pH 8.463, pt -277 mV
b -209 mV, r -208 mV, w -208 mV, y -207 mV
T18 25°C, pH 8.171, pt 4 mV
b -132 mV, r -110 mV, w -143 mV, y -126 mV
1 ml samples taken from T17 & T18 for plate counts. Residuals frozen (T17-504 & T18-504).

Prepared 1L of modified J13 media with 1 ppm ascorbic acid as follows:

NaCl	lot # 947723	1.64850 g
$NaHCO_3$	897789	0.11700 g
$MgSO_4 \cdot 7H_2O$	947409	0.06470 g
NaF	950992	0.00440 g
$NaNO_3$	897183	0.01370 g
lactate	943605	341 ul
ascorbic acid	947749	0.00100 g (≈ 1 ppm)

into 1000 ml sterile 18.1 MΩ H_2O.

Prepared 1L of modified J13 media with 1 ppm yeast extract as follows:

NaCl	lot # 947723	1.64850 g
$NaHCO_3$	897789	0.11700 g
$MgSO_4 \cdot 7H_2O$	947409	0.06470 g
NaF	950992	0.00440 g
$NaNO_3$	897183	0.01370 g
lactate	943605	341 ul
yeast extract	59695JB	0.00100 g (≈ 1 ppm)

into 1000 ml sterile 18.1 MΩ H_2O.

14:30 (82811) T = 23 ≡ 510 hrs
T17 28°C, pH 8.447, pt -123 mV
b -92 mV, r -82 mV, w -78 mV y -73 mV

T18 29°C, pH 8.149, pt -22mV
b -152mV, r -130mV, w -163mV, y -147mV
1ml samples taken from each vessel and residuals frozen (T17-510 & T18-510).

15:30 Flasks Ym-100-6-24 120°C (1 thru 3) and controls 120°C (1&2) were removed from oven and aluminum foil caps were replaced by sterile bering & paper caps. Flask Ym-100-6-24 120°C #1 was broke in the process. Remaining flasks were returned to the oven set at 120°C.

Alice Stone 8/8/96

8/9/96 8:20 (146911) T=41 ≡ 528 hrs
T17 29°C, pH 8.456, pt -261mV
b -242mV, r -237mV, w -239mV, y -239mV
T18 26°C, pH 8.236, pt -17mV
b -147mV, r -124mV, w -157mV, y -141mV
1ml samples taken from each vessel and plate counts performed. Residuals frozen (T17-528 & T18-528)

Results obtained from plate counts on T17 & T18:

Time (hrs)	T17 (CFU/ml)	T18 (CFU/ml)
461	4.00×10^7	3.25×10^7
480	3.58×10^7	2.75×10^7
488	3.67×10^7	3.25×10^7

Ran EIS using Solartron 1260 and EG&G 273 serial no. 41108 potentiostat. Setup as before

T17 red = T17r 529.2	RCT = 4.9MΩ	$C_{DL} = 3.59 \times 10^{-5}$F
T17 white = T17w 529.2	RCT = 3.56MΩ	$C_{DL} = 3.22 \times 10^{-5}$F
T18 red = T18r 529.2	RCT = 6.31MΩ	$C_{DL} = 3.52 \times 10^{-5}$F
T18 white = T18w 529.2	RCT = 11.33MΩ	$C_{DL} = 4.5 \times 10^{-5}$F

15:00 (170840) T=47 ≡ 534 hrs
T17 31°C, pH 8.589, pt -256mV
b -234mV, r -230mV, w -232mV, y -232mV
T18 28°C, pH 8.946, pt -11mV
b -139mV, r -121mV, w -143mV, y -136mV
1ml samples taken from T17 & T18 for plate counts. Residuals frozen. (T17-534 & T18-534).

Alice Stone 8/9/96

8/10/96 10:50 (242255) T=67 ≡ 554 hrs
T17 30°C, pH 8.737, pt -285mV
b -288mV, r -285mV, w -287mV, y -287mV
T18 25°C, pH 8.063, pt -10mV
b -140mV, r -130mV, w -145mV, y -138mV
1ml samples taken from each vessel for plate counts & Residuals frozen. (T17-554 & T18-554).

Flasks Ym-100-6-24 120°C (1&2) and controls 120°C (1&2) were removed from the oven and placed in the RH chamber at 11:30.

Alice Stone 8/10/96

8/11/96 15:45 (346215) T=96 ≡ 583 hrs
T17 30°C, pH 8.814, pt -340mV
b -336mV, r -334mV, w -338mV, y -337mV
T18 28°C, pH 8.020, pt -15mV
b -142mV, r -130mV, w -143mV, y -140mV
1ml samples taken from T17 & T18 for plate counts. Residuals frozen (T17-583 & T18-583)

Biofilm counts on T17 & T18 using MEP #3 (3:06) blue and red faces.
T17 blue = -335mV T17 red = -336mV
T18 blue = -145mV T18 red = -137mV
Electrodes removed and rinsed in 10ml

18.1 mΩ 0.2um filtered H2O. Swabbed and placed in 1ml of sterile PBS. Vortexed on high for 1.5 min and performed plate counts.
T17-583 B5 T17-583 B6
T18-583 B5 T18-583 B6

Flasks Ym-100-6-24 120°C (1&2) and controls 120°C (1&2) were removed from RH chamber. 50 ml of R2B (p.70/138) was aseptically added to each flask and flasks were placed in shaking water bath set at 30°C (16:30).
Alice Stone 8/11/96

8/12/96 8:30 (406588) T=113 ≡ 600hrs
T17 27°C, pH=8.833, pt -359 mV
b -347mV, r -346 mV, w -351 mV, y -350mV
T18 25°C, pH 8.099, pt -10 mV
b -143mV, r -141 mV, w -142 mV, y -140mV
1 ml samples taken from T17 & T18 for plate counts. Residuals frozen. (T17-600 & T18-600)

Removed 100 ul samples from Ym-100-6-24 120°C (1&2) for plate counts @ 14:00.

Results on plate counts from T17 & T18:

Time (hrs)	T17 (CFU/ml)	T18 (CFU/ml)
504	3.00×10^7	3.25×10^7
510	2.92×10^7	3.83×10^7
528	4.58×10^7	2.58×10^7
534	3.42×10^7	2.92×10^7
554	3.25×10^7	3.58×10^7

Results from samples incubated at 130°c for 48 hrs:
Ym-100-6-24 130°C #1 0 CFU/ml
Ym-100-6-24 130°c #2 0 CFU/ml

Ym-100-6-24 130°C #3 1.10×10^8 CFU/ml *
control 130°C #1 7.30×10^8 CFU/ml *
control 130°C #2 0 CFU/ml
* same colony types present in Ym-100-6-24 130°C #3 and control 130°C #1 due to contamination

Plate counts for bacteria present on rocks:
Ym-100-6-24 130°C #1 0 CFU/ml
Ym-100-6-24 130°c #2 0 CFU/ml
Ym-100-6-24 130°c #3 1.92×10^6 CFU/ml

Results from samples incubated at 140°C for 48 hrs:
Ym-100-6-24 140°C #1 5.67×10^4 CFU/ml (mold)
Ym-100-6-24 140°C #2 0 CFU/ml
Ym-100-6-24 140°C #3 0 CFU/ml
control 140°C #1 0 CFU/ml
control 140°C #2 0 CFU/ml
Plate counts for bacteria present on rocks:
Ym-100-6-24 140°C #1 0 CFU/ml
Ym-100-6-24 140°C #2 0 CFU/ml
Ym-100-6-24 140°C #3 0 CFU/ml
Alice Stone 8/12/96

8/13/96 8:50 (494239) T=137 ≡ 624 hrs
T17 27°C, pH 8.818, pt -398 mV
b -371 mV, r -372mV, w -381mV, y -380 mV
T18 25°C, pH 8.099, pt -11 mV
b -141 mV, r -134 mV, w -147mV, y -141 mV
1 ml samples taken from T17 & T18 for plate counts. Residuals frozen. (T17-624 & T18-624).

Removed 100ul samples from Ym-100-6-24 120°C flasks 1&2 for plate counts @ 9:00.

On 8/12/96 two CP vessels were filled with sterile modified J13 media with 1 ppm ascorbic acid and degased overnight with 99.99% N_2.

Removed reaction vessels from anaerobic glove box and placed samples in degased CP vessels. Ran EIS on each sample using same setup as before except now using 1mA.
sample #1 -777mV sample #2 -728mV
a:A516J13A.Z a:A516j13b.Z
Checked pH of media left in each flask.
Results are: Flask #1 7.233 & Flask #2 7.215
pH meter was calibrated using Buffer solution pH 7.00 (lot # 961196-24).

CCP on Sample #1
A516J13A.DAT, - Epit: -764mV Erp: -779mV

Determined the repassivation potential on Samples #1 & #2 (A516 grade 60 carbon steel) in modified J13 media with 1ppm ascorbic acid. Media was degased overnight with 99.99% N_2. Run parameters the same as before. pH of fresh media 7.331.

Data for A516J13A using 2.vew
R_p: 9.808 kΩ Cdl: 0.005539 F R_s: 54 Ω
Fit parameters R_s: 101.4 R_{ct}: 5.026 kΩ Cdl: 0.003016 F bad Bimuth

Data for A516J13B using 2new Cnyquis
R_{sol} = 67.9 Ω R_{ct}: 1.634 kΩ Cdl: 1.529 mF too high freq
R_{sol} = 799 Ω R_{ct} = 1.420 kΩ Cdl = 12.07 mF low freq

1

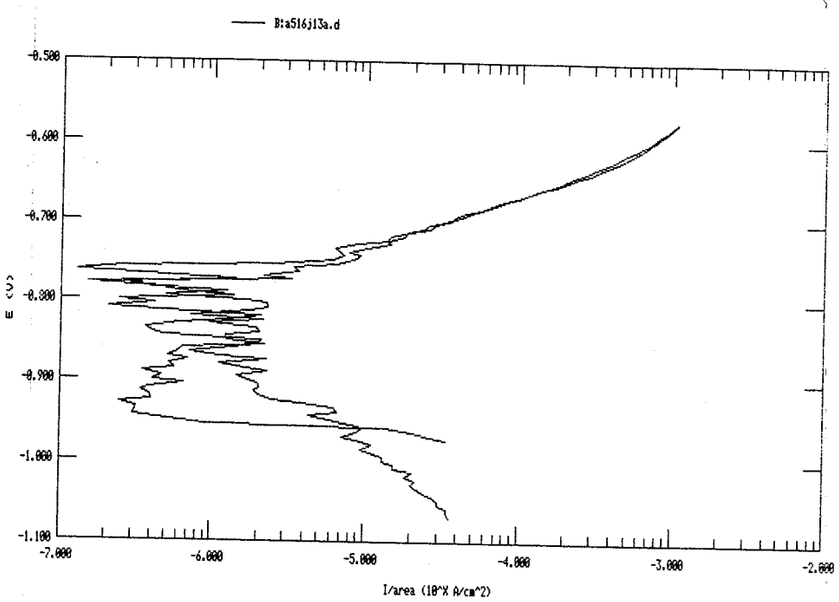
Model 352/252 Corrosion Analysis Software, v. 2.01
CP CYCLIC POLARIZATION
CP PASS vs. R CT PASS
SI 5.000E-03 SR 1.670E-04
FL NONE RT HIGH STABILITY
IT 8.000E-03 ITA 8.000E+00

File Status: NORMAL
IP -0.200 vs. OC
ST 2.993E+01
REF 0.24150 SCE
EW 8.000E+00

Filename: B:a516j13a.dat
Date Run: 03-19-96
ID 20 S
CR AUTO
WRK SOLID
DEN 7.900E+00

Time Run: 09:41:36
V1 0.100 vs. OC
MP 100
AR 8.000E+00
OC -0.774

Pstat: VStat[] Ver 2
FP -0.300 vs. OC
IR NONE
LS YES



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: B:a516j13a.dat
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION
Date Run: 03-19-96

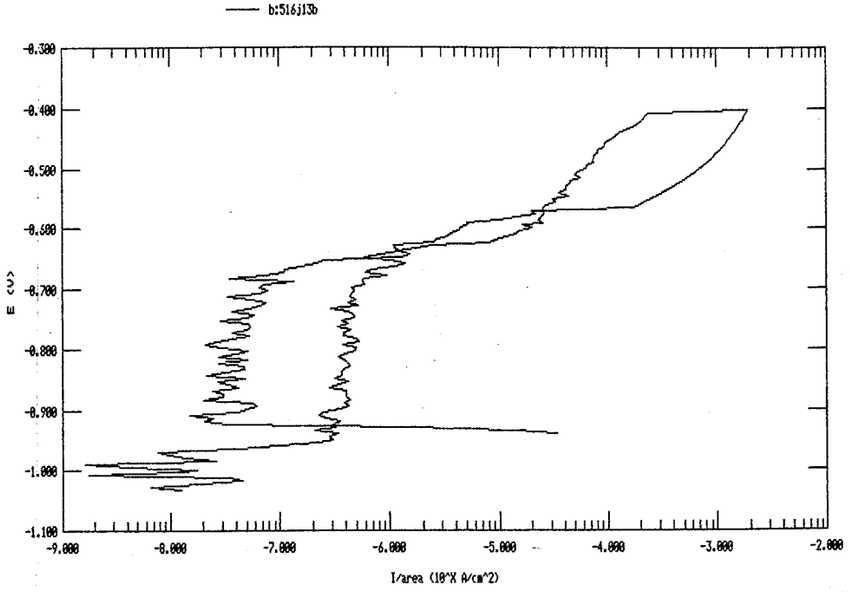
File Status: NORMAL
Time Run: 09:41:36

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	V1	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	MP	100					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7740	V

Alice Stone 8/13/96

2

Model 352/252 Corrosion Analysis Software, v. 2.01 Filename: b:516j13b Pstat: VStat[] Ver 2 CP CYCLIC POLARIZATION
File Status: NORMAL Date Run: 03-19-96 Time Run: 13:59:04
CP PASS vs. R CT PASS IP -0.200 vs. OC ID 20 S VI 0.100 vs. OC FP -0.300 vs. OC
SI 5.000E-03 SR 1.670E-04 ST 2.993E+01 CR AUTO NP 234 IR NONE
FL NONE RT HIGH STABILITY REF 0.24150 SCE WRK SOLID AR 8.000E+00 LS YES
IT 8.000E-05 ITA 8.000E+00 EW 0.000E+00 DEN 7.900E+00 OC -0.738



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: b:516j13b
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION
Date Run: 03-19-96

File Status: NORMAL
Time Run: 13:59:04

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	VI	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	234					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7388	V

Alicia Stone 8/13/96

8/14/96

8:30 (579333) T = 161 ≡ 648 hrs
T17 27°C, pH 8.787, pt -259 mV
b -302 mV, r -304 mV, w -305 mV, y -303 mV
T18 25°C, pH 8.092, pt -11 mV
b -140 mV, r -134 mV, w -147 mV, y -142 mV
1 ml samples taken from T17 & T18 for plate counts.
Residuals frozen (T17-648 & T18-648).

Removed 100 ul samples from Ym-100-6-24 120°C flasks #1 & #2 for plate counts at 9:00.

Discovered pH meter was not reading correctly. Previous pH values were incorrect and pH meter was recalibrated using a two point calibration. Buffer solution pH 10.00 (lot # 960984-24) and buffer solution pH 7.00 (lot # 961196-24) were used as standards. New pH values are as follows:
Flask #1 8.392 Flask #2 8.298

Prepared 1L of modified J13 media with 1ppm YE as follows:

Na_2CO_3	lot # 960685	0.01060 g
$NaHCO_3$	897789	0.08821 g
NaCl	947723	1.64846 g
$NaNO_3$	897183	0.01370 g
NaF	950992	0.00440 g
lactate	943605	341 ul
Yeast extract	59695JB	0.00100 g
$MgSO_4 \cdot 7H_2O$	947409	0.06470 g

into 1000 ml 18.1 MΩ H_2O. pH checked and found to be 8.9.

