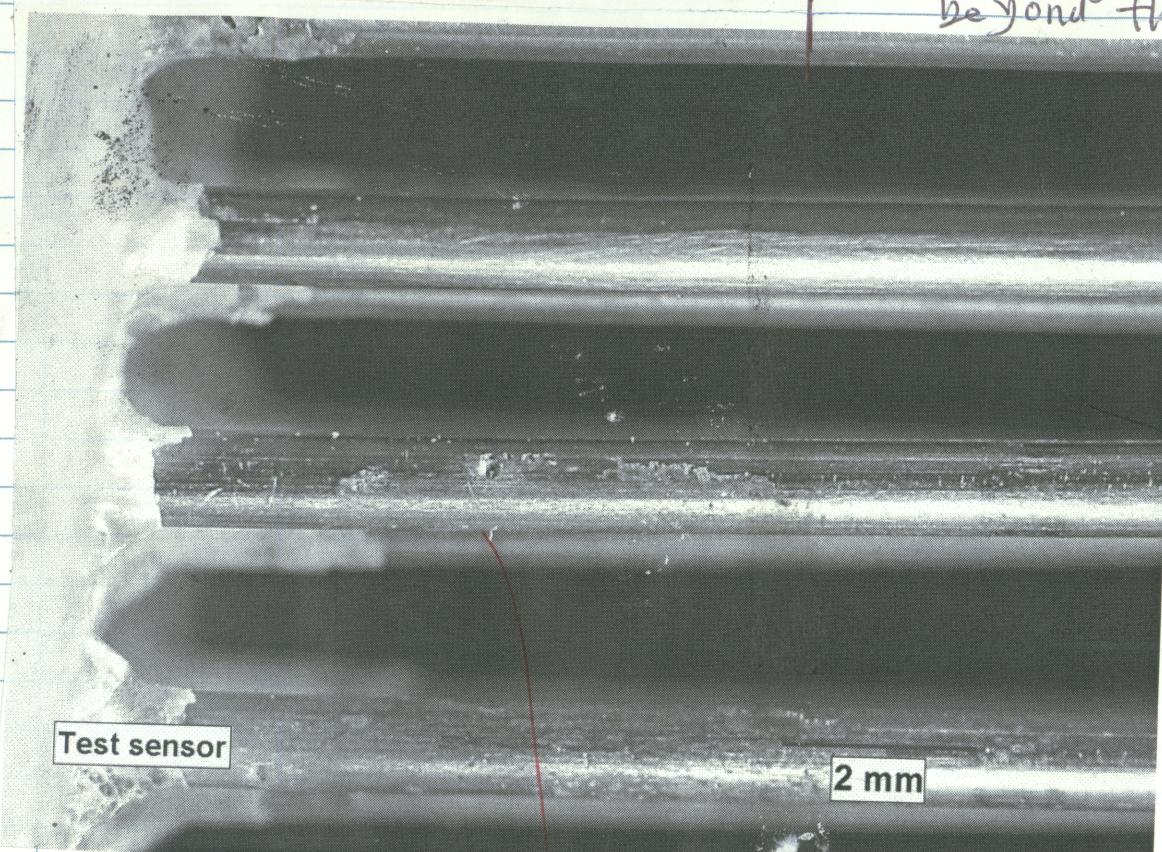


d8474\_TestSensor\_pits\_near\_top

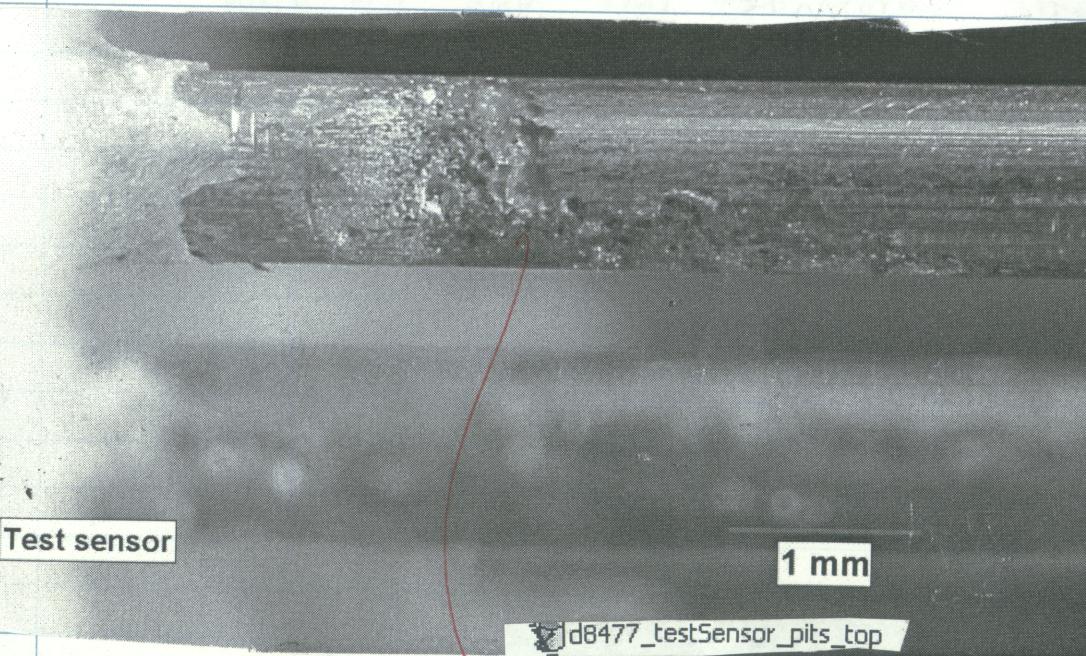
No pitting found  
beyond this line



Test sensor

2 mm

pitting near the epoxy!



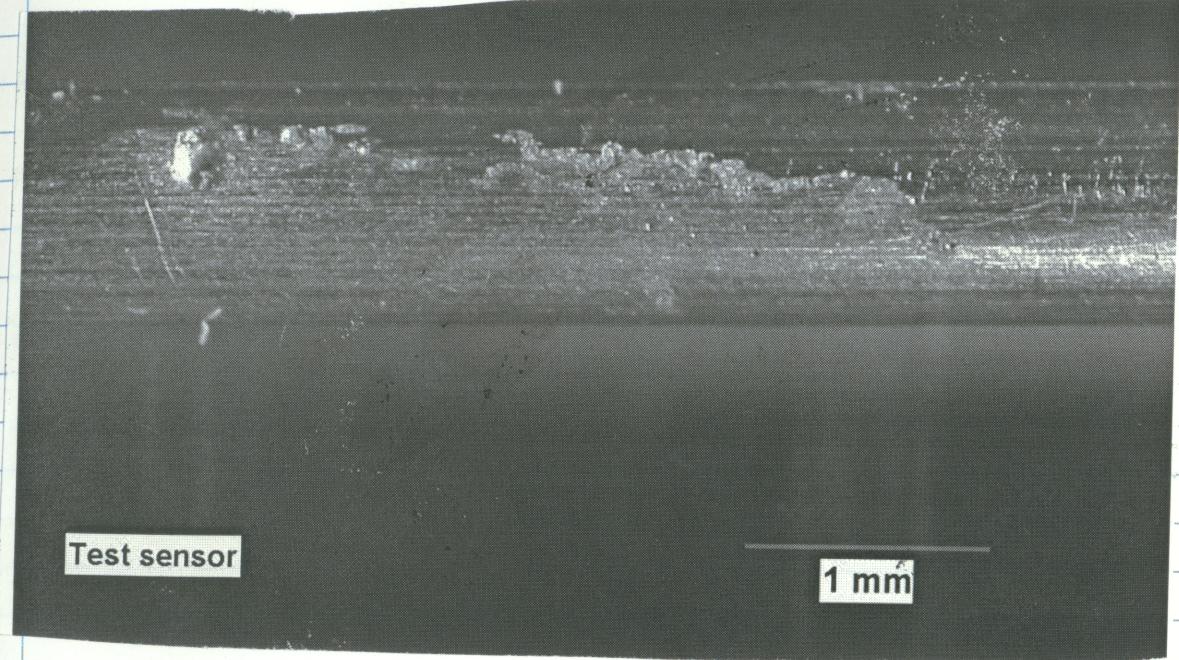
Test sensor

1 mm

d8477\_TestSensor\_pits\_top

J-Y 2117/06

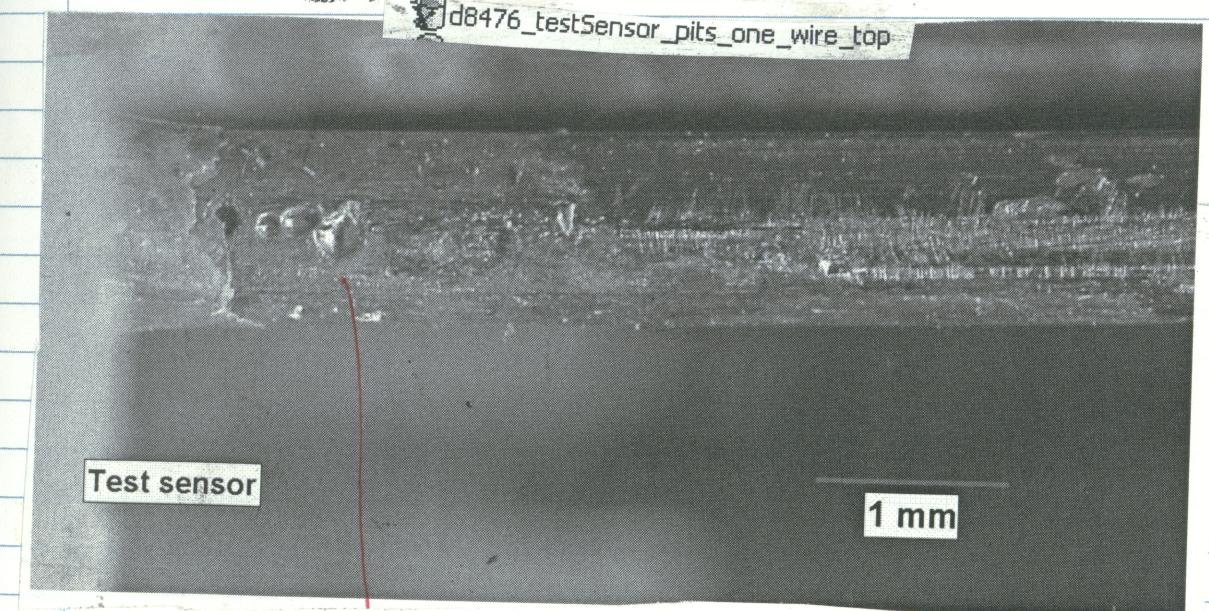
d8475\_testSensor\_pits\_one\_wire\_Lower\_top



Test sensor

1 mm

d8476\_testSensor\_pits\_one\_wire\_top



Test sensor

1 mm

Pitting found only  
near the epoxy J-Y 2117/06

Not found in sections immersed in  
solution.

lt.yang@cnwra.swri.edu

Thursday, July 17<sup>th</sup> meeting with Dr. Yang & Balak  
at SWRI - █ 522-2483 ←

rbecker5@satx.rr.com - 378-5825 - cell  
Geri Becker or 497-7560

Stainless 304 or 304L - L = lower carbon

Examining corrosion in simulated  
seawater - Seawater is used for  
comparisons with published data.

Coupons polished with 600 grit.

- Have not developed corrosion, perhaps  
owing to low cell counts ( $10^4$  to  $10^5$ )

- Is it possible to measure  $H_2S/H_2$  production  
in 10ml aliquots - can the ion chromatograph  
be used?

- Write up protocol for MPN - use ASTM method +  
cite reference.

- Write up recipes for modified reduced chloridite  
medium and Postgate's media B, C, D, E, with ref.  
use 9 ml tubes.

- Baars broth - look up the recipe

- SRB corrosion on 304SS

- Consortium capable of corroding stainless steel?

- Identify possible acid producers for  
experiments

- Prepare modified Baars with differing  
amounts of Ferrous Ammonium Sulfate

note:  
some  
areas  
are  
highlighted  
not crossed  
out

- 2
- Compare growth curve in Baars solution  
Vs. a different medium,

Left SWRI at █ 2½ h travel

July 18, 2003

Spoke with Roger Dykstra - 522-5076  
Scientist/technician who sets up + monitors  
reaction vessels.

Monitored sampling by Roger & Geri and  
watched Geri serially dilute and transfer  
samples.

Roger thinks the electrical potential is  
continuously monitored. He tries to measure  
this manually at least once a day.

The cells are sampled Monday, Wednesday and  
Friday by Geri for serial dilution and  
estimation of growth by visual technique  
(does it look turbid or clear?). Tubes are  
incubated at 31°C for 7 days to determine  
growth.

Left SWRI at █  
2½ h travel

July 21, 2003

10:15 AM - Library work at UTSA  
2:20 PM - QA/QC materials reviewed,  
SRB media reviewed. QA/QC forms signed  
or returned by post on this day.

#### Media observations:

(1) Differential Reinforced Chloridite Medium (DRCM)

Note: some  
areas are  
highlighted  
not crossed  
out

Stuart Barbosa  
4/25/2006

Roger J Dykstra  
10/10/03

Stuart Barbosa  
4/25/2006

Roger J Dykstra  
10/10/03

Guentzel, 1980, Wastewater assay methods. In: Laboratory procedures manual for environmental samples and clinical specimens, C.A. Sorber, Project manager, Center for Applied Research & Technology, VTIA, p. 37-43], as used by Hudson (1989 - Iron Metabolism of Desulfovibrio desulfuricans unpubl. MS thesis, UTSW) should provide good growth, but can't be prepared in advance (see recipe).

Postgate's media B, C and D have the best potential for the first set of experiments (Postgate 1979 - The sulphate-reducing bacteria, Cambridge University Press, Cambridge, 151 p.). 1/24

July 22, 2003

Began [REDACTED] AM - Examining media from literature - transcribed recipes to send to Leitai & Gerin. Stopped at [REDACTED]  
began again at [REDACTED], stopped at [REDACTED]  
3 h

July 23, 2003

Literature review - began [REDACTED]

2 h

July 24<sup>th</sup>, 2003

went to SWRI, arrived [REDACTED]. Darrell Dunn tried to help me find reagents for media. Discussed QA issues with Dunn. Couldn't find reagent so decided to order new reagents. Went through the Fisher catalog to list required reagents. Gave list of required reagents to Roger Dykstra to order. Left SWRI at [REDACTED].  
2 1/2 h total

Stuart Birnbaum  
4/25/2006

Roger J Dykstra  
10/10/03

note: some  
arcs are  
negative  
not  
crossed  
out

### Media recipes

Media that may be used for cultivating sulphate-reducing bacteria

#### Postgate<sup>1</sup> Medium B

KH <sub>2</sub> PO <sub>4</sub>	0.5g	
NH <sub>4</sub> Cl	1.0g	
CaSO <sub>4</sub>	1.0g *	use 1.265 g CaSO <sub>4</sub> ·2H <sub>2</sub> O
MgSO <sub>4</sub> ·7H <sub>2</sub> O	2.0g	
Sodium Lactate	3.5g	= 6 ml of 60% soln
Yeast extract	1.0g	
Ascorbic acid	0.1g	
Thioglycollic acid	0.1g	
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g	

Add above ingredients to tap water (I use deionized water) to make 1 liter. Adjust pH to between pH = 7.0 and 7.5. This medium always contains a precipitate. NaCl should be added for marine strains.

#### Postgate<sup>1</sup> Medium C

KH <sub>2</sub> PO <sub>4</sub>	0.5g	
NH <sub>4</sub> Cl	1.0g	
Na <sub>2</sub> SO <sub>4</sub>	4.5g	
CaCl <sub>2</sub> ·6H <sub>2</sub> O	0.06g	
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.06g	
Sodium lactate	6.0g	= 10 ml of 60% soln
Yeast extract	1.0g	
FeS <sub>0.4</sub> ·7H <sub>2</sub> O	0.004g	
Sodium citrate·2H <sub>2</sub> O	0.3g	

<sup>1</sup> Postgate, J.R., 1979. The sulphate-reducing bacteria, Cambridge University Press, Cambridge, England, 151 pp.

Prepared by Dr. Stuart Birnbaum for Southwest Research Institute, July 22, 2003.

Stuart Birnbaum  
4/25/2006

Roger J Dykstra  
10/10/03

Add ingredients and make up to 1 liter with distilled water and adjust pH to  $7.5 \pm 0.2$ . This medium may be cloudy after autoclaving but should clear after cooling. Add NaCl for salt-water strains.

#### Postgate<sup>1</sup> Medium D

KH <sub>2</sub> PO <sub>4</sub>	0.5g
NH <sub>4</sub> Cl	1.0g
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.1g
MgCl <sub>2</sub> ·6H <sub>2</sub> O	1.6g
Yeast extract	1.0g
FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.004g
Sodium pyruvate	3.5g
Or choline chloride	1.0g

Distilled water to make 1 liter, pH  $7.5 \pm 0.2$ . Sterilize by filtration; add extra NaCl for salt-water strains.

#### Postgate<sup>1</sup> Medium E

KH <sub>2</sub> PO <sub>4</sub>	0.5g
NH <sub>4</sub> Cl	1.0g
Na <sub>2</sub> SO <sub>4</sub>	1.0g
CaCl <sub>2</sub> ·6H <sub>2</sub> O	1.0g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	2.0g
Sodium lactate	3.5g
Yeast extract	1.0g
Ascorbic acid	1.0g
Thioglycolic acid	0.1g

<sup>1</sup> Postgate, J.R., 1979. The sulphate-reducing bacteria, Cambridge University Press, Cambridge, England. 151 pp.

Prepared by Dr. Stuart Birnbaum for Southwest Research Institute, July 22, 2003.

Stuart Birnbaum  
4/25/2006

Roger T. Deamer  
10/10/03

FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.5g
Agar	15.0g

Tap water 1 liter. Boil to dissolve agar and adjust to pH 7.6 with NaOH after boiling. Autoclave and use before it solidifies. Add extra NaCl for salt-water strains.

Medium B is for diagnostic purposes and maintenance of cultures; growth is indicated by blackening of the iron salt.