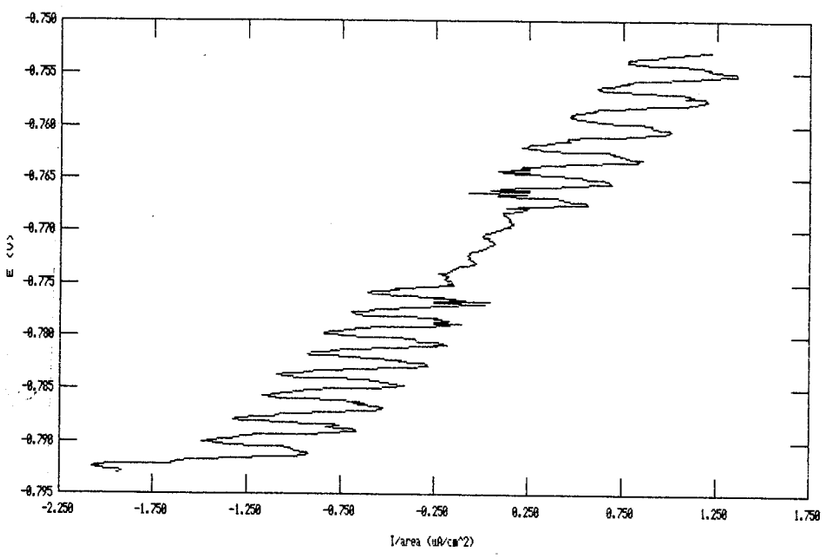
600 ml of mod. J13 media with 1ppm YE (prepared above) and a polished A516 grade 60 carbon steel were placed in a CP vessel and degased with 99.99% N_2 for 1 hr.

Ran linear polarisation on A516 grade 60 as overleaf in degased solution with parameter & results as printed below using quickcalc R_p = 1.7734 K ohm I_{corr} = 1.565 $\mu A/cm^2$ 0.7119 mpy

Model 352/252 Corrosion Analysis Software, v. 2.01 — Filename: B:516j13p9.dat — Pstat: VStat[] Ver 2

LP LINEAR POLARIZATION — File Status: NORMAL — Date Run: 03-20-96 — Time Run: 12:42:04

CP	PASS vs. R	CT	PASS	IP	-0.020 vs. OC	ID	20 S	FP	0.020 vs. OC	SI	1.000E-04
SR	1.000E-04	ST	1.000E+00	CR	AUTO	NP	401	IR	NONE	FL	NONE
RT	HIGH STABILITY	REF	0.00000 User	WRK	SOLID	AR	8.000E+00	LS	YES	EW	2.792E+01
DEN	7.900E+00	OC	-0.773								



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: B:516j13p9.dat
Pstat: VStat[] Ver 2
LP LINEAR POLARIZATION — File Status: NORMAL
Date Run: 03-20-96 — Time Run: 12:42:04

Cond. Time	CT	pass	s	Initial Pot.	IP	-20.00E-3	V oc
Cond. Pot.	CP	pass	V	Final Pot.	FP	20.00E-3	V oc
Initial Delay	ID	20	s				
Scan Rate	SR	100.00E-3	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	0.1000	mV	Step Time	ST	1.000	s
No. of Points	NP	401					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	User 0.0000 V	
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	27.92	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7730	V

8-14-96

Results from plate counts on T17 & T18:

Time (hrs)	T17 (CFU/ml)	T18 (CFU/ml)
583	7.67×10^6	3.50×10^7
600	6.33×10^6	3.75×10^7

Results from biofilm counts on T17 & T18:

Coupon	T17 (CFU/ml)	T18 (CFU/ml)
583 B5	3.58×10^4	3.33×10^5
583 B6	3.09×10^4	3.17×10^5

Ran EIS on A516 Grade 60 in modified J13 media with 1 ppm yeast extract using Solartron 1260 and EG&G 273 serial no. 41108 potentiostat. Same set up as before. saved as a:A516J13C.z R_{ct} = 1.9 kΩ

Prepared 1L of modified J13 media with 1 ppm yeast extract as follows:

Na_2CO_3	lot # 960685	0.01060 g
$NaHCO_3$	897789	0.08821 g
NaCl	947723	1.64846 g
$NaNO_3$	8917183	0.01370 g
NaF	950992	0.00440 g
Lactate	943605	341 ul
yeast extract	5969SJB	0.00100 g
$MgSO_4 \cdot 7H_2O$	947409	0.06470 g

into 1000 ml 18.1 MΩ H_2O pH 8.9

Alice Stone 8/14/96

8/15/96 8:40 (668358) T = 185 ≡ 672 hrs

T17 26°C, pH 8.775, pt -218 mV
b -252 mV, r -252 mV, w -251 mV, y -251 mV

T18 24°C, pH 8.092, pt -11 mV
b -140 mV, r -132 mV, w -147 mV, y -143 mV

1 ml samples taken from each vessel for plate counts. Residuals frozen (T17-672 & T18-672).

Removed 100 ul samples from Ym-100-6-24 120°C flasks #1 & #2 for plate counts at 9:00.

Three CP vessels were filled with approx 600 ml of modified J13 media with 1ppm yeast extract. All openings were covered with autoclave paper and vessels were sterilized along with all of the other accessories. Autoclaved for 1hr at 121°C and 14 psi.

Three A516 Grade 60 carbon steel specimens were polished to 600 grit finish and then weighed. Weights are as follows:

Sample #1 10.76545 g
Sample #2 10.93889 g
Sample #2 10.66550 g

Alice Stone 8/15/96

8/16/96 8:20 (751640) T= 209 ≡ 696 hrs
T17 28°C, pH 8.813, pt -220mV
b-251mV, r -251mV, -251mV, y -250mV
T18 25°C, pH 8.162, pt -11 mV
b-140mV, r -131mV, w -149 mV, y -144mV
1 ml samples taken from T17 & T18 for plate counts and residual frozen. (T17-696 & T18-696).

Removed 100 ul samples from Ym-100-6-24 120°C flasks #1 & #2 for plate counts at 9:00.

Ran EIS using Solartron 1260 and EG&G 273 serial no 41108 potentiostat Set up as before

T17 red = T17r 697.Z Rct = 8.17 mΩ
T17 white = T17w 697.Z Rct = 7.86 mΩ
T18 red = T18r 697.Z Rct = 3.43 mΩ
T18 white = T18w 697Z Rct = 7.35 mΩ

Results from plate counts on T17 & T18:

Time (hrs)	T17 (CFU/ml)	T18 (CFU/ml)
624	1.75×10^7	2.50×10^6
648	1.92×10^7	6.58×10^5

Results on plate counts from Ym-100-6-24 120°C 242 hrs flask #1 & #2:

Time (hrs)	Ym-100-6-24 #1	Ym-100-6-24 #2
22.5	0 CFU/ml	0 CFU/ml
41.5	0 CFU/ml	0 CFU/ml
65.5	2.42×10^7 CFU/ml	3.08×10^7 CFU/ml
89.5	2.50×10^7 CFU/ml	2.33×10^7 CFU/ml

Evaluation of SRB growth on A516 carbon steel at pH 9 & 25 ppm sulphate looking at corrosion rate on Exp. cells (cp vessels pG2) which set up with platinum electrodes & degassed 1hr with 95% N_2 5% H_2 before specimens pG2 were added (pre-weighed) & allowed to equilibrate for 1hr. 2 cells will be inoculated with D. vulgaris & 1 will act as abiotic control EIS will be run to evaluate corrosion rate (R_{ct}) & after approx 2 wks cyclic polarization will be run to determine Exp. initial E_{corr} after 2hr.

vessel #	1	2	3
E_{corr}	-768mV	-686mV	-749 mV

T17 & T18 terminated

Samples 1-3 three were sterilized and added to the CP vessels. Vessels were then degased for 1hr. Ran EIS on samples 1-3. Vessel #1 & #3 were inoculated with 1ml of

D. vulgaris 295779.

EIS on #1 A:1A5163.2
~~#2 A:2A5163.2~~ failed to run salt bridge too weak?
Error too unstable

8-16-96

8/17/96 14:00 Removed 100 ul samples from Ym-100-6-24 120°C flasks #1 & #2 for plate counts.
Alice Stone 8/17/96

8/18/96 Removed 100ul samples from Ym-100-6-24 120°C flasks #1 & #2 for plate counts @ 14:00.
Alice Stone 8/18/96.

8/19/96 Results from plate counts on T17 & T18:

Time (hrs)	T17 (CFU/ml)	T18 (CFU/ml)
672	2.83×10^6	2.92×10^7
696	3.17×10^6	2.08×10^7

Results from plate counts on Ym-100-6-24 120°C flasks #1 & #2:

Time (hrs)	Ym-100-6-24 #1	Ym-100-6-24 #2
113.5	2.00×10^8 CFU/ml	2.00×10^8 CFU/ml

Initial counts from Mod J13 w/1ppm ascorbic acid + D. vulgaris shows 10^{+3} CFU/ml in flask #1 & #2.

Ran EIS on Flasks #1, #2 & #3.
Flask #1 1a51667.Z Rp $1.276 \times 10^5 \Omega$ 9.62KΩ
Flask #2 2a51667.Z Rp 25KΩ
Flask #3 3a51667.Z Rp 7.7KΩ

Removed flasks Ym-100-6-24 120°C (1 & 2) and controls 120°C (1 & 2) from shaking water bath. 100 ul samples were removed for plate counts.

Weighed out 1g of Ym-100-6-24 120°C (1 & 2) and placed in 10 ml of sterile PBS. Rinsed each sample three times with 10 ml sterile PBS and then sonicated for 1 min. 100 ul samples were taken for plate counts.

8-19-96

8/21/96 Run EIS on A516 specimen in J13 + 200 ppm lactate 1 ppm YE 1 & 3 + SRB.

A:1A516114.2 R_{ct} = 145.0 KΩ
A:2A516114.2 R_{ct} = 54 KΩ
A:3A516115.2 R_{ct} = ~~17 KΩ~~ 17 KΩ

Div 06 results from samples taken on 8/12/96 are as follows:

heterotrophic:	Biotic coupon	1.17×10^8 CFU/ml
	Abiotic coupon	1.92×10^6 CFU/ml
	Biotic bulk phase	8.83×10^8 CFU/ml
	Abiotic bulk phase	2.75×10^5 CFU/ml
SRB:	Abiotic coupon	No counts
	Biotic coupon	10^7
	Biotic bulk phase	10^8
	chemostat bulk ph[illegible]	10^8

Alice [illegible] 8/21/96

8-22-96 made 200 ml of 10 [illegible] Tryptophane (DL) weighed out 0.00408g of Sig[illegible] TOPHAN Lot 45H09031 in a 200 ml volumetric [illegible] 200 ml of 18.1 MΩ water prepared for Quick to [illegible] with Ralph Hill Div 15

D. vulgaris 295779 8.16.96

Sample of copper cut approx 1" square & polished by hand to 425 grit and rinsed in acetone & dried in oven at 65°C added 1ml of 10^{-4} solution dropwise & dried to an area approx diameter 1cm dried at 55°C 8.22.96

Results from plate counts on Ym-100-6-24 120°C flasks #1 & #2:

Time (hrs)	Ym-100-6-24 120°C #1	Ym-100-6-24 120°C #2
161.5	2.75×10^8	4.50×10^8
209.5	1.00×10^8	2.75×10^8

Results from samples incubated at 120°C for 288 hrs!

Ym-100-6-24 120°C #1	6.25×10^8 CFU/ml
Ym-100-6-24 120°C #2	6.08×10^8 CFU/ml
control 120°C #1	0 CFU/ml
control 120°C #2	0 CFU/ml

Results from plate counts for bacteria present on rocks:

Ym-100-6-24 120°C #1	2.17×10^7 CFU/ml
Ym-100-6-24 120°C #2	3.33×10^7 CFU/ml

Alice Stone 8/22/96

8/23/96 Weighed out 3X4 g of Ym-100-6-24 and placed in sterilized conical flasks labeled Ym-100-6-24 (1 thru 3). Placed flasks along with controls (1 & 2) in oven at 135°C @ 16:40.

Alice [redacted] 8/23/96

8/25/96 Removed flasks [redacted] 6-24 (1 thru 3) and controls (1&2) from oven at 135°C and placed [redacted] chamber at 16:40.

Alice [redacted] 8/25/96

8/26/96 Ran EIS on Flasks #1, #2, & #3 using same setup as before.

Flask #1	1a516259.2	R_{CT} = 1.02 KΩ
Flask #2	2a516259.2	R_{CT} = 63.2 KΩ
Flask #3	3a516259.2	R_{CT} = 31.8 KΩ

Removed flasks Ym-100-6-24 (1 thru 3) and controls (1&2) 135°C from RH chamber and placed in shaking water bath at 16:40.

Alice Stone 8/26/96

8/27/96 Made 1L of 0.525% sodium hypochlorite solution and pH electrodes were sterilized overnight.

Reaction vessels washed and then setup with drip tubes, media outlets, gas sparge tubes, and all other holes filled with bungs except for one which was covered with autoclave paper. Autoclaved for 1 hr at 14psi and 121°C. MEP's were sonicated for 10 min in absolute acetone and air dried. MEP's were placed in sterilized vessels immediately upon removal from autoclave. Two thermometers were sterilized with isopropanol and placed in vessels (SN 0323007 & SN 0323005). Vessels were then placed in incubator at 70°C

Placed two platinum electrodes in 25% HNO_3 in incubator set at 70°C.

Made 1L of Thiosulfate media as follows:
0.31642g $Na_2S_2O_3$ (2mM) lot # 923931A
1.64859g NaCl " 947723
1ml trace minerals
900ml 18.1MΩ H_2O. Autoclaved for 1 hr at 14 psi and 121°C. Added 100ml of 10X Mn media that was autoclaved for 1 hr at 121°C and 14 psi.

Made 1L of sodium nitrate media as follows:
0.16998g $NaNO_3$ (2mM) lot # 897183
1.64859g NaCl 947723
1ml trace minerals
900ml 18.1MΩ H_2O. Autoclaved for 1hr at 14 psi and 121°C. Added 100ml of 10X Mn media that was autoclaved for 1hr at 121°C and 14 psi.

Alice Stone 8/27/96

8/28/96

Meter #1 Orion EA920 SN 5001A
Meter #2 Orion EA940 SN 2330
pH 4 buffer solution lot # 960230-24
pH 7 buffer solution lot # 961196-24
pH 10 buffer solution lot # 960984-24

Meter #1	pH 4	149.4 mV
meter #2	pH 4	160.6 mV
Meter #1	pH 7	9.5 mV
meter #2	pH 7	17.1 mV
meter #1	pH 10	-139.2 mV
Meter #2	pH 10	-133.1 mV

Reaction vessels taken to CAS for ethylene oxide sterilization.

Alice Stone 8/28/96

8/29/96

Final counts from modified J13 media with 1ppm ascorbic acid and D. vulgaris indicate 10^4 CFU/ml in flask #1 & flask #2.

Made 2 X 500ml 10% TSB as follows:
TSB lot # 58855 1.5g per 500ml 18.1MΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi. TSB solutions were allowed to cool and then electrodes were removed from the sodium hypochlorite solution and placed in sterile TSB solutions.

"Weighed out approx. 3g of Ym-100-6-24 and placed in bunged conical flask. flask was the put in oven set at 120°C at 17:00.

8/30/96

Ran EIS on flasks #1, #2, & #3

flask #1	1a516355.Z	R_{ct} 169-6 kΩ
flask #2	2a516355.Z	R_{ct} 50.2 kΩ
flask #3	3a516355.Z	R_{ct} 36.8 kΩ

Removed flasks Ym-100-6-24 135°C (1 thru 3) and controls 135°C (1 & 2) from shaking water bath. 100ul samples were removed for plate counts

Weighed out 1g of Ym-100-6-24 135°C (1 thru 3) and placed in 10ml of sterile PBS. Rinsed each sample three times with 10ml of sterile PBS and then sonicated for 1 min. 100ul samples were removed for plate counts

Alice Stone 8/30/96

9/3/96 Ran EIS on flasks #1, #2, & #3
flask #1 1a516451.2 Rp 124 KΩ
flask #2 2a516451.2 Rp 52 KΩ
flask #3 3a516451.2 Rp 113 KΩ

Discovered that pH electrodes were not sterile due to the cloudiness present in the 10% TSB. Removed electrodes from 10% TSB and placed in fresh 0.525% sodium hypochlorite solution.