Medium C is for mass culture of organisms for research; the citrate prevents precipitation of the iron salt.

Medium D is a diagnostic medium, testing for sulphate-free growth.

Medium E is for counting populations of organisms as black colonies in deep agar, and for isolation of pure cultures.

#### Differential Reinforced Clostridial Medium (DRCM)<sup>2,3</sup>

Peptone	10.0g
Beef extract	10.0g
Sodium acetate	5.0g
Yeast extract	1.5g
Soluble starch	1.0g
Glucose	1.0g
5% solution L-cysteine	10.0 ml
Distilled H <sub>2</sub> O	990 ml
4% solution sodium sulfite	0.1 ml
7% solution ferric citrate	0.1 ml

<sup>2</sup> Guentzel, M.N., 1980. Wastewater assay methods. In: Laboratory procedures manual for environmental samples and clinical specimens, C.A. Sorber, Project Manager, Center for Applied Research and Technology, UTSA, p. 37-43.

<sup>3</sup> Hudson, L.J., 1989. Iron metabolism of *Desulfovibrio desulfuricans*. Unpublished M.S. thesis, UTSA.

Prepared by Dr. Stuart Birnbaum for Southwest Research Institute, July 22, 2003.

Stuart Birnbaum  
4/25/2006

Roger T. Deamer  
10/10/03

7  
Make a 5% solution of L-cysteine by dissolving 5.0g of L-cysteine in 95 ml distilled H<sub>2</sub>O. Make a 4% solution of sodium sulfite by dissolving 4.0g sodium sulfite in 96 ml distilled H<sub>2</sub>O. Make a 7% solution of ferric citrate by dissolving 7.0g ferric citrate in 93 ml of distilled H<sub>2</sub>O. Dissolve peptone, beef extract, sodium acetate, yeast extract, and starch in water. Boil to dissolve completely. Add glucose and adjust the pH to 7.1 - 7.2 with 10 N NaOH while hot. Filter while hot through Whatman filter paper #2 using a membrane filter apparatus under a vacuum. Use as many filter discs as necessary. Autoclave in a flask at 121°C for 15 minutes. Add the L-cysteine aseptically and dispense 10 ml volumes into sterile 15 x 125 mm screw cap tubes. On the day the medium is to be used, steam the tubes for not more than three minutes. Add 0.1 ml of the 4% sodium sulfite solution and 0.1 ml of the 7% ferric citrate solution to each tube. Solution expires in two weeks.

Rings & Robinson, 1987 - Corrosion of Stainless Steel by Sulfide-Reducing Bacteria. Electrochemical Techniques, Corrosion. NACE, V.44, p 386-396.

#### medium A (g/L)

Sodium lactate 7  
beef extract 1  
peptone 2  
 $MgSO_4 \cdot 2H_2O$  \* 2  
 $Na_2SO_4$  1.5  
 $K_2HPO_4$  0.5  
 $CaCl_2$  0.1  
distilled  $H_2O$  1L

Same as medium A except for heat labile component ( $K_2HPO_4$ ). Instead add 5 mL of Ferric ammonium sulfate made with 7.84 g/100 mL - sterile filter in 0.45 mm

For both, adjust pH to 7.0 ± 0.1

#### heat labile components

Sodium ascorbate  
(Ascorbic acid  $Na_2S_2O_8$ ) 5mL/L  
ammonium Sulfate 5mL/L

Na ascorbic acid = 2.10g/100 mL - sterile filter 0.45 mm  
( $Na_2S_2O_8$ ) = 2.64g/100 mL - sterile filter 0.45 mm

Prepared by Dr. Stuart Birnbaum for Southwest Research Institute, July 22, 2003.

\* does this really exist or is this a typo?

Stuart Birnbaum  
4/25/2006

Bryant Ke  
10/10/03



## ANAEROBE SYSTEMS

PRODUCT: Anaerobic Modified Bar's Broth Medium  
CATALOG #: AS-888

#### Ingredients:

#### I. Bulk components: (Autoclavable) Amount per Liter

Component 1:	
NaCl	5.0 g / L
Sodium Citrate	5.0 g / L
Magnesium Sulfate	2.0 g / L
Ammonium Chloride	1.0 g / L
Calcium Sulfate	1.0 g / L
Distilled Water	400.0 mL / L

Component 2	
Potassium Phosphate, dibasic	0.5 g / L
Distilled Water	200.0 mL / L

Component 3:	
Sodium Lactate	3.5 mL / L
Yeast Extract	1.0 g / L
Distilled Water	400L / L

#### II. Heat labile components:

Ferrous Ammonium Sulfate	1.0 g / L
Distilled water	20.0 mL / L

#### Directions:

1. Into 3 separate containers measure distilled water.
2. Dissolve bulk ingredients into each respective component container.
3. Adjust each container to a pH of 7.5.
4. Dissolve Ferrous ammonium sulfate in distilled water.
5. Autoclave each component for 15 minutes at 121°C.
6. Cool to room temperature.
7. Add sterile heat labile components.
8. Dispense 2 liters into a sterile 2-L container.
9. Label and package each container.

jeremymedinab@anaerobesystems.com

15906 Concord Circle \* Morgan Hill, CA 95037 \* (408) 782 - 7557 Fax (408) 7  
<http://www.anaerobesystems.com>

Stuart Birnbaum  
4/25/2006

Royal K  
10/10/03



## ANAEROBE SYSTEMS

PRODUCT: Anaerobic Modified Baar's Broth  
CATALOG #: AS-888

Ingredients Name	Supplier	Order #
Distilled Water	Municipal Source	
NaCl	Fisher	S271-50
Sodium Citrate	Sigma	S4641
Magnesium Sulfate anhydrous	Sigma	M2643
Ammonium Chloride	Sigma	X4514
Calcium Sulfate dihydrate	Sigma	C7411
Potassium Phosphate, Dibasic	Sigma	P5504
Sodium Lactate *	Sigma	L7022
Yeast Extract	Marcor	I238
NaCl	Fisher	S271-50
Iron Ammonium Sulfate	Sigma	F2262
Ferrous		

\* Ask about the sodium lactate - this number in the sigma catalog is for powder, yet the recipe on preceding page refers to 3.5 g/L.

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http://www.anerobesystems.com

Stuart Birbara  
4/25/2006

Roger T. Potts  
10/10/03

Aug 7, 2003

Arrived SWRI [REDACTED] Met with Liefai and Brian Denby. Discussed reagents, glassware, protocols and procedures for QA of media preparation. Departed SWRI [REDACTED]  
2 1/2 travel

Aug 8, 2003

Arrived SWRI [REDACTED] Am to make media. Didn't have anhydrous calcium sulfate so used calcium sulfate dihydrate instead. Recalculated amount of  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  required as follows:

$$\text{Need } 1\text{g } \text{CaSO}_4 - \text{F.W.} = 136.16$$

$$\text{Have } \text{CaSO}_4 \cdot 2\text{H}_2\text{O} - \text{F.W.} = 172.17$$

SO:

$$\frac{1\text{g}}{136.16} = \frac{X}{172.17}$$

$$X = \frac{172.17}{136.16} = 1.265\text{g}$$

Prepared media B and C (Portugate) - added 3.5 g/L sodium chloride. pH to 7.1 for media B and 7.3 for media C.

Left SWRI at 10:35 to go to UTSA for autoclave - Arrived UTSA at 11:10. Labeled bottles and decanted media for autoclaving. 6mls of media C were decanted by pipette into 10ml screw-caps vials; 20 vials were filled. Approximately 70mls of media C were decanted into 100ml bottles (6 bottles); The remainder of the liter was left in a 1-liter bottle. The same was done for media B (Portugate). The media was delivered to the autoclave at UTSA at 12:45 PM (SWRI work ended at [REDACTED])  
5 1/2 travel

Noted some arcs are

highlight

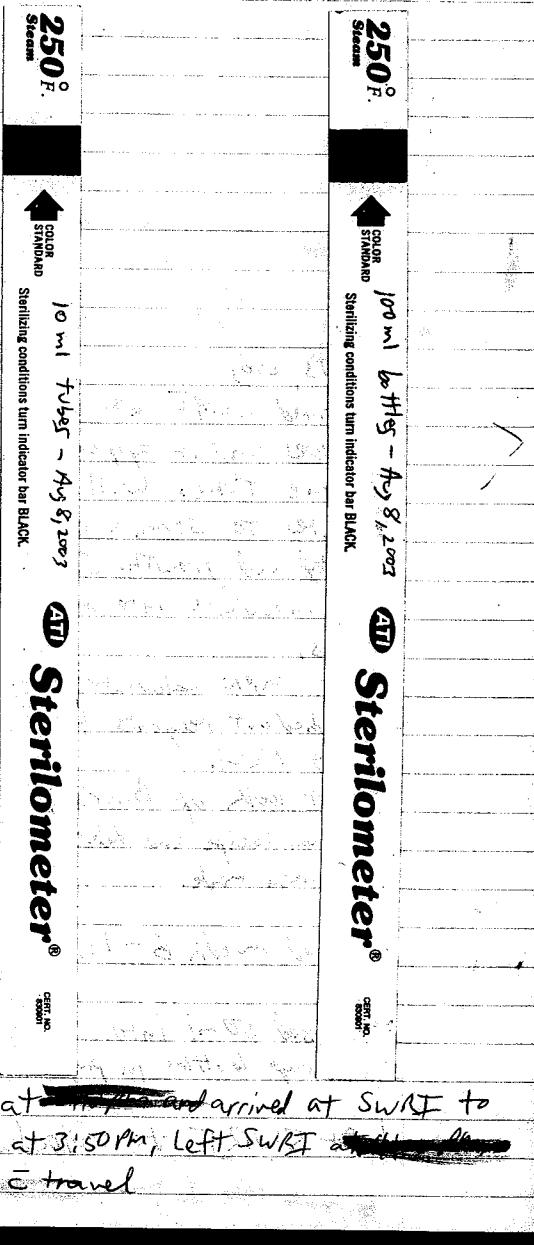
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out

Stuart Birbara  
4/25/2006

Roger T. Potts  
10/10/03

SWRI work began again at 3:00pm. Picked up the autoclaved media. Used Sterilometer strips to Q/A the autoclaving process. Labeled strips are attached below.



Left UTSA at [redacted] and arrived at SWRI to deliver media at 3:50pm. Left SWRI at [redacted] 4:42 pm travel

note: some areas  
are highlight  
not  
crossed  
out

Aug 11, 2003

Arrived SWRI at [redacted] am, met with Geri Becker - examined cell transfers (no growth yet) and examined media prepared on Aug 8. No growth in uninoculated media indicates it is sterile and good for use in growth experiments. Discussed MPN and optical growth measurements in preparation of growth curve experiment II. Examined media reagents needed. Left SWRI at [redacted]. 1 dram = 3.697 ml, A 3 dram bottle = 11.09 ml, 1/5 dram = 5.54 ml, 2 1/2 dram

note: some areas  
are highlight  
not crossed  
out.

Aug 13, 2003

Arrived SWRI at [redacted]. Geri checked cultures and it appears that we have growth in one tube. Will prepare Postgate media B plates to streak cells to check for purity and growth. If pure culture we will inoculate into media C and media B tubes.

Get MPN calculator for Geri.  
Weighed out reagents for Postgate B plus 5.0g NaCl.  
must look up Baar's broth medium to see original recipe and determine what modifications have been made.

Prepared media B - 1 liter - adjusted pH to 7.0.

Dispensed 50ml into 10 - 100ml pyrex screw cup bottles in preparation for autoclaving.

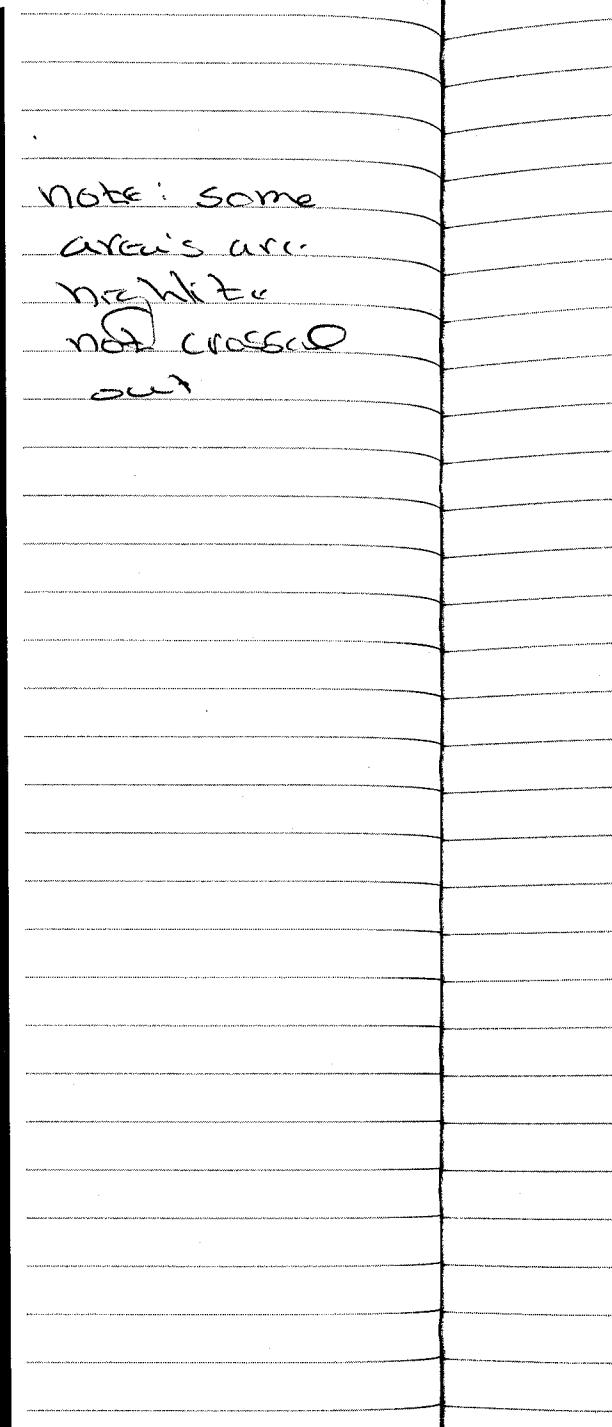
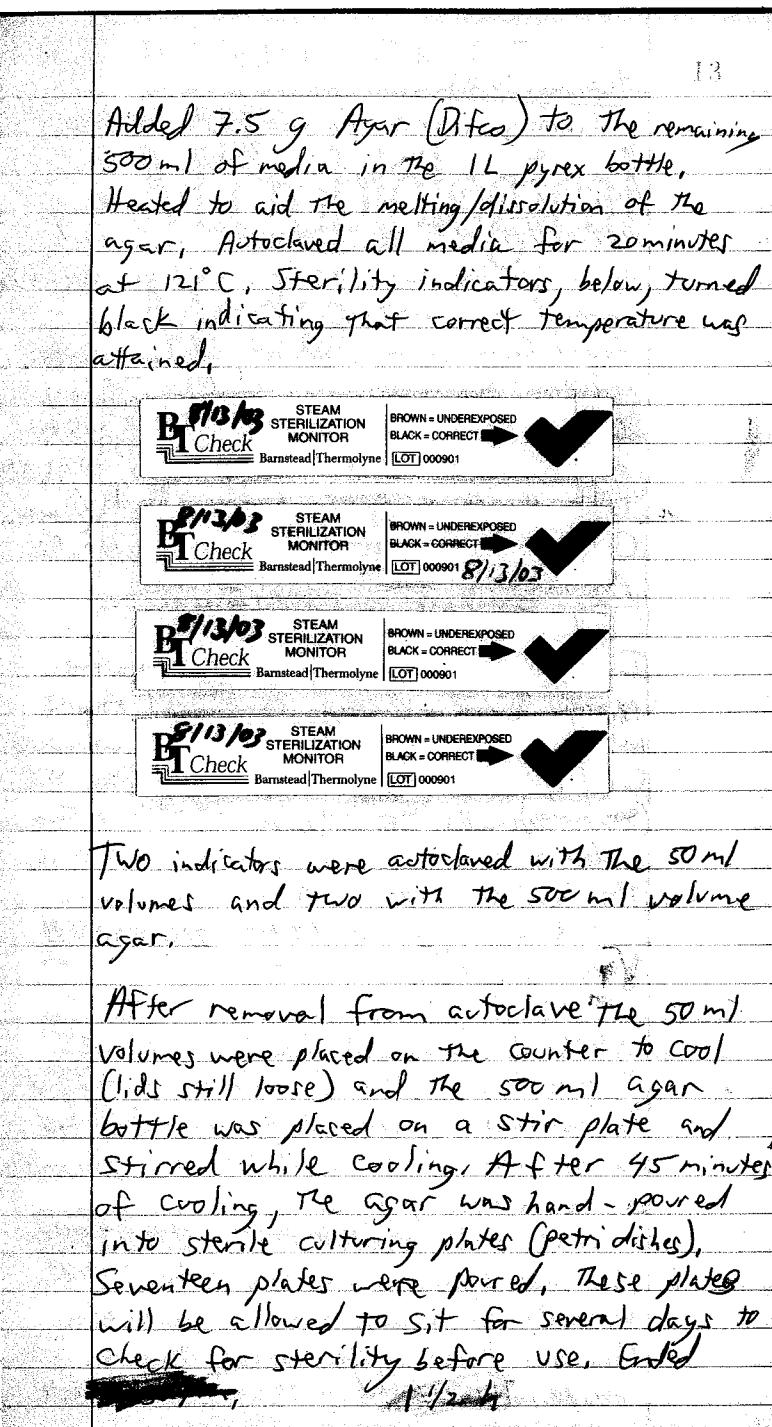
Left SWRI to autoclave at 2:30pm  
6 L travel

Stuart Barber  
9/25/2006

Roger JP  
10/10/03

Stuart Barber  
4/25/2006

Roger JP  
10/10/05



Aug. 14, 2003  
Library work from [REDACTED] + [REDACTED] Am,  
17/2/03

Aug. 15, 2003  
Arrived SWRI at [REDACTED]. Labeled agar plates, checked growth in incubator, worked on literature. Found formulae for Baer's medium for sulfate reducers, Baer's medium for sulfate reducers modified, and Baer's medium for sulfate reducers, modified with 2.5% NaCl (Atlas, R.M., 1993, Handbook of microbiological media (Lawrence C. Parks, editor), CRC Press, Boca Raton, FL, pgs 102-103,

I need to confirm with Anaerobe Systems exactly how they prepare the modified Baer's medium. They report 1.0 g/L ferrous ammonium sulfate, but the recipe as reported by Atlas (1993) (see above) calls for 1.0g/20 mL. This is then added to 1L for a final volume of 1020 mL. The final concentration of ferrous ammonium sulfate would thus be  $9.8 \times 10^{-4}$  g/L which is very low  $[Fe^{2+}]$ .