Ran linear and cyclic polarizations on flasks #1, #2, & #3. which contain A516 Grade 60 in modified J13 media with 1ppm yeast extract. pt potential -299mV. Sample #3 - 686mV
linear polarizations saved as:
flask #3 3a516451.dat
flask #2 2a516451.dat
flask #1 1a516451.dat

3a516451.dat Rp 79.83Ω I_{corr} 34 μA cm-2 ? Rp calc.
2a516451.dat
1a516451.dat

On 8/29/96 1ml samples were removed from flasks #1, #2, & #3 and placed in lactate/acetate media to check for the presence of SRB's.

Results from SRB check on 8/29/96 indicate SRBs in flask #1 only.

1 ml samples removed from flasks #1, #2, & #3 and placed in lactate/acetate media to check for SRBs. Sample from flask #1 was diluted to 10^{-7}.

CP Results saved as:
sample #1 1516451.dat pH 9.0 pt -254 mV WE -762 mV
sample #2 2516451.dat pH 9.5
sample #3 3516451.dat Epit -4.58mV Ecp -718mV

Performed Aw counts Ym-100-6-24 using Aqua Lab CX-2 SN0493785 calibrated with:
MgCl 0.362 ± 0.000 (Greenspan 0.331 ± 0.002)
NaCl 0.774 ± 0.000 (Greenspan 0.755 ± 0.001)
NaOH 0.088 ± 0.000 (Greenspan 0.089 ± 0.024)
Ym-100-6-24 0.072 ± 0.000

Placed sample Ym-100-6-24 in RH chamber at 14:00.

Results from samples incubated at 135°C for 48hrs:

Ym-100-6-24	135°C	#1	1.92×10^8 CFU/ml
Ym-100-6-24	135°C	#2	1.92×10^8 CFU/ml
Ym-100-6-24	135°C	#3	1.50×10^8 CFU/ml
control		#1	2.58×10^8 CFU/ml
control		#2	0 CFU/ml

Results for plate counts for bacteria present on rocks:

Ym-100-6-24	135°C	1.00×10^6 CFU/ml
Ym-100-6-24	135°C	1.08×10^6 CFU/ml
Ym-100-6-24	135°C	1.25×10^6 CFU/ml

Performed Aw counts on Ym-100-6-24 after rehydration using Aqua Lab CX-2 SN0493785.
Ym-100-6-24 0.896 ± 0.000

pitted 10-8-96

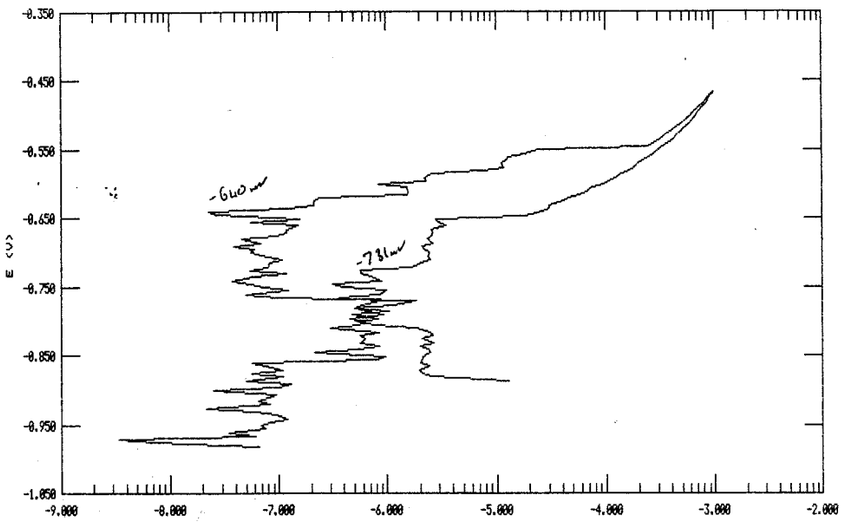
Sample #1 Abiotic

Model 352/252 Corrosion Analysis Software, v. 2.01 — Filename: B:1516451.dat — Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION — File Status: NORMAL — Date Run: 03-26-96 — Time Run: 13:10:39

CP	PASS vs. R	CT	PASS	IP	-0.200 vs. OC	ID	20 S
SI	5.000E-03	SR	1.670E-04	ST	2.993E+01	CR	AUTO
FL	NONE	RT	HIGH STABILITY	REF	0.24150 SCE	WRK	SOLID
IT	8.000E-03	ITA	8.000E+00	EW	0.000E+00	DEN	7.900E+00

V1	0.100 vs. OC	FP	-0.300 vs. OC
NP	188	IR	NONE
AR	8.000E+00	LS	YES
OC	-0.686		

B:1516451.da

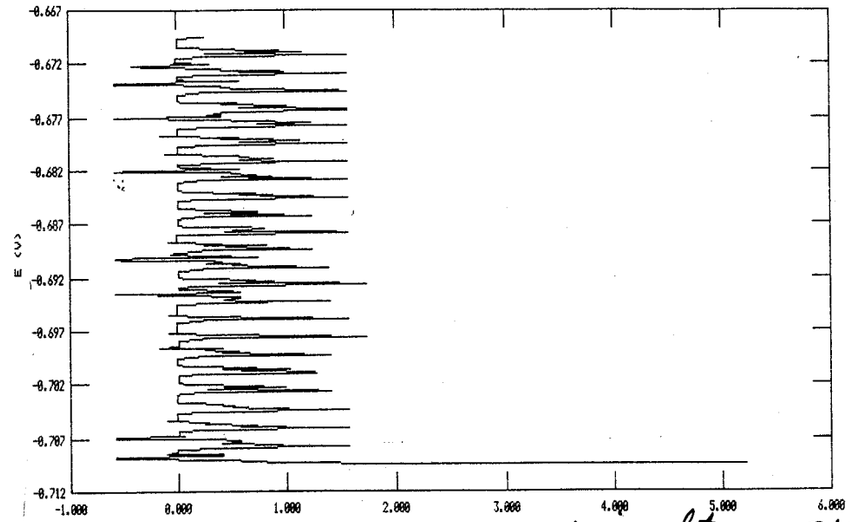


Model 352/252 Corrosion Analysis Software, v. 2.01 — Filename: B:1a516451.dat — Pstat: VStat[] Ver 2
LP LINEAR POLARIZATION — File Status: NORMAL — Date Run: 03-26-96 — Time Run: 12:56:25

CP	PASS vs. R	CT	PASS	IP	-0.020 vs. OC	ID	20 S
SR	1.000E-04	ST	1.000E+00	CR	AUTO	NP	401
RT	HIGH STABILITY	REF	0.24150 SCE	WRK	SOLID	AR	8.000E+00
DEN	7.900E+00	OC	-0.698				

FP	0.020 vs. OC	SI	1.000E-04
IR	NONE	FL	NONE
LS	YES	EW	2.792E+01

B:1a516451.d



Alice Stone 9/4/96

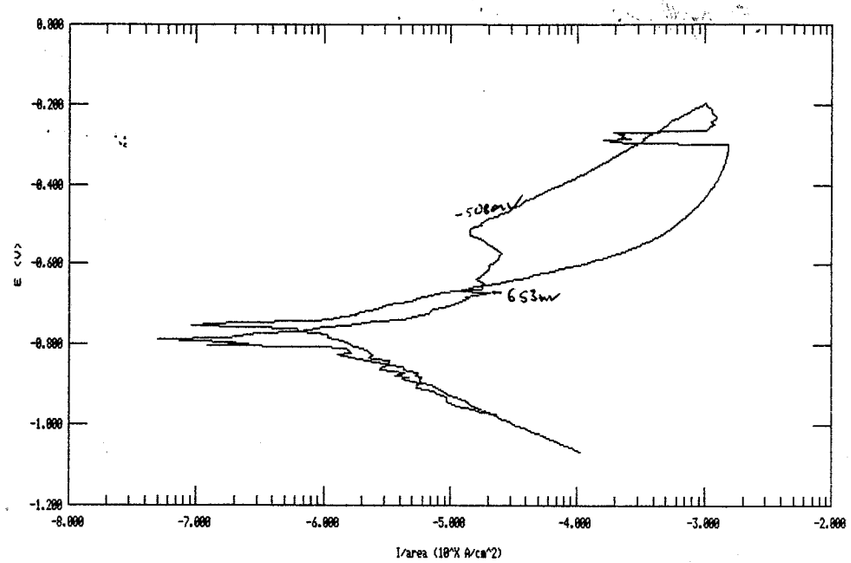
Sample #2 Abiotic

Model 352/252 Corrosion Analysis Software, v. 2.01 — Filename: B:2516451.dat — Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION — File Status: NORMAL — Date Run: 03-26-96 — Time Run: 15:24:24

CP	PASS vs. R	CT	PASS	IP	-0.200 vs. OC	ID	20 S
SI	5.000E-03	SR	1.670E-04	ST	2.993E+01	CR	AUTO
FL	NONE	RT	HIGH STABILITY	REF	0.24150 SCE	WRK	SOLID
IT	8.000E-03	ITA	8.000E+00	EW	0.000E+00	DEN	7.900E+00

V1	0.100 vs. OC	FP	-0.300 vs. OC
NP	338	IR	NONE
AR	8.000E+00	LS	YES
OC	-0.773		

B:2516451.da

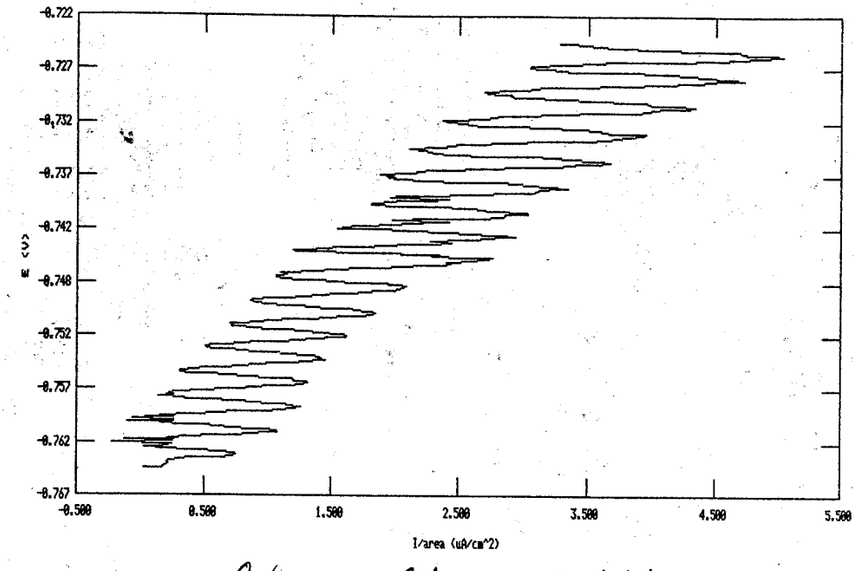


Model 352/252 Corrosion Analysis Software, v. 2.01 — Filename: B:2a516451.dat — Pstat: VStat[] Ver 2
LP LINEAR POLARIZATION — File Status: NORMAL — Date Run: 03-26-96 — Time Run: 15:13:59

CP	PASS vs. R	CT	PASS	IP	-0.020 vs. OC	ID	20 S
SR	1.000E-04	ST	1.000E+00	CR	AUTO	NP	401
RT	HIGH STABILITY	REF	0.24150 SCE	WRK	SOLID	AR	8.000E+00
DEN	7.900E+00	OC	-0.745				

FP	0.020 vs. OC	SI	1.000E-04
IR	NONE	FL	NONE
LS	YES	EW	2.792E+01

B:2a516451.d



Alice Stone 9/4/96

Date 10-8-96

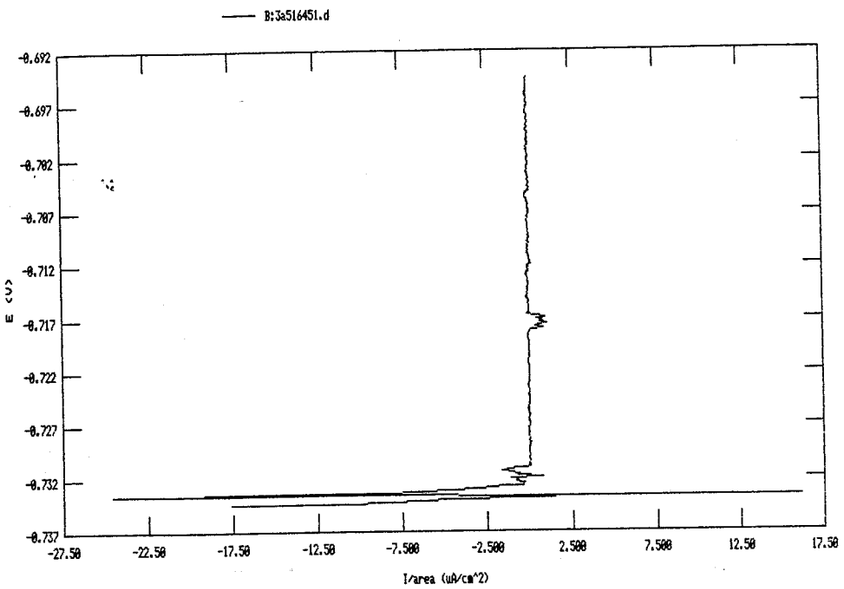
Sample #3 ABIOTIC

Model 352/252 Corrosion Analysis Software, v. 2.01
LP LINEAR POLARIZATION
CP PASS vs. R — CT PASS
SR 1.000E-04 — ST 1.000E+00
RT HIGH STABILITY — REF 0.24150 SCE
DEN 7.900E+00 — OC -0.715

File Status: NORMAL
IP -0.020 vs. OC
CR AUTO
WRK SOLID

Filename: B:3a516451.dat
Date Run: 03-26-96 — Time Run: 09:37:13
ID 20 S
NP 401
AR 8.000E+00
LS YES

Pstat: VStat[] Ver 2
SI 1.000E-04
FL NONE
EW 2.792E+01



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: B:3a516451.dat
Pstat: VStat[] Ver 2
LP LINEAR POLARIZATION — File Status: NORMAL
Date Run: 03-26-96 — Time Run: 09:37:13

Cond. Time	CT	pass	s	Initial Pot.	IP	-20.00E-3	V oc
Cond. Pot.	CP	pass	V	Final Pot.	FP	20.00E-3	V oc
Initial Delay	ID	20	s				
Scan Rate	SR	100.00E-3	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	0.1000	mV	Step Time	ST	1.000	s
No. of Points	NP	401					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	27.92	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7150	V

Alice Stone 9/4/96

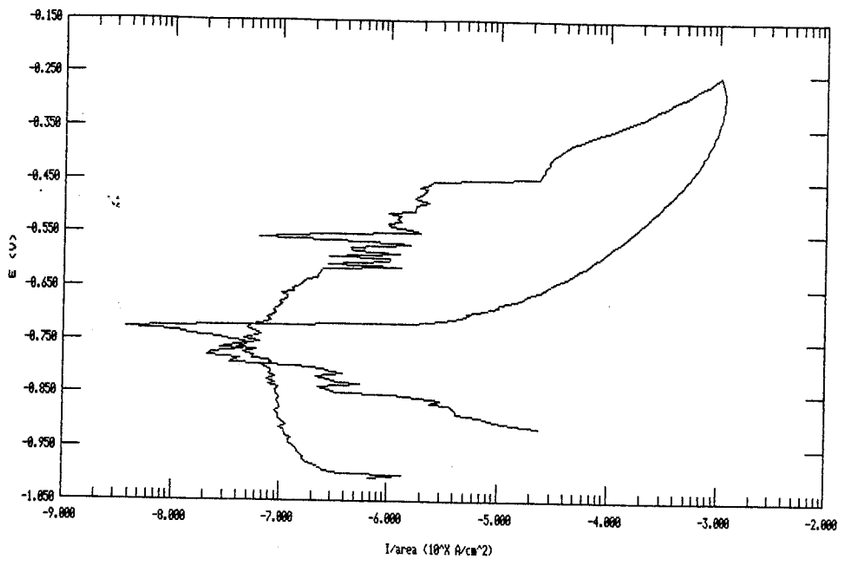
Model 352/252 Corrosion Analysis Software, v. 2.01
CP CYCLIC POLARIZATION
CP PASS vs. R — CT PASS
SI 5.000E-03 — SR 1.670E-04
FL NONE — RT HIGH STABILITY
IT 8.000E-03 — ITA 8.000E+00

File Status: NORMAL
IP -0.200 vs. OC
ST 2.993E+01
REF 0.24150 SCE
EW 0.000E+00

Filename: B:3516451.dat
Date Run: 03-26-96 — Time Run: 09:56:40
ID 20 S
CR AUTO
WRK SOLID
DEN 7.900E+00

Pstat: VStat[] Ver 2
VI 0.100 vs. OC
NP 284
AR 8.000E+00
OC -0.713

FP -0.300 vs. OC
IR NONE
LS YES



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: B:3516451.dat
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION — File Status: NORMAL
Date Run: 03-26-96 — Time Run: 09:56:40

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	VI	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	284					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7130	V

Alice Stone 9/4/96

9/5/96 Made 2 X 500ml 10% TSB as follows:
TSB lot # 58855 1.5 g per 500 ml 18.1 MΩ H_2O. Autoclaved for 20 min at 121°C and 14 psi. pH electrodes were then placed in 10% TSB solutions.