Spoke to Jeremy McDonald at Anaerobe Systems regarding the composition of modified Baer's solution media. He determined I had an old formula. He faxed the new information as seen in following pages.

Left SWRI [REDACTED] 4/2/03 travel

The first 3 pages is the suggested Baer's medium as cited in the Handbook of microbiological media. This is followed by the current Anaerobe Systems recipe. This is pasted in to show the FAX date.

note: some arcs are highlight not crossed out.

Stuart Barbare  
4/25/2006

Royce P  
10/10/03

Stuart Barbare  
4/25/2006

Royce P  
10/10/03

HANDBOOK OF  
**Microbiological  
Media**

15

By  
**RONALD M. ATLAS**  
Edited by  
**LAWRENCE C. PARKS**



CRC Press  
Boca Raton Ann Arbor London Tokyo

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Printed on acid-free paper

Stuart Barber  
4/25/2006  
Rogert Pyle  
10/10/03

Baar's Medium  
for Sulfate Reducers

Information potentially subject to copyright protection was redacted from this location. The redacted material is from the information on p. 124 of this scientific notebook.

Baar's Medium  
for Sulfate Reducers, Modified

Stuart Barber  
4/25/2006  
Rogert Pyle  
10/10/03

18



## ANAEROBE SYSTEMS

## Product Insert

## ANAEROBIC MODIFIED BAAR'S BROTH MEDIUM (BAAR'S)

Products:

Information potentially subject to copyright protection was redacted from this location. The redacted material is from the information on p. 124 of this scientific notebook.

Baar's Medium  
for Sulfate Reducers, Modified  
with 2.5% NaCl  
Composition per 1020mL:

Page 1 of 3  
Anaerobe Systems  
15906 Concord Circle Morgan Hill, CA 95037 408 782 7557 Fax 408 782 3031  
<http://www.anaerobesystems.com>

Stuart Beiber  
4/25/2006

Roger D. Peden  
10/10/03

Stuart Beiber  
4/25/2006

Roger D. Peden  
10/10/03

Procedure

Crossed Out Lines

DOC-A-NOTES  
P2

Information potentially subject to copyright protection was redacted from this location. The redacted material is from the information on p. 124 of this scientific notebook.

Page 2 of 3

Anaerobe Systems  
15906 Concord Circle Morgan Hill CA 95037 408 782 7557 Fax 408 782 3031  
<http://www.anabio.com>

Roger J. H.  
10/10/03

Stuart Bumban  
4/25/2006

Stuart Bumban  
4/25/2006

Roger J. H.  
10/10/03

20

08/15/2003 09:17 FAX 408 782 3031

ANAEROBE SYSTEMS

DOC-A-NOTES

P2

004/004

## References

1. Dowell, V. R., Jr. and T. M. Hawkins. 1974. *Laboratory Methods in Anaerobic Bacteriology*. CDC Laboratory Manual. USDEHW C. D. C. Atlanta, GA. 30333.
2. Holdeman, L. V., F. P. Cato and W. E. C. Moore. 1971. *Anaerobe Laboratory Manual*. Virginia Polytechnic Institute and State University. Blacksburg, VA. 24061.
3. Jostinske-Sonne, H. R., Summanen, P., Citron, D. M., Barron, E. J., Wexler, H. M. and S. M. Finegold. 2002. *Wadsworth - KTL Anaerobic Bacteriology Manual*. Star Publishing Co., Belmont, CA 94002.
4. Englekirk, P. G., Duben-Englekirk, J. and Dowell, V. R. 1992. *Principles and Practice of Clinical Anaerobic Bacteriology*. Star Publishing Co., Belmont, Ca 94002.
5. Krieg, N. R. and Holt, J. G. (editors). 1984. *Bergey's Manual of Systematic Bacteriology Volume 1*. Williams & Wilkins, Baltimore, MD 21202.
6. QA for commercially prepared microbiological culture media - Second Edition, Approved Standard. NCCLS document M22-A2. NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898.

Issued Date: 5/1/2003

21.

Aug 18, 2003

Began work at [REDACTED] - prepared 2 dram vials for autoclaving to be used for MPN. 80 vials were autoclaved at 121°C for 20 minutes, allowed to cool, and caps tightened. Arrived at SWRI at 1:00 PM. Inoculated 3 tubes with *D. vulgaris* from broth - 3 tubes of Postgate B. These were 12 ml tubes containing 6 ml of media, one mL of inoculum was added and the tubes were topped up to full with sterile media B. These 3 tubes were placed in a Bio-Bag and sealed, autoclaved, and placed in the incubator. I also plated out on media B plates the stock culture and the test-cell culture. The plates were either incubated aerobically or placed in a Bio-Bag for anaerobic incubation.

Went to the supply room with Brian to see what volume a 1 dram vial holds to full. It is exactly 5 mL, so this is the preferred vial for the MPN. Need to label and autoclave a box of 1 dram vials.

Left SWRI at [REDACTED] - 6:46 PM

Worked on calculations of Fe in differing media [REDACTED] 1 hr

After deciding to use 1 dram vials rather than 2 dram vials, I labeled 144 vials with autoclave tape in preparation to autoclave. [REDACTED] 1 hr

Stuart Birbar  
4/25/2006

Roger T. Pm  
10/10/03

NOTE: Some areas are very little, no creases out.

August 19, 2003

Started 9:00 AM - Ended 12:00 PM

Conducted library work to explore protocols for sulfide analysis. Used the following references:

- Standard Methods for the examination of water and wastewater, 17<sup>th</sup> edition (1998) and 20<sup>th</sup> edition (1998)
- Annual Book of ASTM Standards (2000)

Also examined Standard Methods in chemical Analysis, but this provided no additional insight.

The water and wastewater book described many protocols, but most are either gravimetric ~~or titrations~~ or titrations, all seem very laborious. However, the methylene blue titration has been developed into a Hach test kit and holds promise.

The ASTM method suggests using an ion specific electrode (ISE) - a protocol also described in Water & wastewater. These two methods may serve as a good complementary check on each other.

The ASTM and Hach Methylene blue techniques are on the following pages.  
[REDACTED] 3 hours

Stuart Birbar  
4/25/2006

Roger T. Pm  
10/10/03

August 20, 2003

23

Started at [REDACTED] in library, Then to SWRI.  
Arrived SWRI at [REDACTED], Read results of the  
inoculation on 8/18/03.

- Three tubes inoculated with the subisolate are turbid - Two Tubes more turbid than the third, but this may be due to the differing size of the inoculum.
  - The media B bottle used to "top up" after inoculation is clear indicating aseptic transfer.
  - The ~~8/18/03~~ anaerobic spread plate from the sub shows no growth suggesting pure culture - need to continue with incubation (no facultative contamination).
  - The aerobic spread plate from the test cell shows abundant growth.
  - The anaerobic spread plates from the sub culture show no growth.
  - The anaerobic spread plate from the test cell shows no growth.
- Ended at [REDACTED] 5 hours with travel

August 22, 2003

Arrived SWRI at [REDACTED]. Aseptically transferred ~~8/19/03~~ of media B into 1 dram vials - 30 vials done. Met with Lietal to discuss sulfide measurement techniques. Decided upon the Hach and ASTM ISE methods. Looked up required supplies in Fisher catalog for ISE and reagents. Called Hach to verify needed reagents. Gave Brian necessary information to order supplies. Left at [REDACTED] 3 hours

24

August 23, 2003,  $a^{30} \text{ to } 11^{30} \text{ atm}$   
Worked on Fe concentration in different media as follows:

Iron calculations for different media.

Four media were examined and their iron content, as ferrous ( $\text{Fe}^{2+}$ ) iron was calculated for comparisons. The four media are Modified Baar's Media (currently being used in the corrosion experiments), Postgate's Media B and C, and Medium B of Ringas and Robinson (1987) who determined that corrosion took place in the presence of the SRB. Please note that Ringas and Robinson conducted potentiostatic tests only in sterile media (no SRB) and in their Medium A (no iron media).

Modified Baar's medium uses 1.0 g/L ferrous ammonium sulfate,  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 2\text{H}_2\text{O}$ , as a reductant and iron source. The gram formula weight for ferrous ammonium sulfate is 392.1. The gram formula weight for iron is 55.85. Therefore, the percent iron in ferrous ammonium sulfate =  $55.85/392.1 = 0.1424$ , or 14.24% per gram of ferrous ammonium sulfate.

Postgate's Medium B and Medium C use ferrous sulfate,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , with a gram formula weight of 278.01. Again, using 55.85 as the gram formula weight for iron yields the following:  $55.85/278.01 = 0.2009$ , or 20.09% iron per gram of ferrous sulfate.

Medium B of Ringas and Robinson (1987) also uses ferrous ammonium sulfate, and the calculation above applies.

#### Modified Baar's Media

The weight percent of iron in the Modified Baar's Medium is determined by multiplying the weight of ferrous ammonium sulfate added to the medium by the percent iron in the ferrous ammonium sulfate. This is:

$1.0 \text{ g/L ferrous ammonium sulfate} \times 0.1424 = 0.1424 \text{ g Fe/L}$ . This can be represented as moles of Fe by dividing this result by the gram formula weight of Fe:  $0.1424/55.85 = 2.5 \times 10^{-3} \text{ M}$ .

#### Postgate's Medium B

The weight percent of iron in the Postgate's Medium B is determined by multiplying the weight of ferrous sulfate added to the medium by the percent iron in the ferrous sulfate. This is:

$0.5 \text{ g/L ferrous sulfate} \times 0.2 = 0.1 \text{ g Fe/L}$ . This can be represented as moles of Fe by dividing this result by the gram formula weight of Fe:  $0.1/55.85 = 1.8 \times 10^{-3} \text{ M}$ .

#### Postgate's Medium C

The weight percent of iron in the Postgate's Medium C is determined by multiplying the weight of ferrous sulfate added to the medium by the percent iron in the ferrous sulfate. This is:

$0.004 \text{ g/L ferrous sulfate} \times 0.2 = 0.0008 \text{ g Fe/L}$ . This can be represented as moles of Fe by dividing this result by the gram formula weight of Fe:  $0.0008/55.85 = 1.4 \times 10^{-5} \text{ M}$ .

Stuart Birbara  
4/25/2006

Roger J. Poirier  
10/10/03

Stuart Birbara  
4/25/2006

Roger J. Poirier  
10/10/03

25

Ringas and Robinson (1987) Medium B

The weight percent of iron in the Ringas and Robinson (1987) Medium B is determined by multiplying the weight of ferrous ammonium sulfate added to the medium by the percent iron in the ferrous ammonium sulfate. This is a bit more complex since they presented their medium in a manner that differs from the microbiology literature. They dissolved 7.84 g ferrous ammonium sulfate in 100 mL water, and then added 5 mL of this solution to the media. Although not specific, I assume the final volume of their media was 1L.

I calculated this differently than the above calculations. 7.84g of ferrous ammonium sulfate /100mL = 0.0784g/mL of ferrous ammonium sulfate. They used 5 mL so 5mL x 0.0784g/mL of ferrous ammonium sulfate = 0.392g/L of ferrous ammonium sulfate (5 mL were added to 1L). This value must be multiplied by the percent of iron in the ferrous ammonium sulfate as follows:  $0.392 \times 0.1424 = 0.056$  g Fe/L. This can be represented as moles of Fe by dividing this result by the gram formula weight of Fe:  $0.056/55.85 = 1.0 \times 10^{-3}$  M.

SUMMARY

MEDIUM	Fe SOURCE	g Fe/L	Moles Fe
Modified Baar's	ferrous ammonium sulfate	0.14 g Fe/L	$2.55 \times 10^{-3}$ M
Postgate's Medium B	ferrous sulfate	0.10 g Fe/L	$1.8 \times 10^{-3}$ M
Postgate's Medium C	ferrous sulfate	0.0008 g Fe/L	$1.4 \times 10^{-3}$ M
Medium B (Ringas and Robinson (1987))	ferrous ammonium sulfate	0.056 g Fe/L	$1.0 \times 10^{-3}$ M

This table summarizes the iron content of the different media we discussed. Note that the currently used Modified Baar's medium has the highest iron content, providing 2.5 times the amount of iron than the medium used by Ringas and Robinson (1987). It is also noteworthy that Ringas and Robinson (1987) did not use their Medium B for their potentiostatic tests, they used their Medium A which is iron free.

2 hours

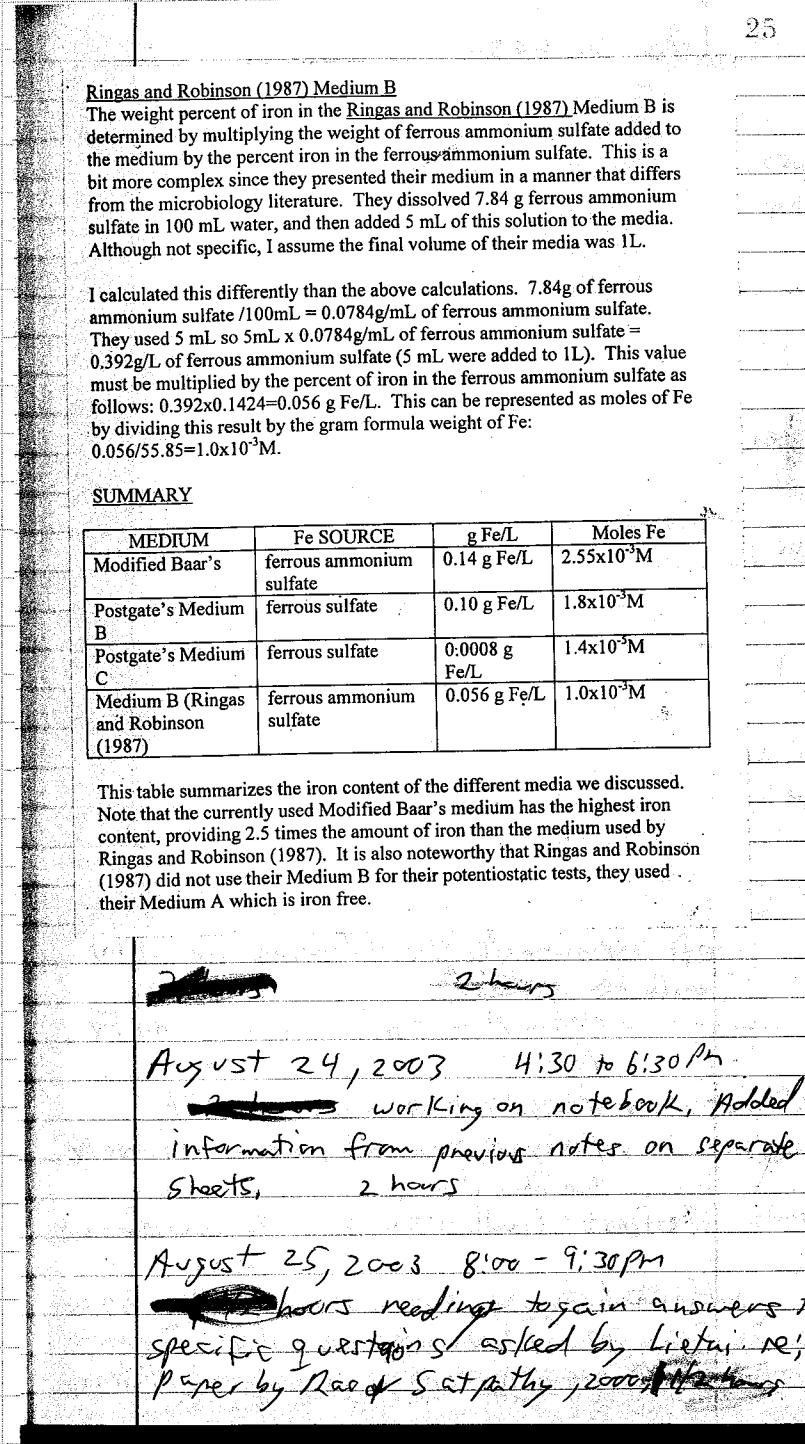
2 hours

August 24, 2003 4:30 to 6:30 pm

~~2 hours~~ working on notebook, Added information from previous notes on separate sheets, 2 hours

August 25, 2003 8:00 - 9:30 pm

~~2 hours~~ reading to gain answers to specific questions asked by Lietti re: paper by Rao & Sathpathy, 2000. ~~Answers~~



26

26

Aug 26, 2003

Arrived SURT 2:00 pm, meeting with Lietti and Geri Becker to discuss literature of corrosion, corrosion tests, and to plan the next stage of experiments. They explained the current protocol as follows,

To 500 mL of 0.5M NaCl solution, they add 40 mL total nutrients as:

- 10mL anaerobic nutrient broth with Vibrio cells (the slime-former)
- 30mL modified Baar's media - no SRB

This incubates for 7 days at room temp. After 7 days, 10 mL of modified Baar's with SRB is inoculated.

Every 2-3 days 10 mL is removed from the test cell and 10mL of fresh modified Baar's medium is added.