Made 2 X 1L of modified J13 media with 1ppm yeast extract and dextrose as the carbon source as follows:

Na_2CO_3	lot # 960685	0.01060 g
$NaHCO_3$	897789	0.08821 g
NaCl	947723	1.64846 g
$NaNO_3$	8917183	0.01370 g
NaF	950992	0.00440 g
$MgSO_4 \cdot 7H_2O$	947409	0.06470 g
yeast extract	59695JB	0.00100 g
dextrose	64682JA	0.20000 g

per 1L 18.1 MΩ H_2O. Autoclaved for 1hr at 121°C and 14 psi. Media was discarded because dextrose carmelized and pH was too low.

9/6/96 Made 2 X 1L of modified J13 media with dextrose as the carbon source and 1ppm yeast extract as follows:

Na_2CO_3	lot # 960685	0.01060 g
$NaHCO_3$	897789	0.08821 g
NaCl	947723	1.64846 g
$NaNO_3$	8917183	0.01370 g
NaF	950992	0.00440 g
$MgSO_4 \cdot 7H_2O$	947409	0.06470 g
yeast extract	59695JB	0.00100 g

per 990 ml 18.1 MΩ H_2O.

dextrose	64682JA	0.20000 g

per 10ml 18.1 MΩ H_2O.

Autoclave modified J13 media and dextrose solutions for 1hr at 14 psi and 121°C.
pH of modified J13 media before autoclaving 8.997
pH of dextrose solution before autoclaving 8.962

Dextrose solutions carmelized after autoclaving and were discarded. pH was 5.152

Dextrose solution was remade by placing 0.20000 g dextrose (lot # 64682) in 2 X 10ml of 18.1 MΩ H_2O. 10 ml of dextrose solution was filter sterilized and added to modified J13 media made above. pH 8.989

Three CP vessels were filled with approx. 600 ml of modified J13 media with dextrose as the carbon source and 1ppm of yeast extract. All openings were covered with autoclave paper and vessels were sterilized along with all other accessories. Autoclaved for 1hr at 121°C and 14 psi. Glassware was allowed to cool in autoclave and then was immediately assembled upon removal.

Three A516 Grade 60 carbon steel specimens were polished to 600 grit finish and then weighed. Results are as follows:

sample #4	10.81850 g
sample #5	10.90120 g
sample #6	10.70680 g

Alice Stone 9/6/96

9/9/96 sterilized samples 4, 5, & 6 and placed in CP vessels. Degased vessels with 99.99% N_2 for 1 hr. Ran EIS on each sample. saved as 4a5161.z, 5a5161.z, & 6a5161.z

9/10/96

sample #4 ~~Rp~~ R_{CT} 28 KΩ
sample #5 R_{CT_1} 0.169 KΩ R_{CT_2} 16 KΩ
sample #6 R_{CT} 29.5 KΩ

Inoculated vessels #5 & #6 by placing a loopfull of YMI-1, YMI-4, YMI-5-2, YMI-6, YMI-9, YMI-10, Ym-10-A-04, Ym-10-H-12, YM-10-H-05, Ym-10-Q-06, & Ym-10-M-02 in 3 ml of Mod. J13 media with dextrose and 1 ppm yeast extract. 1 ml of bacterial suspension was added to vessel #5 & #6 at 11:00.

Characterization of Yucca Mountain Isolates

Test	YMI-1	YMI-2	YMI-3	YMI-4	YMI-5-1	YMI-5-2	YMI-6	YMI-7	YMI-8	YMI-9	YMI-10
pigmentation	white	clear	white	cream	pink	yellow	yellow	off-white	yellow	white	white
morphology	irregular	irregular	irregular	circular	punctiform	circular	circular	circular	circular	irregular	circular
shape	short rods	long thin rods	rods in chains	med-long rods	short rods	very short rods	very short rods	rods in chains	spiral rods	short rods	short rods in chains
Gram stain	+	+	+	+	-	-	-	-	-	+	+
aerobic	+	+	+	+	+	+	+	+	+	+	+
anaerobic	+	-	-	+	-	+	+	-	-	+	+
motility	-	-	-	-	-	-	-	-	-	+	+
catalase	-	-	-	-	+	+	+	+	+	+	+
oxidase	-	-	-	-	-	-	-	-	-	-	-
glucose (acid)	+	+	+	+	+	+	+	+	+	+	+
carbohydrates (F/O/-)	-	F	-	O	O	-	O	-	O	F	O
spore former	+	+	+	+	-	-	-	+	+	+	+
polymer producer	++	++	++	+	-	-	-	+	+	++	++

Alice Stone 9/11/96

9/11/96 pH electrodes still not sterile. 10% TSB is cloudy indicating bacterial growth. removed electrodes from 10% TSB and placed in fresh 0.525% sodium hypochlorite solution at 9:00.

Test	YM-10-A-04	YM-10-H-12	YM-10-H-05	YM-10-Q-06	YM-10-M-02
pigmentation	bright yellow	orange-yellow	yellow	orange	cloudy white
morphology	punctiform	circular	punctiform	circular	punctiform
shape	very short rods	very short rods	very short rods	very short rods	short rods
Gram stain	+	-	+	+	+
aerobic	+	+	+	+	+
anaerobic	+	-	-	-	-
motility	-	-	-	-	-
catalase	-	-	-	+	-
oxidase	-	-	-	-	-
glucose (acid)	+	+	+	+	+
carbohydrates (F/O/-)	F	O	F	F	-
spore former	-	-	-	-	-
polymer producer	+	+++	+++	+	+

Alice Stone 9/11/96

9/12/96 Ran EIS on samples #4, #5, & #6 using same setup as before. T=96 hrs
sample #4 4a51648.Z R_{ct} 76 mΩ - 71 KΩ low freq removed.
sample #5 5a51648.Z R_{ct} 2.2 KΩ
sample #6 6a51648.Z R_{ct} 22 KΩ

Made 2 x 500 ml 10% TSB as follows: TSB lot # 58855 1.50 g per 500 ml 18.1 MΩ H_2O. Autoclaved for 20 min at 121°C and 14 psi. pH electrodes were then placed in 10% TSB solutions.

Initial counts from modified J13 media with 1 ppm yeast extract and sample #1 and D. vulgaris show 10^{-2} CFU/ml. Dilution series will continually be checked for any changes.

1 ml samples were removed from flasks #4, #5, & #6 and placed in lactate/acetate media to check for APB.

Alice Stone 9/12/96

9/16/96 Ran EIS on samples #4, #5, & #6 using same setup as before.

sample #4 a:4a516196.z R_{ct} = 68.1 kΩ

sample #5 a:5a516196.z R_{ct} = 1.81 kΩ

sample #6 a:6a516196.z R_{ct} = 30.6 kΩ

pH electrodes still not sterile. Removed from 10% TSB and placed in sodium hypochlorite solution.

Alice Stone 9/16/96

9/17/96 Removed 100ul samples from vessels #4, #5, #6 and also removed 100ul samples from #4, #5, & #6 in lactate/lacetate media from 9/12/96. Performed serial dilutions and plate counts on R2A.

Made 2 x 500 ml 10% TSB. Autoclaved for 45 min at 121°C and 14 psi. pH electrodes were removed from sodium hypochlorite solutions and placed in 10% TSB

Alice Stone 9/17/96

9/18/96 Ran EIS on samples #4, #5, #6 using same setup as before

sample #4 a:4a516244.z 32 kΩ

sample #5 a:5a516244.z 1.35 kΩ 2nd Warburg.

sample #6 a 6a516244.z 15.7 kΩ

Freeze dried Ym-10-H-12, Ym-10-H-05, & Ym-10-M-02.

Alice Stone 9/18/96

9/19/96 Results from plate counts on samples #4, #5, & #6 are as follows:

	sample #4	sample #5	sample #6
vessel	0 CFU/ml	5.00×10^6 CFU/ml	7.58×10^5 CFU/ml
lactate/acetate	0 CFU/ml	4.67×10^6 CFU/ml	7.67×10^5 CFU/ml

Made 1 Liter of 9K media as follows:

$(NH_4)SO_4$	lot# 953346	3.0g
KCl	946305A	0.1g
K_2HPO_4	950082	0.5g
$MgSO_4 \cdot 7H_2O$	947409	0.5g
$Ca(NO_3)_2$	4236 KENC	0.01g

added to 700ml of 18.1 MΩ H_2O. 1ml of 10N H_2SO_4 was added to adjust pH to desired range. Solution was then autoclaved for 45 min at 14 psi and 121°C.

$FeSO_4 \cdot 7H_2O$	946178	44.22g

added to 300ml of 18.1 MΩ H_2O and stirred. Solution was filter sterilized with an ACRO 50 0.2um lot # 1705 filter. The two solutions were combined.

Freeze dried YmI-10, Ym-10-A-04, & Ym-10-Q-06.

Alice Stone 9/19/96

9/20/96 Ran EIS on samples #4, #5, & #6 using same setup as before:

sample #4 a:4a516292..z

sample #5 a:5a516292.z

sample #6 a:6a516292.z

Setup a 500 ml Kimax vessel with a gas sparge stone, air in, air out, media drip tube, and media out tube. Added 475 ml 18.1 MΩ H_2O and autoclaved for 45 min at 121°C and 14 psi. Upon cooling 25 ml of 9K

solution was asceptically added making the resulting solution 5% 9k. Added thiobacillus at 11:00 and airated with compressed air.

Ran cyclic polarizations on samples #4, #5, & #6 which contain A516 Grade 60 carbon steel in Modified J13 with 1000 ppm Cl^-, 1ppm YE, & dextrose.

	#4	#5	#6
pH final	9.728	9.600	8.515
pt	-388mV	-543 mV	-354 mV
sample	-782mV	-776mV	-755 mV
saved as	b:4516292.dat	b:5516292.dat	b.6516292.dat

Freeze dried YmI-9, YmI-8, & YmI-7

Div 06 Results are as follows:

Abiotic swabs	0
Biotic swabs	10^4
Abiotic planktonic	0
Biotic planktonic	10^5
Thio planktonic	10^7

Removed 100ul samples from vessels #4, #5, & #6 for plate counts. (292 hrs)

1/2 Sample Ym-100-5-16a was crushed and fractionated to 2-4mm. Now called Ym-100-7-24.

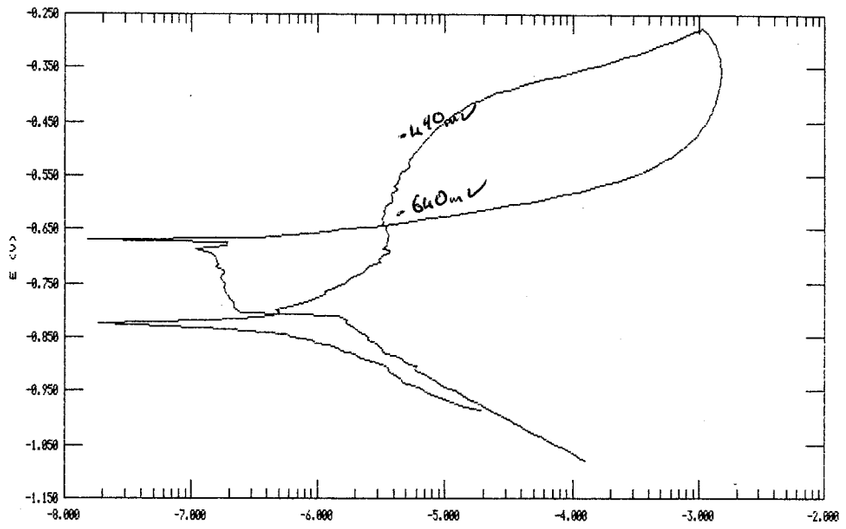
Samples #4, #5, & #6 were weighed and the results are as follows:

sample #4	10.76039g
sample #5	10.85417g
sample #6	10.67003g

sample #4 Abiotic

Model 352/252 Corrosion Analysis Software, v. 2.01 Filename: b:4516292 Pstat: VStat[] Ver 2 CP CYCLIC POLARIZATION
File Status: NORMAL Date Run: 04-04-96 Time Run: 12:22:23

CP	PASS vs. R	CT	PASS	IP	-0.200 vs. OC	ID	20 S
SI	5.000E-03	SR	1.670E-04	ST	2.993E+01	CR	AUTO
FL	NONE	RT	HIGH STABILITY	REF	0.24150 SCE	WRK	SOLID
IT	8.000E-03	ITA	8.000E+00	EW	0.000E+00	DEN	7.900E+00
VI	0.100 vs. OC	FP	-0.300 vs. OC				
MP	304	IR	NONE				
AR	8.000E+00	LS	YES				
OC	-0.785						



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: b:4516292
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION
Date Run: 04-04-96
File Status: NORMAL
Time Run: 12:22:23

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	V1	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	MP	304					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7850	V

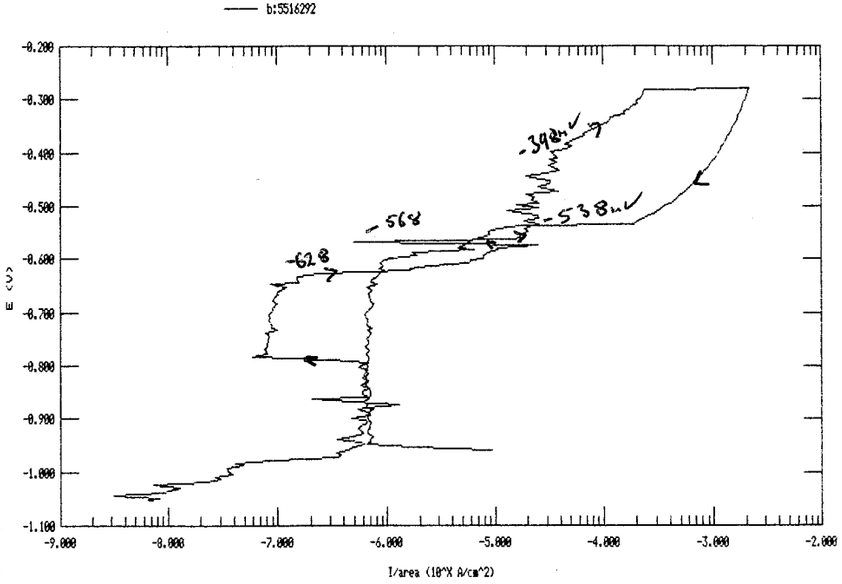
Alicia Stone 9/20/96

9/21/96

Sample #5 BIOTIC

Model 352/252 Corrosion Analysis Software, v. 2.01 Filename: b:5516292 Pstat: VStat[] Ver 2 CP CYCLIC POLARIZATION
File Status: NORMAL Date Run: 04-04-96 Time Run: 15:11:36

CP	PASS vs. R	CT	PASS		IP	-0.200 vs. OC	ID	20 S		VI	0.100 vs. OC	FP	-0.300 vs. OC
SI	5.000E-03	SR	1.670E-04		ST	2.993E+01	CR	AUTO		NP	292	IR	NONE
FL	NONE	RT	HIGH STABILITY		REF	0.24150 SCE	WRK	SOLID		AR	8.000E+00	LS	YES
IT	8.000E-03	ITA	8.000E+00		EW	0.000E+00	DEN	7.900E+00		OC	-0.758		



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: b:5516292
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION File Status: NORMAL
Date Run: 04-04-96 Time Run: 15:11:36

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	VI	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	292					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7580	V

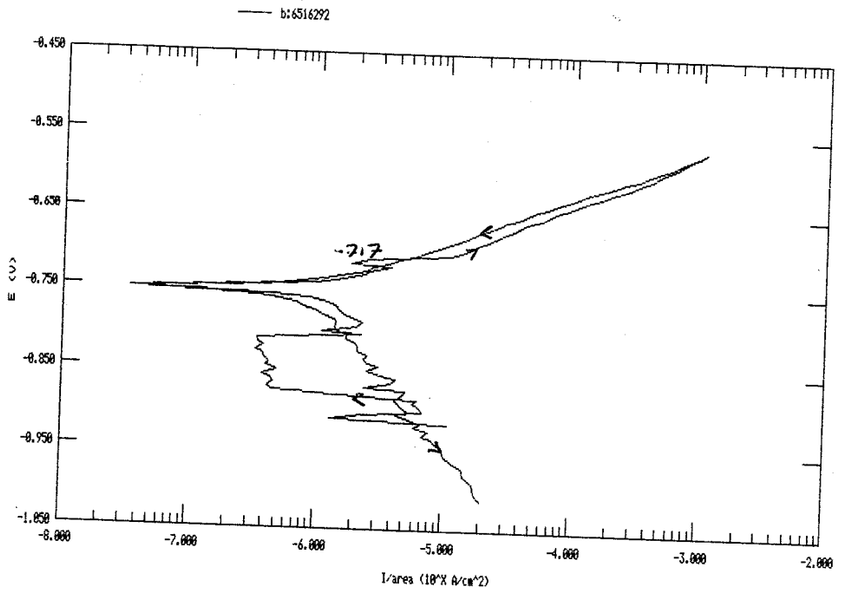
Weighed out 3 X 4g of Ym-100-7-24 and placed in sterilized conical flasks labeled Ym-100-7-24 (1 thru 3). Placed these flasks along with control 120°C (1 ea) in oven set at 120°C @ 4:00.

Weighed out 2 X 4g of Ym-100-7-24 and placed in sterilized conical flask labeled Ym-100-7-24 80°C (1 ea). Added 50 ml of R2B to these flasks and also to one control 80° flask. Place all flasks in RH chamber set at 80°C @ 4:00.

Sample #6 BIOTIC

Model 352/252 Corrosion Analysis Software, v. 2.01 Filename: b:6516292 Pstat: VStat[] Ver 2 CP CYCLIC POLARIZATION
File Status: NORMAL Date Run: 04-05-96 Time Run: 11:54:28

CP	PASS vs. R	CT	PASS		IP	-0.200 vs. OC	ID	20 S		VI	0.100 vs. OC	FP	-0.300 vs. OC
SI	5.000E-03	SR	1.670E-04		ST	2.993E+01	CR	AUTO		NP	160	IR	NONE
FL	NONE	RT	HIGH STABILITY		REF	0.24150 SCE	WRK	SOLID		AR	8.000E+00	LS	YES
IT	8.000E-03	ITA	8.000E+00		EW	0.000E+00	DEN	7.900E+00		OC	-0.717		



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: b:6516292
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION File Status: NORMAL
Date Run: 04-05-96 Time Run: 11:54:28

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	VI	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	160					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7170	V

Alice Stone 9/21/96

9/23/96

Results from plate counts on samples #4, #5, & #6. T=292 hrs

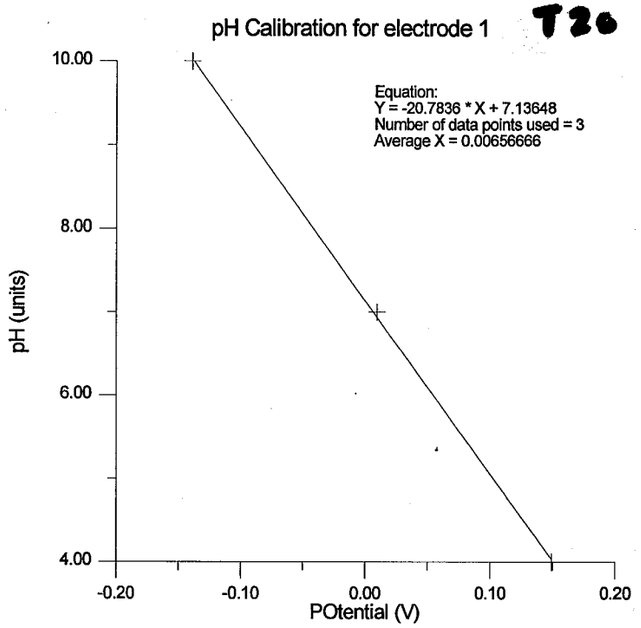
sample #4 0 cfu/ml
sample #5 5.00×10^{-5} cfu/ml
sample #6 8.92×10^{-5} cfu/ml

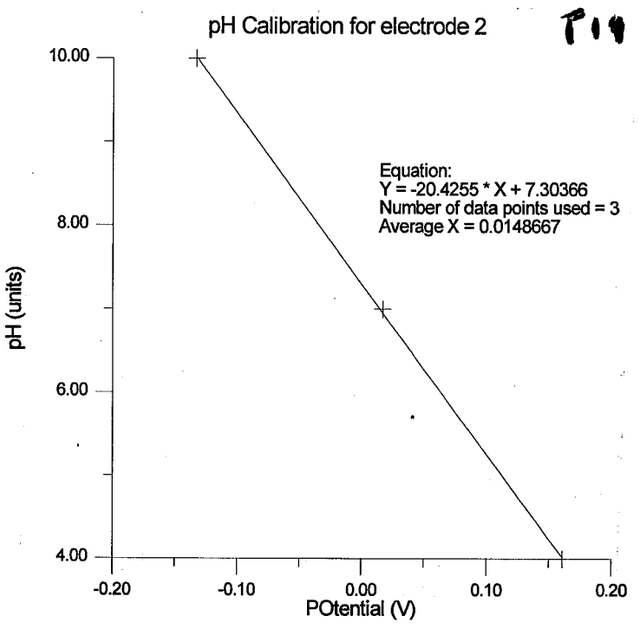
Removed flasks Ym-100-7-24 (1 thru 3) 120°C and Controls (1 & 2) 120°C from oven. Broke Ym-100-7-24 #3 120°C. placed other 4 flasks in RH chamber set at 30°C.

Removed flask Ym-100-7-24 (1 & 2) 80°C and control #1 80°C from RH chamber and placed in incubator/oven set at 80°C.

Made 1L of Thiosulfate media as follows:
0.31642 g $Na_2S_2O_3$ (2mM) lot # 923931A
1.64859 g. NaCl 947723
1 ml trace minerals
added to 900 ml 18.1 MΩ H_2O. Autoclaved for 1 hr. at 14 psi and 121°C. Added 100 ml of 10 x Mn media that was autoclaved for 1 hr at 121°C and 14 psi.

Removed sterile reference electrodes from 10% TSB and placed in vessels (T19 & T20) assembled on p. 67/182. Both vessels where filled with 1 L Thiosulfate media prepared on p. 68/182 & p. 86/182 & gased with 95% N_2 and 5% H_2. Abiotic run began.





Alice Stone 9/23/96

9/24/96

Removed flasks Ym-100-7-24 (1 & 2) 120°C and controls (1 & 2) 120°C from RH chamber and added 50 ml of R2B to each. Placed flasks in shaking water bath @ 2:00. Took 100 µl samples from flasks Ym-100-7-24 (1 & 2) and performed plate counts.

Removed flasks Ym-100-7-24 (1 & 2) 80°C and control #1 80°C from incubator and returned ~~to~~ RH chamber set at 80°C @ 2:00.

Made 2 x 1L of modified J13 media with 6 ppm Ce^{-}, 1 ppm yeast extract, and dextrose as the carbon source as follows:

Na_2CO_3	lot # 960685	0.01060 g
$NaHCO_3$	897789	0.08821 g A.S.
NaCl	947723	~~0.01546~~ g 0.01058 g
$NaNO_3$	8917183	0.01370 g
NaF	950992	0.00440 g
$MgSO_4 \cdot 7H_2O$	947409	0.06470 g
yeast extract	59695JB	0.00100 g
dextrose	64682JA	0.20000 g

All items except for dextrose where placed in 990 ml 18.1 MΩ H_2O. Autoclaved for 1hr at 121°C and 14psi. Dextrose was placed in 10 ml of 18.1 MΩ H_2O and filter sterilized into each media bottle with 990 ml of the above solution.

Removed 100ul samples from flasks Ym-100-7-24 120°C (1 & 2) at 5:00 and performed plate counts.

Freeze dried samples YmI-5-1, YmI-5-2, & YmI-6.

Alice Stone 9/24/96

9/25/96

Removed 100 ul samples from Ym-100-7-24 120°C (1 & 2) and Controls 120°C (1 & 2) @ 8:00. Performed plate counts.

Data logger started @ 9:15.

Removed 100 ul samples from Ym-100-7-24 120°C (1 & 2) and performed plate counts @ 11:00

Freeze dried samples YmI-4, YmI-3, & YmI-2

Removed 100ul samples from Ym-100-7-24 120°C (1 & 2) and performed plate counts. @ 2:00

Removed 100ul samples from Ym-100-7-24 120° (1 & 2) and performed plate counts @ 5:00

Three CP vessels with all openings covered with autoclave paper were sterilized with all other accessories. Autoclaved for 1hr at 121°C and 14psi. Glassware was allowed to cool in autoclave and then was immediately assembled upon removal. Platnum electrodes were also sterilized with 70% isopropanol and added to each vessel.

Alice Stone 9/25/96

9/26/96

Removed 100ul samples from Ym-100-7-24 120°C (1 & 2) and controls 120°C (1 & 2) @ 8:00. & performed plate counts.

Freeze dried sample YmI-1.

Removed 100ul samples from Ym-100-7-24 120°C (1 & 2) @ 11:00 and performed plate counts.

Results from plate counts performed on samples incubated at 120°C for 48 hours:

Date	Time	Control #1	control #2	Ym-100-7-24 #1	Ym-100-7-24 #2
9/24	2:00	0 cfu/ml	0 cfu/ml	0 cfu/ml	0 cfu/ml
9/24	5:00	–	–	0 cfu/ml	0 cfu/ml
9/25	8:00	0 cfu/ml	0 cfu/ml	0 cfu/ml	0 cfu/ml
9/25	11:00	–	–	0 cfu/ml	0 cfu/ml
9/25	2:00	–	–	3.00×10^4	3.08×10^4
9/25	5:00	0 cfu/ml	0 cfu/ml	8.17×10^5	7.92×10^6

Weighed out 2x 4g of Ym-100-7-24 and placed in sterilized conical flasks labeled Ym-100-7-24 120°C (4&5). Placed these flasks along with control 120°C (3&4) in oven set @ 120°C @ 4:30.

Removed 100ul samples from Ym-100-7-24 120°C (1&2) and controls 120°C (1&2) and performed plate counts @ 5:00.

Alice Stone 9/26/96

9/27/96

Results from plate counts performed on samples incubated at 120°C for 48 hours:

date	time	control #1	control #2	Ym-100-7-24 #1	Ym-100-7-24 #2
9/26	8:00	0 cfu/ml	0 cfu/ml	3.00×10^8 cfu/ml	3.17×10^8 cfu/ml
9/26	11:00	–	–	5.17×10^7 cfu/ml	4.83×10^7 cfu/ml
9/26	2:00	–	–	7.33×10^7 cfu/ml	7.50×10^7 cfu/ml
9/26	5:00	0 cfu/ml	0 cfu/ml	8.00×10^7 cfu/ml	7.58×10^7 cfu/ml

Three A516 Grade 60 carbon steel specimens were polished to a 600 grit finish and then weighed. Results are as follows:

sample #7 10.67227 g
sample #8 10.61585 g
sample #9 10.73661 g

Three CP vessels were filled with approx. 600ml of modified J13 media with dextrose as the carbon source, 1ppm yeast extract, & 6ppm Cl^- which was previously sterilized. Sterilized samples #7, #8, & #9 and place one in each vessel @ 16:00. pH of media 9.001

A.S. 9/27/96

Inoculated vessels ~~#7~~, #8, & #9 by placing a loopfull of YmI-1 thru YmI-10, Ym-10-A-04, Ym-10-H-12, Ym-10-H-05, Ym-10-Q-06, & Ym-10-M-02 in 3ml of Modified J13 media with dextrose, 1ppm yeast extract, and 6ppm Cl^-. 1ml of bacterial suspension was added to vessels #9 & #8 @ 16:30.

Alice Stone 9/27/96

9/30/96

Ran EIS on samples #7, #8, & #9

sample 7a: 7a51672.Z R_ct 1.145 kΩ C= 55.4 mF
sample 8a: 8a51672.Z R_ct 0.720 kΩ C= 29.6 mF
sample 9a: 9a51672.Z R_ct 1.927 kΩ C= 37.5 mF

Removed flasks Ym-100-7-24 80°C (1&2) and control 80°C #1 from RH chamber and performed streak plates on each. Flasks were then placed in incubator set at 80°C @ 14:00.

Removed flasks Ym-100-7-24 120°C (4&5) and controls 120°C ~~(4&5)~~ (3&4) from the oven and placed in RH chamber at 14:00.

15:00 (452888) T= 126 hrs.
T19 30°C, pt -136mV, pH 7.285
b -127mV, r -155mV, w -167mV, y -107mV
T20 28°C, pt -161mV, pH 8.005
b -198mV, r -110mV, w -193mV, y -187mV

Removed 100ul samples from T19 & T20 to check for contamination. Plated out on LBA2 @ 15:00. (T19-126 & T20-126)

Preparation of samples to be inoculated into vessels T19 & T20 as follows: removed 24 ml of sterile media for inoculum preparation and placed in sterile mc tubes. Added 1 loopfull of SP200 to each mc tube and vortexed for 1 min. T19 & T20 vessels were inoculated @ 16:00.

Removed 100ul samples from T19 & T20 for plate counts. (T19-128 & T20-128)

17:00 (459299) T = 128 hrs
T19 30°C, pt -120 mV, pH 8.000
b -120 mV, r -155 mV, w -169 mV, y -110 mV
T20 28°C, pt -157 mV, pH 7.291
b -192 mV, r -109 mV, w -193 mV, y -183 mV
Alice Stone 9/30/96

10/1/96 9:00 (517599) T = 144 hrs
T19 28°C, pt -435 mV, pH 7.411
b -127 mV, r -169 mV, w -171 mV, y -102 mV
T20 28°C, pt -342 mV, pH 8.093
b -430 mV, r -226 mV, w -408 mV, y -365 mV
horrible sulfide smell very evident!
Removed 1 ml samples from vessels T19 & T20 for plate counts. Residuals frozen as T19-144 & T20-144.

Ran EIS on T19 & T20 — 190 kΩ 10-4-96
T19 red = T19r144.Z — 140 kΩ
T19 white = T~~20~~19w144.Z — ~~205~~ 192 kΩ 10-4-96

T20 red = T20r144.Z 2.05 kΩ
T20 white = T20w144.Z 337 kΩ

Removed flasks Ym-100-7-24 80°C (1&2) and control 80°C #1 from incubator and added 50 ml R2B to each flask. Flasks where then placed in RH chamber set at 80°C @ 10:00.

Removed flasks Ym-100-7-24 120°C (4&5) and controls 120°C (3&4) from the RH chamber and added 50 ml of sterile R2B to each flask. Flasks where then placed in shaking water bath @ 10:00.

Removed 100ul samples from Ym-100-7-24 120°C (4&5) and controls 120°C (3&4) and performed plate counts @ 11:00 after 1 hr in shaking water bath.

Removed 100ul samples from Ym-100-7-24 120°C (4&5) and performed plate counts @ 14:00 after 4 hrs in shaking water bath.

15:00 (538817) T = 150 hrs
T19 32°C, pt -455 mV, pH 8.282
b -168 mV, r -211 mV, w -188 mV, y -127 mV
T20 29°C, pt -416 mV, pH 7.592
b -425 mV, r -329 mV, w -429 mV, y -415 mV
Removed 1 ml samples from vessels T19 & T20 for plate counts. Residuals frozen as T19-150 & T20-150

Prepared 500 ml of 5% 9K media as follows: Autoclaved 475 ml of 18.1 MΩ H_2O and then added 25 ml of 9K. Media was then placed in Div 06 500 ml Kimax vessel.

Prepared 2 x 500 ml of 5% lactate/acetate media as follows: Autoclaved 2x 475 ml 18.1 MΩ H_2O for 45 min at 121°C and 14 psi. Added 2 x 25 ml of lactate/acetate (p. 80/138) media to each. 500 ml of 5% lactate/acetate was added to two Div 06 500 ml Kimax vessels.