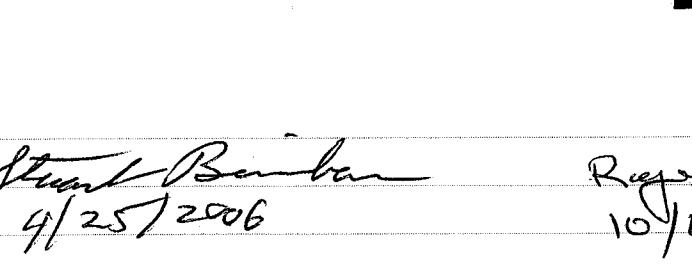
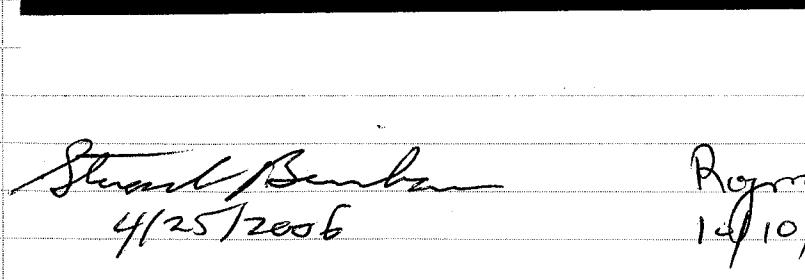
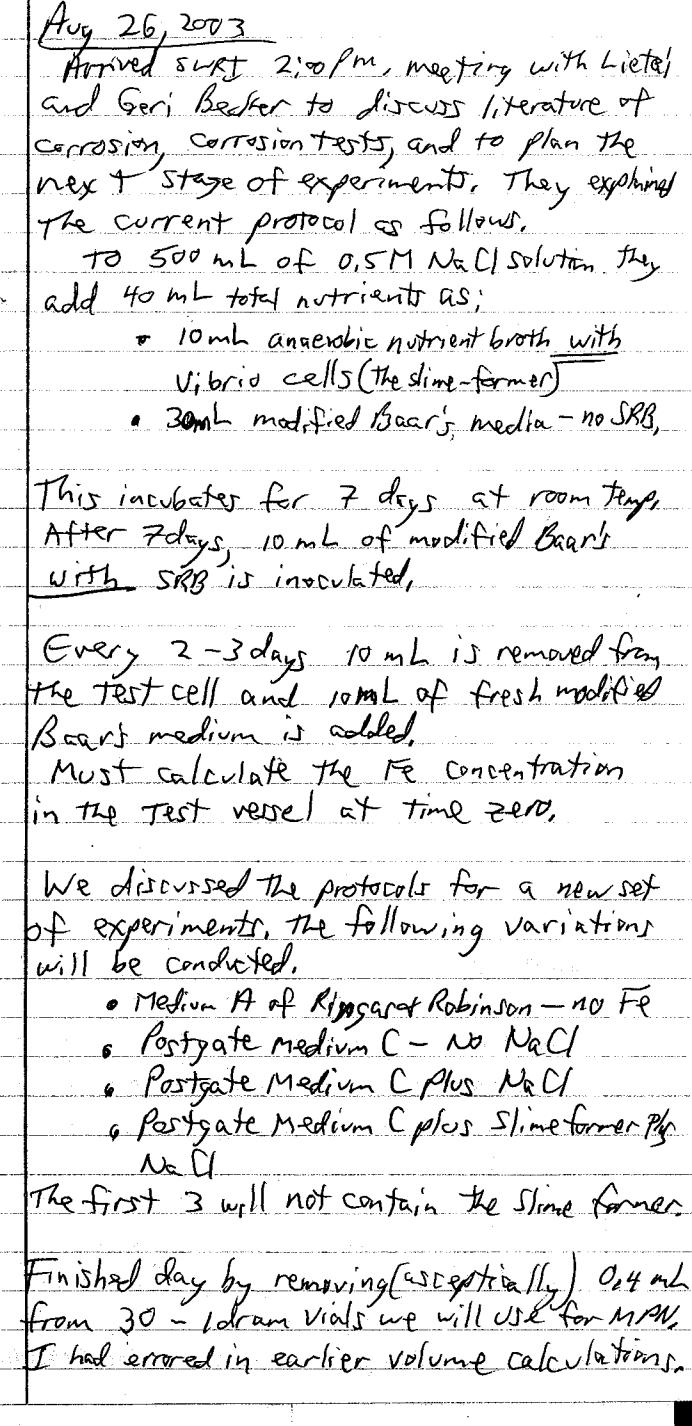
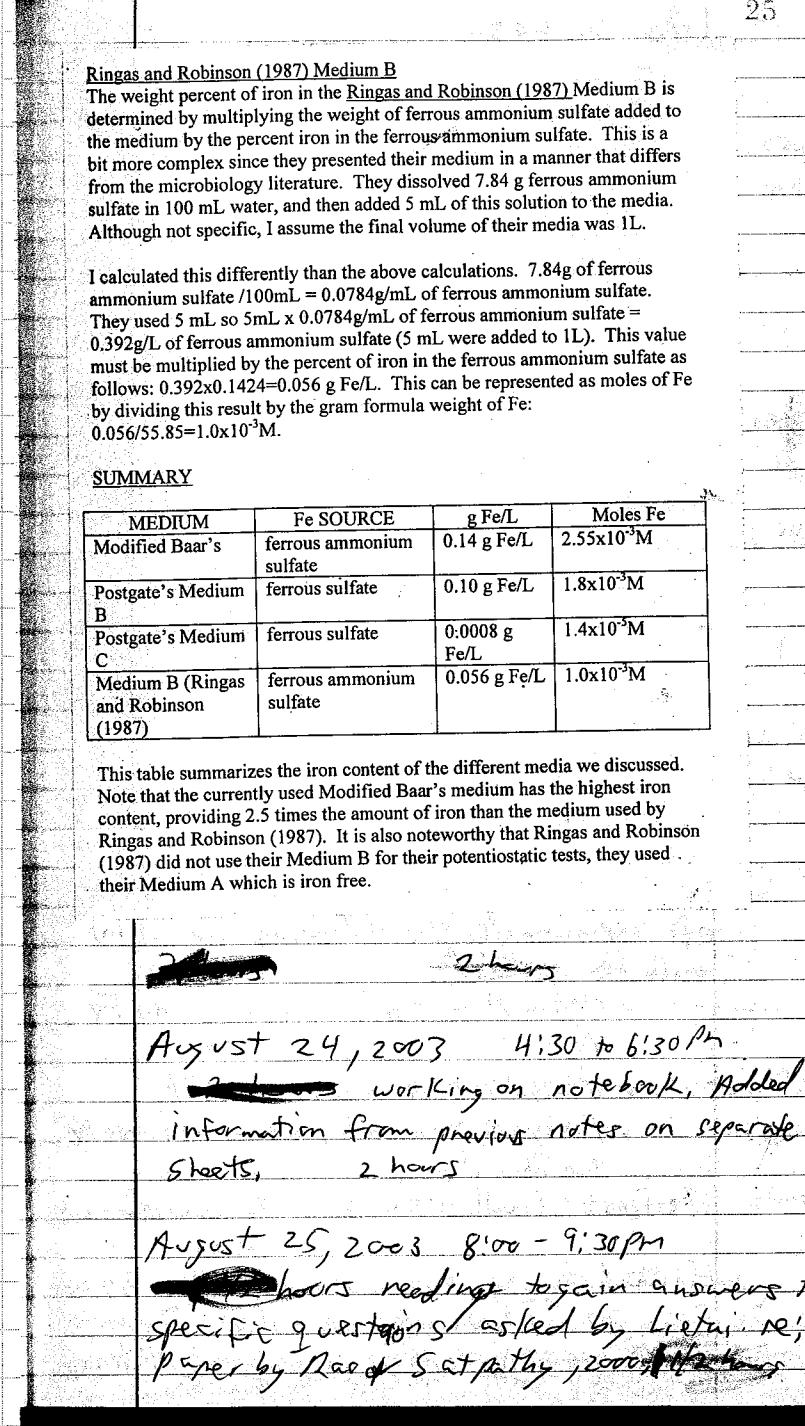
Must calculate the Fe concentration in the test vessel at time zero.

We discussed the protocols for a new set of experiments. The following variations will be conducted.

- Medium A of Ringast Robinson - no Fe
- Postgate medium C - no NaCl
- Postgate Medium C plus NaCl
- Postgate Medium C plus Slime former plus NaCl

The first 3 will not contain the slime former.

Finished day by removing (accidentally) 0.4 mL from 30 - 1 dram vials we will use for MPN. I had erred in earlier volume calculations.



Aug 27, 2003 at SWRI

27

Started 7:00 am - media preparation  
made 3L of postgate medium C and  
adjusted pH to 7.4. Decanted into 7x50mL  
bottles - 6 with 420mL or one with 280mL - spilled  
 $\approx$  200mL.

prepared 1L of Medium A of Rings  
and Robinson, 1987 as follows:

Per Liter -	grams
Sodium lactate	7 ( $= 11.7 \text{ mL of } 6\text{M} \text{ sol}$ )
Beef extract	1
peptone	2
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	2 ( $= 3.15 \text{ g of } \text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ )
$\text{Na}_2\text{SO}_4$	1.5
$\text{K}_2\text{HPO}_4$	0.5 <sup>(5.6)</sup> <sub>see pg 7 my notes</sub> SJR
$\text{CaCl}_2$	0.1 ( $= 1.97 \text{ NO } \text{ g of } \text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ )

\* There is no such chemical in Sigma or Aldrich. I assume it is a typo and should be  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and used 2.0g of this. See next paragraph!  
Apparently Ricca can custom produce a dihydrate salt of  $\text{MgSO}_4$ . Therefore, I used 3.15g of heptahydrate to provide an equal concentration of  $\text{MgSO}_4$  as the dihydrate.