Removed 100 ul samples from Ym-100-7-24 120°C (4&5) and controls 120°C (3&4) and performed plate counts @ 17:00 after 7 hrs in shaking water bath.

Alice Stone 10/1/96

10/2/96 Removed 100 ul samples from Ym-100-7-24 120°C (4&5) and controls 120°C (3&4) and performed plate counts @ 8:20 after ~ 21 hrs in shaking water bath.

9:00 (603662) T= 168 hrs
T19 30°C, pt -470 mV, pH 8.745
b -375 mV, r -383 mV, w -360 mV, y -363 mV
T20 27°C, pt -431 mV, pH 8.545
b -416 mV, r -351 mV, w -430 mV, y -424 mV
Removed 1 ml samples from T19 & T20 and performed plate counts. Residuals frozen as T19-168 & T20-168.

Ran EIS on samples #7, #8, & #9:
sample 7 a:7a516120.z R_{ct} = 1.744 kΩ C = 5.74×10^{-4}
sample 8 a:8a516120.z R_{ct} = 0.863 kΩ C = 3.28×10^{-4}
sample 9 a:9a516120.z R_{ct} = 1.273 kΩ C = 2.68×10^{-4}

Made biofilm counts on T19 & T20 @ 10:00 (607928) T= 169 hrs using MEP #1 (12:00) blue and red.
T19 blue = -376 mV T19 red = -382 mV
T20 red = -350 mV T20 blue = -416 mV

Electrodes removed and rinsed in 10 ml filter sterilized 18.1 MΩ H_2O. Swabbed coupons with sterile cotton swabs and placed in 1 ml of sterile PBS. Vortexed on high for 1.5 min and performed plate counts.
T19-169B1 & T19-169R1
T20-169B1 & T20-169R1

Removed 100 ul samples from Ym-100-7-24 120°C (4&5) and performed plate counts @ 10:00 after 23 hrs in shaking water bath.

Made 1000 ml of 100x modified J13 media with 6 ppm Cl^- as follows into 1 L 18.1 MΩ H_2O.

Na_2CO_3	Lot # 960685	1.06000 g
$NaHCO_3$	897789	8.82100 g
NaCl	947723	1.05800 g
$NaNO_3$	8917183	1.37000 g
NaF	950992	0.44000 g
$MgSO_4 \cdot 7H_2O$	947409	6.47000 g

Autoclaved for 1 hr at 121°C and 14 psi

Results from plate counts on T19 & T20:

Time (hrs)	T19 (cfu/ml)	T20 (cfu/ml)
126	0	0
128	2.75×10^6	3.33×10^6
144	3.00×10^6	2.08×10^7
150	5.58×10^6	1.67×10^7

500 ml Kimax vessel containing 5% 9K media was inoculated with Thiobacillus @ 2:00. (Div 06)

500 ml Kimax vessel containing 5% lactate/acetate media was inoculated with acid producing bacteria @ 3:00 (Div 06)

Run Ells of [illegible] 10-2-96

Removed 100 ul samples from Ym-100-7-24 120°C (4 & 5) and performed plate counts @ 2:00 after 26 hours in shaking water bath.

15:00 (625010) T=174 hrs.
T19 34°C, pt -433 mV, pH 8.706
b -385 mV, r -388 mV, w -366 mV, y -364 mV
T20 30°C, pt -463 mV, pH 8.814
b -424 mV, r -353 mV, w -432 mV, y -426 mV
Removed 1 ml samples from each vessel and performed plate counts. Residuals frozen as T19-174 & T20-174.

Results from streak plates done on Ym-100-7-24 80°C (1 & 2) and control 80°C #1 are as follows
Ym-100-7-24 80°C #1 no growth
Ym-100-7-24 80°C #2 no growth
control 80°C #1 no growth
Alice Stone 10/2/96

10/3/96 Removed 100 ul samples from Ym-100-7-24 120°C (4 & 5) and control 120°C (3 & 4) and performed plate counts @ 8:00 after 48 hours in shaking water bath.

9:00 (690293) T=192 hrs
T19 30°C, pt -411 mV, pH 8.935
b -381 mV, r -380 mV, w -345 mV, y -338 mV
T20 28°C, pt -449 mV, pH 8.775
b -440 mV, r -359 mV, w -431 mV, y -431 mV
Removed 1 ml samples from each vessel and performed plate counts. Residuals frozen as T19-192 & T20-192.

Results from plate counts on T19 & T20:

Time (hrs)	T19 (cfu/ml)	T20 (cfu/ml)
168	5.25×10^7	4.42×10^7
174	3.17×10^7	3.33×10^7

Results from biofilm counts on T19 & T20:

Time (hrs)	T19 (cfu/ml)	T20 (cfu/ml)
169 R1	5.67×10^4	5.50×10^4
169 B1	5.58×10^4	5.58×10^4

Results from plate counts on Ym-100-7-24 120°C (4 & 5) and controls 120°C (3 & 4) incubated at 120°C for 120 hrs:

date	time	control #1	control #2	Ym-100-7-24 #4	Ym-100-7-24 #5
10/1	11:00	0 cfu/ml	0 cfu/ml	0 cfu/ml	0 cfu/ml
10/1	2:00	0 cfu/ml	0 cfu/ml	0 cfu/ml	0 cfu/ml
10/1	5:00	0 cfu/ml	0 cfu/ml	7.17×10^3 cfu/ml	7.08×10^3 cfu/ml
10/2	8:00	0 cfu/ml	0 cfu/ml	4.17×10^5 cfu/ml	4.33×10^5 cfu/ml
10/2	10:06	0 cfu/ml	0 cfu/ml	5.92×10^6 cfu/ml	5.75×10^6 cfu/ml
10/2	2:00	0 cfu/ml	0 cfu/ml	5.17×10^7 cfu/ml	5.50×10^7 cfu/ml
10/2	5:00	0 cfu/ml	0 cfu/ml	6.83×10^7 cfu/ml	7.08×10^7 cfu/ml

100x Modified J13 media with 6ppm Cl^- prepared on p. 95/182 was discarded because the carbonate precipitated out of solution in autoclave.

Made 1L of 10x modified J13 media with 6 ppm Cl^- as follows:

Na_2CO_3	lot # 960685	0.10600 g
$NaHCO_3$	897789	0.88210 g
NaCl	947723	0.10580 g
$NaNO_3$	8917183	0.13700 g
NaF	950992	0.04400 g
$MgSO_4 \cdot 7H_2O$	947409	0.64700 g

into 1L 18.1MΩ H_2O. Autoclaved for 1 hr at 121°C and 14 psi.

2:00 (708876) T = 197 hrs
T19 30°C, pH 8.953, pt -419 mV
b -378 mV, r -377 mV, w -336 mV, y -329 mV
T20 28°C, pH 8.798, pt -420 mV
b -441 mV, r -368 mV, w -431 mV, y -434 mV
Removed 1 ml samples from each vessel and performed plate counts. Residuals frozen as T19-197 & T20-197.
Alice Stone 10/3/96

10/4/96 8:36 (775258) T = 215 hrs
T19 30°C, pt -429 mV, pH 8.971
b -371 mV, r -374 mV, w -300 mV, y -278 mV
T20 28°C, pt -430 mV, pH 8.770
b -455 mV, r -372 mV, y -439 mV, w -428 mV
Removed 1 ml from each vessel and performed plate counts. Residuals frozen as T19-215 & T20-215.

Ran EIS on T19 & T20:
T19 red = T19r215.Z R_{ct} 356 kΩ
T19 white = T19w215.Z R_{ct} 130 kΩ
T20 red = T20r215.Z R_{ct} 152 kΩ
T20 white = T20w215.Z R_{ct} 319 kΩ

2:00 (794276) T = 221 hrs
T19 30°C, pt -446 mV, pH 8.977
b -360 mV, r -365 mV, w -286 mV, y -249 mV
T20 28°C, pt -428 mV, pH 8.788
b -454 mV, r -373 mV, w -428 mV, y -436 mV
Removed 1 ml from each vessel and performed plate counts. Residuals frozen as T19-221 & T20-221.

Results from plate counts on T19 & T20:

Time (hrs)	T19 (cfu/ml)	T20 (cfu/ml)
192	4.83×10^5	5.67×10^5
197	6.50×10^5	5.50×10^5

Made biofilm counts on T19 & T20 @ 3:00 (797852) T = 222 hrs using MEP#2 blue and red:
T19 red = -366 mV T19 blue = -360 mV
T20 red = -372 mV T20 blue = -455 mV
Electrodes were removed and rinsed in 10 ml filtered sterilized 18.1 MΩ H_2O. Swabbed coupons with sterile cotton swabs and placed each in 1 ml of sterile PBS. Vortexed on high for 1.5 min and performed plate counts.
T19-222 B2 T19-222 R2
T20-222 B2 T20-222 R2
Alice Stone 10/4/96

10/5/96 12:30 (875549) T = 234 hrs
T19 30°C, pt -420 mV, pH 8.741
b -340 mV, r -351 mV, y -161 mV, w -240 mV
T20 28°C, pt -420 mV, pH 8.998
b -450 mV, r -375 mV, y -442, w -434 mV
Removed 1 ml from each vessel and performed plate counts. Residuals frozen as T19-234 & T20-234.

Results from plate counts on T19 & T20:

Time (hrs)	T19 (cfu/ml)	T20 (cfu/ml)
215	2.42×10^5	2.83×10^5
221	1.33×10^5	1.67×10^5

Alice Stone 10/5/96

10/7/96

900 (1035634) T = 288 hrs
T19 27°C, pt -400 mV, pH 8.961
b -311 mV, r -327 mV, w -220 mV, y -101 mV
T20 27°C, pt -370 mV, pH 8.667
b -472 mV, r -376 mV, w -439 mV, y -449 mV
Removed 1 ml samples from each vessel for plate counts. Residuals frozen and labeled as T19-288 & T20-288.

Ran EIS on samples #7, #8, & #9:
sample #7 a: 7a516240.z R_{CT} = 917.7 Ω C = 57 mF
sample #8 a: 8a516240.z R_{CT} = 651 Ω C = 68 mF
sample #9 a: 9a516240.z R_{CT} = 408 Ω C = 2.7 mF

Results from plate counts on T19 & T20:

Time (hrs)	T19 (cfu/ml)	T20 (cfu/ml)
234	2.42×10^5	2.33×10^5

Results from biofilm counts on T19 & T20:

Time (Hrs)	T19 (cfu/ml)	T20 (cfu/ml)
222B2	3.08×10^4	3.33×10^4
222R2	3.00×10^4	2.50×10^4

Removed 100ul samples from vessels #7, #8, & #9 and performed plate counts.

2:30 (1055971) T = 293 hrs
T19 33°C, pt -400 mV, pH 8.962
b -338 mV, r -341 mV, w -216 mV, y -135 mV
T20 30°C, pt -401 mV, pH 8.773
b -470 mV, r -371 mV, w -444 mV, y -447 mV
Removed 1 ml samples from each vessel and performed plate counts. Residuals frozen and labeled as T19-293 & T20-293
Alice Stone 10/7/96

10/8/96

9:00 (1121819) T = 312 hrs
T19 30°C, pt -383 mV, pH 8.508
b -293 mV, r -323 mV, w -194 mV, y -99 mV
T20 28°C, pt -337 mV, pH 8.918
b -469 mV, r -377 mV, w -443 mV, y -446 mV
Removed 1 ml samples from each vessel for plate counts. Residuals frozen and labeled as T19-312 & T20-312.

Ran EIS on vessels T19 & T20:
T19 red = T19r312.z R_{ct} = 243 KΩ
T19 white = T19w312.z R_{ct} = 68 KΩ
T20 red = T20r312.z R_{ct} = 138 KΩ 2 phase angles.
T20 white = T20w312.z R_{ct} = 35 KΩ 2 phase angles
While running EIS on vessel T19 the solution turned a brownish-orange color which could possibly be the result of iron precipitating out of solution.

Results from plate counts on T19 & T20:

Time (hrs)	T19 (cfu/ml)	T20 (cfu/ml)
288	8.92×10^4	7.25×10^4
293	9.33×10^4	8.08×10^4

2:00 (1140673) T = 317 hrs
T19 34°C, pt ~~-344~~ -557 A.S. mV, pH 8.950
b -323 mV, r -312 mV, w -256 mV, y -231 mV
T20 30°C, pt ~~-557~~ -341 A.S. mV, pH 8.657
b -646 mV, r -541 mV, w -583 mV, y -608 mV
Removed 1 ml samples from each vessel and performed plate counts. Residuals frozen and labeled as T19-317 & T20-317.

Made 2 x 1 L of modified J13 media with 6 ppm Cl^-, 1 ppm yeast extract, and lactate as the carbon source as follows:

Na_2CO_3	lot # 960685	0.01060 g
$NaHCO_3$	897789	0.08821 g
NaCl	947723	0.01058 g
$NaNO_3$	8917183	0.01370 g
NaF	950992	0.00440 g
$MgSO_4 \cdot 7H_2O$	947409	0.06470 g
yeast extract	59695JB	0.00100 g
lactate	943605	341 ul

per 1 L 18.1 MΩ H_2O. Autoclaved for 1hr at 121°C and 14 psi.

Alice Stone 10/8/96

10/9/96 Ran EIS on samples #7, #8, & #9:

sample #7 7a516288.2 R_{CT} = 1.188 KΩ C = 75.6 mF
sample #8 8a516288.2 R_{CT} = 831Ω C = 85.7 mF
sample #9 9a516288.2 R_{CT} = 981Ω C = 50.0 mF

Removed 100 ul samples from vessels #7, #8, & #9 and performed plate counts.

Checked pH in vessels #7, #8, & #9. Results are as follows:

	initial pH	pH before degasing	pH after degasing
#7	9.001	A.S. ~~6.989~~ 7.140	8.957
#8	9.001	6.653	9.237
#9	9.001	6.684	9.138

9:20 (1209886) T = 336 hrs
T19 27°C, pt -381 mV, pH 8.880
b -339, r -341 mV, w -314 mV, y -306 mV
T20 26°C, pt -441 mV, pH 8.506
b -617 mV, r -404 mV, w -531 mV, y -524 mV
Removed 1ml samples from each vessel for plate counts. Residuals frozen T19-336 & T20-336.

Ran cyclic polarizations on samples #7, #8, & #9 which contain A516 Grade 60 carbon steel in Modified J13 media with 1 ppm YE, 6 ppm Cl^-, & dextrose. Vessels #8 & #9 are inoculated with APB while #7 is the sterile control.

	#7	#8	#9
pt	-322 mV	-363 mV	-352 mV
sample	-764 mV	-756 mV	-765 mV
saved as	7a516288.dat	8a516288.dat	9a516288.dat

3:00 (1230217) T = 342 hrs
T19 32°C, pt -418 mV, pH 8.865
b -340 mV, r -340 mV, w -315 mV, y -307 mV
T20 29°C, pt -390 mV, pH 8.540
b -609 mV, r -393 mV, w -529 mV, y -510 mV
Removed 1ml samples from each vessel for plate counts. Residuals frozen and labeled as T19-342 & T20-342.

Results from plate counts performed on vessels #7, #8, & #9. T = 240 hrs

#7	0 cfu/ml
#8	2.92×10^6 cfu/ml
#9	2.08×10^6 cfu/ml

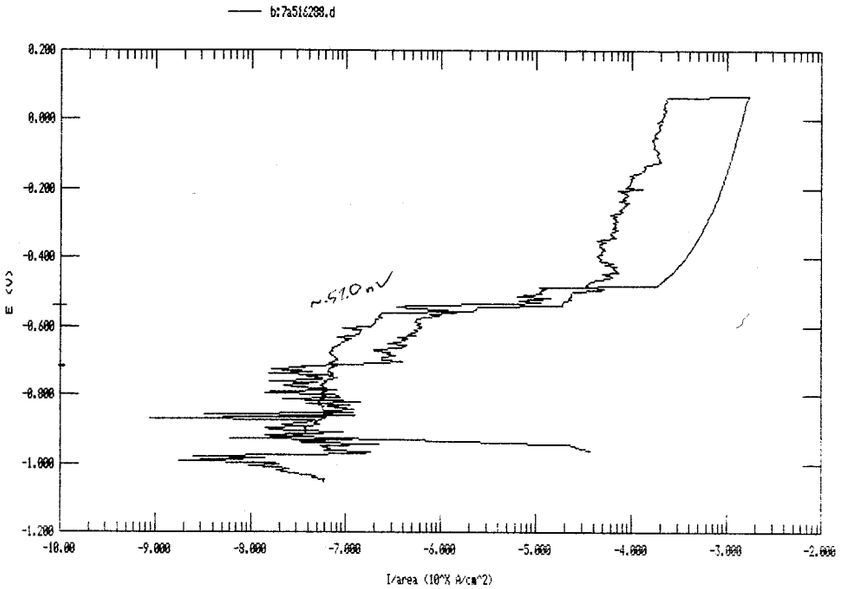
Results from plate counts on T19 & T20:

Time (hrs)	T19 (cfu/ml)	T20 (cfu/ml)
312	1.67×10^5	2.08×10^5
317	2.08×10^5	1.92×10^5

Sample #7 (abiotic)

*Model 352/252 Corrosion Analysis Software, v. 2.01 Filename: b:7a516288.dat Pstat: VStat[] Ver 2

CP	CYCLIC POLARIZATION			File Status:	NORMAL	Date Run:	04-13-96	Time Run:	09:50:06		
CP	PASS vs. R	CT	PASS	IP	-0.200 vs. OC	ID	20 S	V1	0.100 vs. OC	FP	-0.300 vs. OC
SI	5.000E-03	SR	1.670E-04	ST	2.993E+01	CR	AUTO	NP	432	IR	NONE
FL	NONE	RT	HIGH STABILITY	REF	0.24150 SCE	WRK	SOLID	AR	8.000E+00	LS	YES
IT	8.000E-03	ITA	8.000E+00	EW	0.000E+00	DEN	7.900E+00	OC	-0.761		



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: b:7a516288.dat
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION
Date Run: 04-13-96

File Status: NORMAL
Time Run: 09:50:06

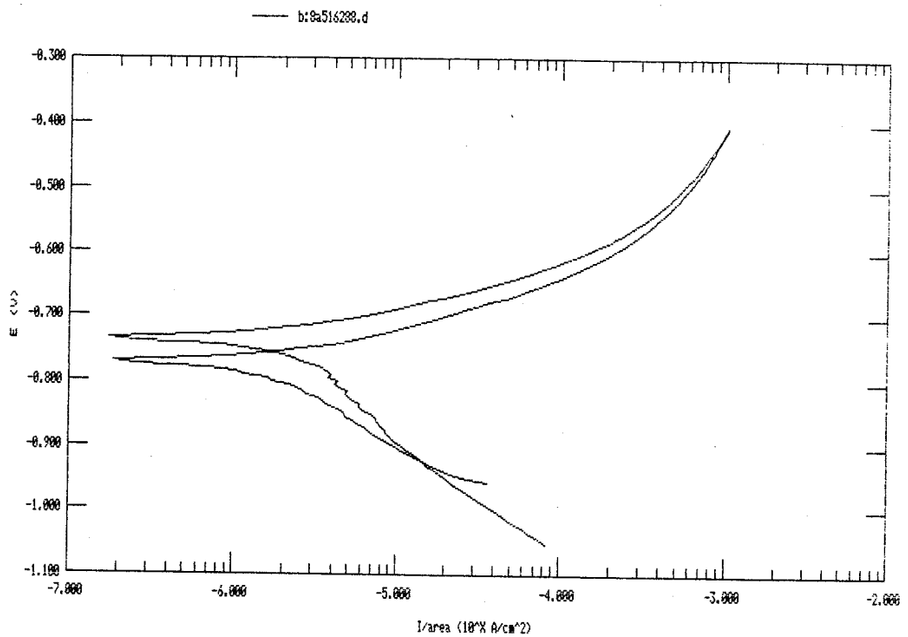
Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	V1	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	432					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7610	V

Alice Stone 10/9/96

Sample #8 (BIOTIC)

*Model 352/252 Corrosion Analysis Software, v. 2.01 Filename: b:8a516288.dat Pstat: VStat[] Ver 2

CP	CYCLIC POLARIZATION			File Status:	NORMAL	Date Run:	04-13-96	Time Run:	13:35:13		
CP	PASS vs. R	CT	PASS	IP	-0.200 vs. OC	ID	20 S	V1	0.100 vs. OC	FP	-0.300 vs. OC
SI	5.000E-03	SR	1.670E-04	ST	2.993E+01	CR	AUTO	NP	240	IR	NONE
FL	NONE	RT	HIGH STABILITY	REF	0.24150 SCE	WRK	SOLID	AR	8.000E+00	LS	YES
IT	8.000E-03	ITA	8.000E+00	EW	0.000E+00	DEN	7.900E+00	OC	-0.756		



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: b:8a516288.dat
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION
Date Run: 04-13-96

File Status: NORMAL
Time Run: 13:35:13

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	V1	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	240					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7560	V

Alice Stone 10/9/96

Sample # 9 (BIOTIC)

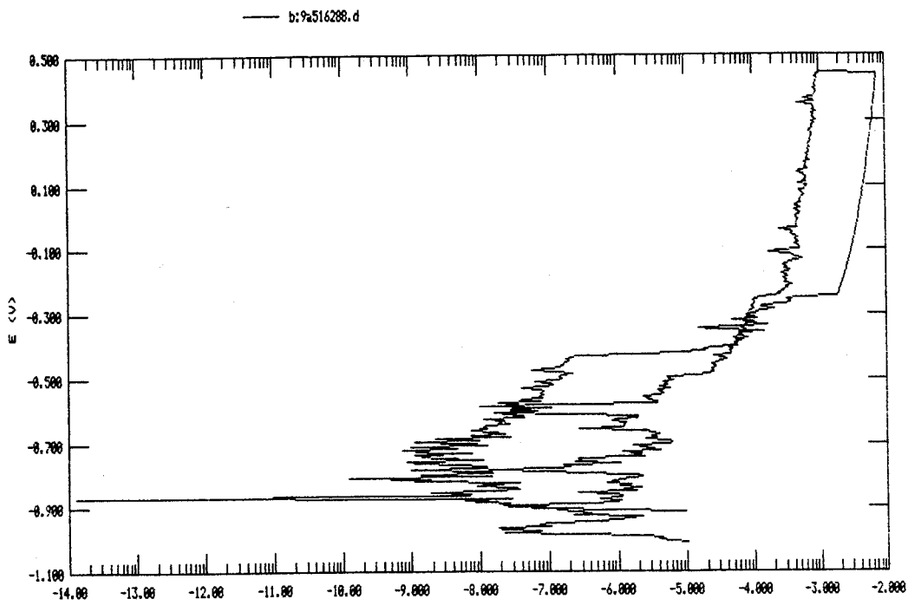
Model 352/252 Corrosion Analysis Software, v. 2.01
CP CYCLIC POLARIZATION
CP PASS vs. R CT PASS
SI 5.000E-03 SR 1.670E-04
FL NONE RT HIGH STABILITY
IT 8.000E-03 ITA 8.000E+00

File Status: NORMAL
IP -0.200 vs. OC
ST 2.993E+01
EW 0.000E+00

Filename: b:9a516288.dat
Date Run: 10-09-96
ID 20 S
CR AUTO
WRK SOLID
DEN 7.900E+00

Time Run: 15:37:12
V1 0.100 vs. OC
NP 564
AR 8.000E+00
OC -0.715

Pstat: VStat[] Ver 2
FP -0.300 vs. OC
IR NONE
LS YES



Model 352/252 Corrosion Analysis Software, v. 2.01
Filename: b:9a516288.dat
Pstat: VStat[] Ver 2
CP CYCLIC POLARIZATION
Date Run: 10-09-96
File Status: NORMAL
Time Run: 15:37:12

Cond. Time	CT	pass	s	Initial Pot.	IP	-0.2000	V oc
Cond. Pot.	CP	pass	V	Vertex 1 Pot.	V1	0.1000	V oc
Initial Delay	ID	20	s	I Threshold	IT	1.0000E-3	A/cm^2
				Final Pot.	FP	-0.3000	V oc
Scan Rate	SR	0.1670	mV/s	Curr. Range	CR	Auto	
Scan Incr.	SI	5.000	mV	Step Time	ST	29.93	s
No. of Points	NP	564					
				GI Time Const.	TC	Off	
Line Sync.	LS	yes		IR Mode	IR	none	
Rise Time	RT	high stability		Filter	FL	Off	
Working Elec.	WE	Solid		Ref. Elec.	RE	SCE 0.2415	V
Sample Area	AR	8.000	cm^2	Equiv. Wt.	EW	0.0000	g
Density	DE	7.900	g/ml	Open Circuit	OC	-0.7150	V

alice Stone 10/9/96

E_{pit} = -430 mV SCE

E_{rp} = -585 mV SCE

10/10/96 9:25 (1296578) T= 360 hrs.

T19 29°C, pt -394 mV, pH 8.783
b -346 mV, r -344 mV, w -326 mV, y -321 mV

T20 27°C, pt -388 mV, pH 8.391
b -607 mV, r -382 mV, w -525 mV, y -489 mV

Removed 1 ml samples from each vessel for plate counts. Residuals frozen and labeled as T19-360 & T20-360.

Results from plate counts on T19 & T20:

Time (hrs)	T19 (cfu/ml)	T20 (cfu/ml)
336	4.08×10^4	3.67×10^4
342	4.00×10^4	3.83×10^4

Three CP vessels were filled with approx. 600 ml modified J13 with 6 ppm Cl^-, 1 ppm Y^{E}, and lactate (p. 102/182). All openings on CP vessels where covered with autoclave paper. Vessels along with all accessories where autoclaved for 1 hr at 121°C & 14 psi. Glassware was allowed to cool and then was immediately assembled upon removal. Platinum electrodes were sterilized with 70% isopropanol and add to the vessels. Three A516 Grade 60 carbon steel specimens were polished to a 600 grit finish and then sterilized with 70% isopropanol and added to the vessels. Vessels were degased with 95% N_2 & 5% H_2; pH 9.20

Final weights of samples # 7, #8, & #9

Specimen	Initial Weight (g)	Final Weight (g)
Sample #7	10.67227	10.52778
Sample #8	10.61585	10.59507
Sample #9	10.73661	10.59102

10/11/96 added 1ml of SRB culture to vessels 11 & 12, changed out N_2/H_2 cylinder to ensure gas supply for weekend.

10/11/96

10/14/96 Ran EIS on white T19, white T20 & Red T20

T19wend.z

T20wend.z (red)

T20whend.z (white

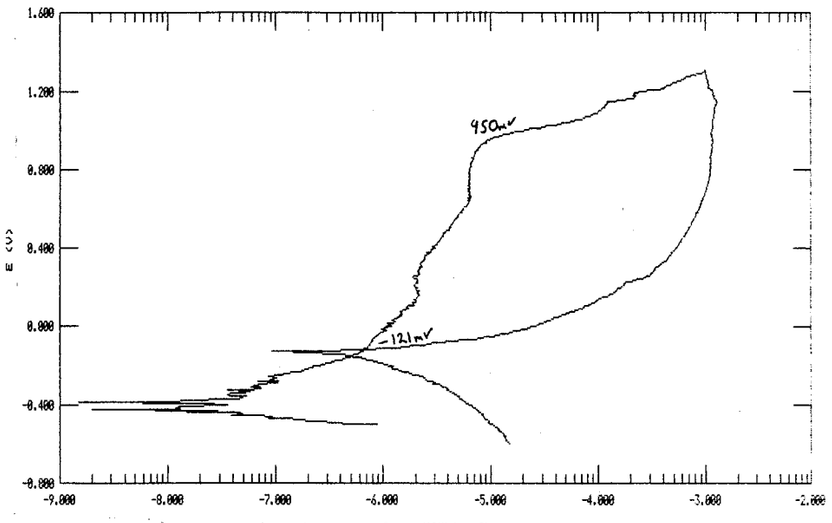
cyclic polarization using std setup run on T19 white & T20 red.

10/14/96

Model J52/252 Corrosion Analysis Software, v. 2.01 Filename: b:t20red.dat Pstat: VStat[] Ver 2

CP CYCLIC POLARIZATION		File Status: NORMAL	Date Run: 10-11-96	Time Run: 14:23:44			
CP PASS vs. R	CT PASS	IP -0.200 vs. OC	ID 20 S	VI 0.100 vs. OC	FP -0.300 vs. OC		
SI 5.000E-03	SR 1.670E-04	ST 2.993E+01	CR AUTO	NP 742	IR NONE		
FL NONE	RT HIGH STABILITY	REF 0.24150 SCE	WRK SOLID	AR 2.000E+00	LS YES		
IT 2.000E-03	ITA 2.000E+00	EW 0.000E+00	DEN 7.900E+00	OC -0.381			

—— b:t20red.dat

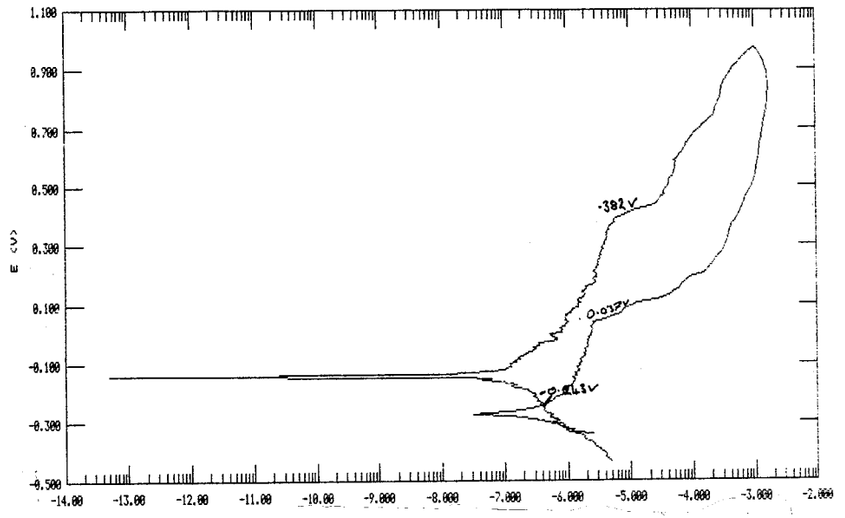


10/14/96

Model J52/252 Corrosion Analysis Software, v. 2.01 Filename: B:t19white.dat Pstat: VStat[] Ver 2

CP CYCLIC POLARIZATION		File Status: NORMAL	Date Run: 10-11-96	Time Run: 08:56:39			
CP PASS vs. R	CT PASS	IP -0.200 vs. OC	ID 20 S	VI 0.100 vs. OC	FP -0.300 vs. OC		
SI 5.000E-03	SR 1.670E-04	ST 2.993E+01	CR AUTO	NP 542	IR NONE		
FL NONE	RT HIGH STABILITY	REF 0.24150 SCE	WRK SOLID	AR 2.000E+00	LS YES		
IT 2.000E-03	ITA 2.000E+00	EW 0.000E+00	DEN 7.900E+00	OC -0.138			

—— B:t19white.d



10/14/96

10/16/96 Ran EIS on samples #10, #11, & #12

sample #10 10a516144.z

sample #11 11a516144.z

sample #12 12a516144.z

Results from plate counts on vessels #7, #8, & #9 are as follows:

#7	0 cfu/ml
#8	1.67×10^6 cfu/ml
#9	1.50×10^6 cfu/ml

Removed 1000ml samples from vessels #10, #11, & #12 and placed in lactate/lactate media for SRB counts. @ 11:00

Made 1L of modified J13 media with 6 ppm Cl^-, 1 ppm yeast extract, and lactate as the carbon source as follows:

Na_2CO_3	lot # 960685	0.01060 g
$NaHCO_3$	897789	0.08821 g
NaCl	947723	0.01058 g
$NaNO_3$	8917183	0.01370 g
NaF	950992	0.00440 g
$MgSO_4 \cdot 7H_2O$	947409	0.06470 g
yeast extract	59695JB	0.00100 g
lactate	943605	341 ul

per 1L 18.1 MΩ H_2O.

Placed approximately 200 ml of the above modified JB media into 2 x 250 ml conical flasks to compare pH values between aerated and nonaerated media. One flask was gased with compressed air for 1 hr and the pH value was recorded
pH of media that was not gased: 9.231
pH of media gased with compressed air: 9.650
Alice Stone 10/16/96

10/17/96 Observations made from electrodes in T19 & T20 are as follows:

T19

MEP1 sulfide present on top of electrode
- red - orange-yellow film
- blue - patchy orange-yellow film
- yellow - very thick orange-yellow film covering coupon
- white - very thick orange-yellow film covering coupon with some pits present

MEP2
- red - patchy orange-yellow film
- blue -
- yellow -
- white -

MEP3 sulfide present on top of electrode
- red - very thick orange-yellow film
- blue - very thick orange-yellow film
- yellow -
- white - slight orange-yellow film

MEP4 (center) sulfide present on top of electrode
- red - heavy pitting & slight orange-yellow film
- blue -
- yellow - slight orange-yellow covering
- white - heavy pitting & slight orange-yellow film

T20

MEP1 sulfide present on top of electrode
- red - thin film covering coupon
- blue - thin orange film present
- yellow - thin film covering coupon
- white - thin film covering coupon

MEP2
- red - black spots present on coupon
- blue - black spots present on coupon
- yellow - slight pitting present
- white - patchy orange film present

MEP3 sulfide on top of electrode
- red - many black spots (patches) present
- blue -
- yellow - thin film covering electrode
- white -

MEP4 (center) sulfide present on top of electrode
- red - orange film covering part of coupon & pits present
- blue -
- yellow - orange film present
- white - black spots & pits present

10/18/96 Ran EIS on samples #10, #11, & #12
- sample #10 10a516192.z
- sample #11 11a516192.z
- sample #12 12a516192.z

Made 4 L of 10% lactate/acetate media as follows: place 3600 ml of 18.1 MΩ H_2O in plastic bottle and added 400 ml lactate/acetate media (p 80/138). Autoclaved for 2 hrs at 121°C and 14 psi.

Alice Stone 10/18/96

10/22/96 Ran EIS on samples #10, #11, & #12

sample #10 10a516288.z

sample #11 11a516288.z

sample #12 12a516288.z

Alice Stone 10/22/96

1/11/96 Setting up chemostats using J13-water to culture YM isolates for use as inoculum in FIR&D program. made up 1 L of 10x J13 as follows

$NaHCO_3$	897789	1.170g	≈ 85 ppm HCO_3^-
NaCl	947723	0.10580g	≈ 6 ppm Cl^-
$NaNO_3$	8917183	0.1370g	≈ 10 ppm NO_3^-
NaF	950992	0.04400g	≈ 2 ppm F^-
$MgSO_4 \cdot 7H_2O$	947409	0.64700g	≈ 25 ppm SO_4^-

autoclaved for 1 hr at 121°C 14 psi.

[signature] 1/11/96

[initials] 5/11/96 assembled 500 ml chemostat for FIR&D with drip tube weir tube, sparge tube & air outlet, filled with 500 ml of 18.1 MΩ H_2O + 100 ml of 10x J13 -(glucose), autoclaved for 1 hr at 120°C @ 15 psi

Made up 4 L of J13 (3600 ml H_2O + 400 10x J13 p112) in carboy & autoclaved for 2 hr at 120°C & 15 psi

Took 10 ml of medium for chemostats to sterile test tube

& inoculated with a Yucca Mtn isolates YMI-1 thru 80 & penny dry isolate from plate made 10/21/96 (p 82 SWS 138) inoculate 500 µl of the solution in chemostat with air flow @ 16:00 @ 16:20 made plate count using dilution method as described elsewhere. (R2A8 medium)

made up 8 dw to give 500 ppm glucose in vessel as follows

250 mg in 10 ml of water & filter sterilised (0.2 µm)

[signature] 5/11/96

5/11/96 took 900 µl sample from chemostat (no flow) for plate counts @ 8:20

took 900 µl sample from chemostat for plate counts on R2A8 @ 9:20

took 900 µl sample from chemostat for plate count on R2A8 @ 10:20 [signature] 5/11/96

took 900 µl sample from chemostat for plate count on R2A8 at 11:20

took 900 µl sample from chemostat for plate count on R2A8 at 12:20

took 900 µl sample from chemostat for plate count on R2A8 at 13:20

M. Hill 5/11/96

took 900 µl sample from chemostat for plate count on R2A8 at 14:20

M. Hill 5/11/96

took 900 µl sample from chemostat for plate count on R2A8 at 15:20 to determine growth rate & µmax.

900 µl sample for plate count on R2A8 @ 16:20

[signature] 5/11/96

6/11/96 8:20 900 µl Sample for plate counts on R2A9

9:20 900 µl " Sample for plate counts on R2A9

10:20 900 µl sample for plate counts on R2A9
M. Hill 11/6/96

11:20 900 µl sample for plate counts on R2A9

12:20 900 µl sample for plate counts on R2A9

13:20 900 µl sample for plate counts on R2A9
M. Hill 11/6/96

Two packed bed reactors set up with 60 ml of 125-90 µm Sand each + autoclaved after 45 min @ 120°C + 14psi; 60 ml measured in graduated cylinde S/N 841367 CSWB
11/6/96

14:20 900 µl sample for plate counts on R2A9
M. Hill 11/6/96

16:20 900 µl sample for plate counts on R2A9
M. Hill 11/6/96

8/11/96 Made up a 100 x basal of J-13 to feed all vessels in 1800 project for a while made in 1000 ml volumetric flask with

$NaHCO_3$	897789	11.70 g	≈ 85ppm HCO_3^-
NaCl	951450	10580 g	≈ 6ppm Cl^-
$NaNO_3$	897183	1.3700 g	≈ 10ppm NO_3^-
NaF	950992	0.4400 g	≈ 2ppm F^-
$MgSO_4 \cdot 7H_2O$	947409	6.470 g	≈ 25ppm SO_4^-

+ stored at 4°C

made 4 l of medium for chemostats 3600 ml of 18.1 MΩ H_2O + 40 ml of 100 x base autoclaved 2 hr @ 14psi 121°C added 2 g of glucose in 10 ml of H_2O filter Sterilised to give 500 ppm

made 20 l of medium for 2 stainless steel static beds using 19.8 l of

18.1 MΩ H_2O + 200 ml of 100 x J13 base filter sterilised at 5psi through Sartobran capsular filter (0.2 µm) stock medium added at 200 ppm.
8/11/96

13/11/96 counts from plates made on chemostat

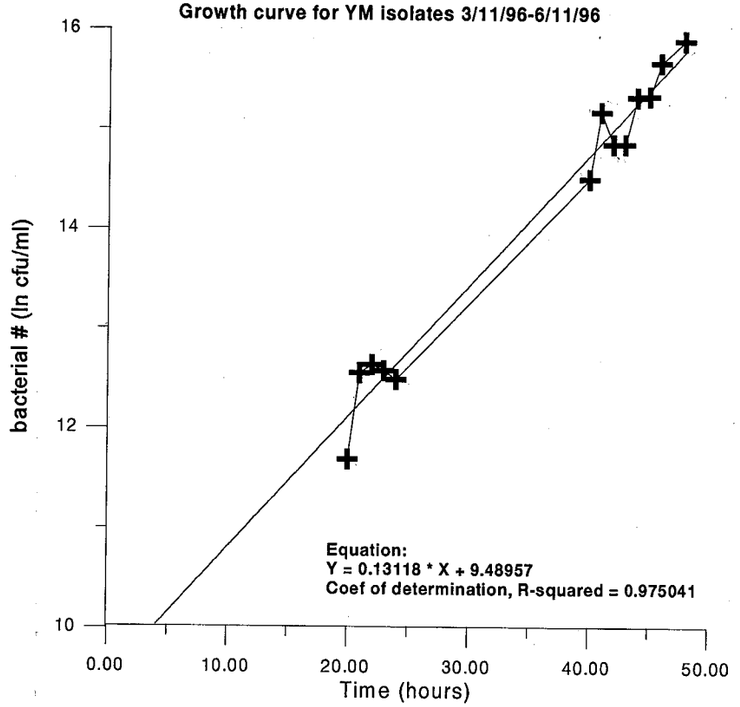
3/11/96	16:20	5.66×10^4 cfu ml^{-1}
5/11/96	12:20	1.19×10^5 cfu ml^{-1}
5/11/96	13:20	2.83×10^5 cfu ml^{-1}
5/11/96	14:20	3.08×10^5 cfu ml^{-1}
5/11/96	15:20	2.91×10^5 cfu ml^{-1}
5/11/96	16:20	2.66×10^5 cfu ml^{-1}
6/11/96	8:20	2.00×10^6 cfu ml^{-1}
6/11/96	9:20	3.91×10^6 cfu ml^{-1}
6/11/96	10:20	2.83×10^6 cfu ml^{-1}
6/11/96	11:20	2.83×10^6 cfu ml^{-1}
6/11/96	12:20	4.53×10^6 cfu ml^{-1}
6/11/96	13:20	4.58×10^6 cfu ml^{-1}
6/11/96	14:20	6.41×10^6 cfu ml^{-1}
6/11/96	16:20	8.00×10^6 cfu ml^{-1}
13/11/96

14/11/96 Made 20 L of medium sterilised J13 with 20 L of 18.1 MΩ H_2O + 200 ml of 100 x J13 (p114) and 4 g of Dextrose filter Sterilised to vessels packed Bed 1+2 in Bldg 51.
11/11/96

21/11/96 plate count on chemostats for Packed on R2A9 ; used as inoculum to packed bed #2 (5 ml)

Made 20 L of medium as per 14/11/96

plate count on Packed at 15:27 on R2A9 plate



21-11-96

3-12-96 Made up x100 stock of basal J-13 for packed bed reactors in FIR+D project used 1000 ml volumetric flask.

$NaHCO_3$	897789	11.70 g	≈ 85 ppm HCO_3^-	~~11.70178 g~~
NaCl	951450	1.0580 g	≈ 6 ppm Cl^-	1.05897 g
$NaNO_3$	~~897~~138	1.3700 g	≈ 10 ppm NO_3^-	1.37037 g
NaF	950992	0.4400 g	≈ 2 ppm F^-	0.44004 g
$MgSO_4 \cdot 7H_2O$	947409	6.470 g	≈ 25 ppm $SO_4^=$	6.47015 g

3-12-96

12/11/96 Made up x100 stock of basal J-13 for packed bed reactors in FIR&D project used 1000 mL volumetric flask.

$NaHCO_3$	897789	11.70 g	≈ 85 ppm HCO_3^-	11.70092 g
NaCl	951450	1.0580 g	≈ 6 ppm Cl^-	1.05798 g
$NaNO_3$	897183	1.3700 g	≈ 10 ppm NO_3^-	1.37012 g
NaF	950992	0.4400 g	≈ 2 ppm F^-	~~0.44013 g~~ m.Hill 12/12/96 0.44002 g
$MgSO_4 \cdot 7H_2O$	947409	6.470 g	≈ 25 ppm $SO_4^=$	6.47012 g

M. Hill 12/11/96

16/12/96 counts for Div 06 MIC IR+D

Abiotic Swab	2×10^4 cfu ml^{-1}
Abiotic clams	2.08×10^5 cfu ml^{-1}
ym clams	3.25×10^7 cfu ml^{-1}
Biotic clams	5.75×10^6 cfu ml^{-1}
Biotic Swab	1.12×10^6 cfu ml^{-1}

#2	10-12-96	16:25	2.58×10^5 cfu ml^{-1}
	11-12-96	16:45	4.58×10^5 cfu ml^{-1}
	13-12-96	16:15	3.41×10^5 cfu ml^{-1}
inoc	12-12-96		5.00×10^7 cfu ml^{-1}
2A	12-12-96	16:00	5.83×10^4 cfu ml^{-1}
2B	12-12-96	16:00	4.75×10^4 cfu ml^{-1}
2C	12-12-96	16:00	4.83×10^4 cfu ml^{-1}
20A	12-12-96	16:00	5.25×10^4 cfu ml^{-1}
20B	12-12-96	16:00	7.33×10^4 cfu ml^{-1}
20C	12-12-96	16:00	3.91×10^4 cfu ml^{-1}
200A	12-12-96	16:00	1.90×10^6 cfu ml^{-1}
200B	12-12-96	16:00	2.83×10^6 cfu ml^{-1}
200C	12-12-96	16:00	2.33×10^6 cfu ml^{-1}
2B	13-12-96	16:15	2.33×10^5 cfu ml^{-1}
2C	13-12-96	16:15	1.50×10^5 cfu ml^{-1}
20A	13-12-96	16:15	2.00×10^5 cfu ml^{-1}
200A	13-12-96	16:15	2.33×10^6 cfu ml^{-1}

16-12-96

Made up x100 stock of basal J-13 for packed bed reactors in FIR&D project. Used 2000 mL volumetric flask.

$NaHCO_3$	897789	23.4g ≈ 85ppm HCO_3'	23.40086g
NaCl	951450	2.116g ≈ 6ppm Cl'	2.11650g
$NaNO_3$	897183	2.74g ≈ 10ppm NO_3'	2.74075g
NaF	950992	0.88g ≈ 2ppm F^-	0.88025g
$MgSO_4 \cdot 7H_2O$	947409	12.94g ≈ 25ppm SO_4'	12.94084g

M. Hill 07/12/96

8/12/96 Dissolved one (1) sigma Phosphate buffered Saline tablet (lot # 45H8917) in 200 ml of nanopure water & autoclaved 2g of Sodium pyrophosphate (lot # 64H1002) until dissolved, dispensed into 2 x 40 ml aliquots & 2 x 60 ml aliquotes in 100 ml medium bottles & autoclaved to sterilise

made 400 ml of PBS (2 tabs) lot # 45H8917 in 400 ml Nanopure water dispensed in 20 ml aliquots & autoclave.

7/12/96 Div 06 Thio chemo - 10^7 cfu ml^{-1}
Thio Biotic chemo 10^2 cfu ml^{-1}
" " Swab 10^3 cfu ml^{-1}
Abiotics Sterile

SRB
Abiotic ∅
SRB chemo 10^7
Biotic Swab 10^4
" chemo 10^5 27-12-96

31/12/96 Made up x100 stock of basal J-13 for packed bed reactors in FIR&D project. Used 2000mL volumetric flask.

$NaHCO_3$	897789	23.4g ≈ 85ppm HCO_3'	23.40072g
NaCl	951450	2.116g ≈ 6ppm Cl'	2.11626g
$NaNO_3$	897183	2.74g ≈ 10ppm NO_3'	2.74057g

NaF 950992 0.88g ≈ 2ppm F^- 0.88039g 3 M. Hill 31/12/96
$MgSO_4 \cdot 7H_2O$ 947409 ≈ 25ppm SO_4' 12.94017g
M. Hill 23/11/97 12.94g
M. Hill 31/12/96

8-1-97 made 10ml of 0.5M Na_2EDTA (lot # 953443) 1.86g
+ 10ml of 5M NaCl (lot # 951450) 2.92g
8-1-97

23/1/97 Made up x100 stock of basal J-13 for packed bed reactors in FIR&D project. Used 2000mL volumetric flask.

$NaHCO_3$	897789	23.4g ≈ 85ppm HCO_3'	23.40055g
NaCl	951450	2.116g ≈ 6ppm Cl'	2.11600g
$NaNO_3$	897183	2.74g ≈ 10ppm NO_3'	2.74086g
NaF	950992	0.88g ≈ 2ppm F^-	0.88084g
$MgSO_4 \cdot 7H_2O$	947409	12.94g ≈ 25ppm SO_4'	12.94002g

M. Hill 23/1/97

29-1-97 Div 06
YMI-chemo - 4.58×10^7 cfu ml^{-1}
Biotic Swab MAB - 1.00×10^5 cfu ml^{-1}
Biotic Swab MAB - 3.50×10^6 cfu ml^{-1}
Abio Swab MAB - 1.16×10^4 cfu ml^{-1}
" chemo MAB - 1.66×10^5 cfu ml^{-1}
Biotic chemo SRB 10^5 cfu ml^{-1}
SRB chemo 10^7 cfu ml.
Biotic Swab SRB 0
Abiotic Swab SRB 0
Abiotic chemo SRB 0
Thio chemo 10^4 cfu ml.
11/3/97

I have reviewed this scientific notebook and find it in compliance with QAP-001. There is sufficient information regarding procedures used for conducting tests, acquiring and analyzing data so that another qualified individual could repeat the activity.

N. Sridhar 2/24/97

Narasi Sridhar
Manager, Engineered Barrier System and Waste Solidification System

N. Sridhar