Aug 29, 2003

Arrived SWRI at noon. Prepared medium A of Rings & Robinson in preparation of new experiments. Prepared medium A as per formula above. Recalculated  $\text{MgSO}_4$  concentration as above.

Discussed aseptic techniques with Lietai, Brian and Roger regarding new experiments. Bok medium to UTSA to autoclave. Removed from autoclave at 4:30 pm, total 4 hours.

Stuart Bumba  
4/25/2003

Roger T R  
4/10/03

Sept 1, 2003

Worked on notebook and calculate [Fe] in test vessels. See below.

#### Iron concentration in test vessels

Received clarification from Lietai and Geri regarding the protocol for the test vessels. To 500 mL of 0.5M NaCl solution they add a total of 40 mL of nutrients as follows:

- 10 mL of nutrient broth with vibrio cells (the slime former)
- 30 mL of modified Baar's medium without SRB

This incubates for 7 days before inoculation with 10 mL of modified Baar's medium with SRB.

Therefore, when the experiment begins, the total volume of modified Baar's Medium is 40 mL and the total volume is 550 mL. The calculation of the amount of iron in the test vessel at time zero for the experiment is as follows:

- Modified Baar's medium contains 0.14 g Fe/L.
- This equals  $1.4 \times 10^{-4}$  g Fe/mL.
- 40 mL of Modified Baar's is added to the vessel and thus provides  $40 \text{ mL} \times 1.4 \times 10^{-4} \text{ g Fe/mL}$  for a total of  $5.6 \times 10^{-3}$  g Fe to 550 mL of fluid.
- $5.6 \times 10^{-3}$  g Fe to 550 mL of fluid equals  $1.02 \times 10^{-2}$  g Fe/L. This is calculated by multiplying the  $5.6 \times 10^{-3}$  g Fe by 1.82 (1.82 is the conversion factor to equate to 1L and is obtained by dividing 1000 mL by the 550 mL used [1000 mL/550 mL])\*\*
- Molarity is determined by dividing  $1.02 \times 10^{-2}$  g Fe/L by the formula weight for Fe (55.85) to equal  $1.82 \times 10^{-4}$  M.

$$** \left( \frac{5.6 \times 10^{-3} \text{ g Fe}}{550 \text{ mL}} \right) \left( \frac{1000 \text{ mL}}{L} \right) = 1.02 \times 10^{-2} \text{ g Fe/L}$$

Sept 3, 2003 - Started at SWRI at 8:00. Met with Lietai to discuss Fe calculations and suggested measuring [Fe] at time zero for new experiments, perhaps by AA.

Met with Brian and Roger to go over sterilization procedures for new experiments. Tested autoclave in lab and worked on the

Stuart Bumba  
4/25/2003

Roger T R  
4/10/03

design of the  $H_2S$  trap, finished at 13:00,<sup>29</sup>

Sept. 5, 2003, Arrived SWRI at 8:00, Worked on experimental design for new experiments. Worked on apparatus sterilization for new experiments. Finished at 13:00.

Sept. 8, 2003, started at SWRI at 8:00. Prepared media B Plates for SRB verification. Worked on notes and reading. Worked on experiment setup. Finished at 16:00.  
88 9/1/2003  
15:00

Sept. 9, 2003, Worked from 13:00 to 18:00 on reading and notebook.

Sept. 10, 2003, began at SWRI at 7:30. Worked with Roger to set up experiments. Aseptically added media to test vessels. Aseptically added sterile DI water to gas trap. Aseptically added NaCl (0.5M) solution to electrode tube.

Rings media A required the heat-labile components of Ammonium Sulfate and L-Ascorbic acid to be prepared and sterile filtered through a 0.2  $\mu m$  sterile filter using a syringe.

Made 0.5 kg 9/1/2003  
Heat sterilized NaCl in oven at 170°C for 2 hours to add to media to make 0.5 M NaCl for two of the four tests. Finished at 3:30pm (15:30) 21.91 g NaCl / 750 mL makes 0.5 M NaCl

Sept. 12, 2003 - Started at SWRI at 7:30 - Prepared Rings medium A and transferred

approximately 9mL into each of 40 - 13 dram vials. Ready to autoclave.

Worked with Geri Becker to aseptically add NaCl to two test vessels to make 0.5 M NaCl concentration.

Had conference with Brian regarding protocols for the sulfide ISE. Done at 11:30.

Sept. 15, 2003 - Began at 1:30 pm at SWRI - Prepared vials for mPN - Discussed Sulfide analysis with Brian and discussed glassware requirements for next set of experiments. Done at 3:30 pm 2 hours

Sept. 17, 2003 Began at SWRI at 8:00 am. Autoclaved vials for mPN. Prepared media B for mPN. Prepared media B with 0.25 0.25 M NaCl since 2 test vessels are at 0.5 M NaCl and two are with NO NaCl. Added 14.81 g NaCl per liter. Adjusted pH to 7.2 - Autoclaved 121°C for 15 minutes. Finished 2 pm - 6 hours

Sept. 19, 2003 - Began at SWRI at 7:30. Aseptically transferred 9mL media B (Autoclave) into 120 vials in prep for mPN. Cultures arrived from Anaerobic Systems and Hach supplies for sulfide analysis arrived. Done at 11:30 AM. 4 hours.

Sept. 22, 2003 - Review of manuscript. 2.5 hours 18:00 - 20:30.

Stuart Bruban  
9/25/2003

Project No.         
10/10/03

Stuart Bruban Project No.         
9/25/2003 10/10/03

Oct. 1, 2003 - Started at SWRI at 8:00 AM. 31  
 Worked on calibrating sulfide analysis techniques. Two protocols will be followed: (1) a sulfide ion specific electrode protocol (ASTM D4658-92, reapproved 1996) and (2) The Hach method 8131 Methylene Blue method adapted from the Standard Methods for the Examination of Water and Wastewater.

The ISFE wasn't calibrated properly - The titration was unsuccessful. Must be repeated.

The Hach method works well and is very sensitive. The maximum concentration for the procedure is 0.73 mg/L. I first transferred 1ml of media C with cells into a 25 mL cuvette and added H<sub>2</sub>O to total 25 mL. This was over the limit. I next transferred 0.1mL and diluted to 25mL. This gave a reading of 0.68 mg/L. Multiply by 250 (the dilution factor) to get 170 mg/L sulfide. However, this was blanked against deionized water rather than media C. So it may not be a valid number. On the other hand, only 0.1 mL of media with cells was used, so perhaps the H<sub>2</sub>O blank is appropriate. Must check this out. Ended 4:00 PM (16:00) 8 hours

Oct. 3, 2003. - Started at SWRI at 10:00  
 Met with Lietzai - explored ways to analyze for iron. Possible methods are in the Hach Manual.  
 (1) Total Fe 0 to 3,000 mg/L - Ferrocection reagent pillows cat no. 21057-69  
 (2) Fe 0 to 1,300 mg/L - Ferrozine Iron reagent solution pillows Cat # 2301-66

Also transferred 10 mL of cells into fresh media C, 10 mL into fresh media B, and did a streak plate and spread plate on media B plates.

Stuart Birbaw  
4/25/2006

Report The  
10/10/03

Placed plates in anaerobic bag to incubate. Placed inoculated media to incubate. Ended 12:30 - 2.5 hours

Oct. 6, 2003 - started at SWRI at 11:00. Added NaCl (0.5M) and reference electrodes to test vessels. Discussed methods to add platinum electrodes to the test vessels. Finished at 12:30 - 1.5 hours.

Oct. 8, 2003 - Arrived at SWRI at 8:30. Added platinum electrodes to test vessels. Drilled holes in rubber bungs using an electric drill with the drill bit sterilized in alcohol and flamed. The area drilled was swabbed with alcohol prior to drilling. The drilling stopped before penetration of the bung. An 18g sterile needle from a syringe was used to complete penetration. The platinum electrode was swabbed as it was inserted into the vessel.

Afterwards I transferred cells from media C into 500mL bottle of media C. I read the Klett for the inoculum which is 30 KV. The Klett after inoculating (from the 500mL bottle) is 16 KV.

Did serial dilution to  $10^{-10}$  and transferred into 5-tube MPN from  $10^5$  to  $10^{-10}$  ( $\sim 5\text{mL}$   $10^{-11}$  to  $10^{-9}$  serially diluted vials), placed all MPN and serial dilution vials in Biobags and incubator.

Worked on Notebook. Ended at 15:30. 7 hours

Stuart Birbaw  
4/25/2006

Report The  
10/10/03

10/20/03  
1300

Removed  $\approx$  11 mL from each vessel  
10mL from each vessel will be sent to chemistry for iron content analysis.

10/20/03  
See ~~Specia~~<sup>R&P</sup> page for other work performed.

10/21/03

Sent 1 sample to chemistry  
See attached sheet on page 143

Results see page 168.

J.yd 11/10/03

10/21/03

Monday, October 20, 2003

Prepared to inoculate test vessels A (Ringer's A media), B (Postgate Media C without NaCl) and C (Postgate Media C with 0.5M NaCl) with SRB. First recorded the Klett absorption of the stock culture used to inoculate. The optical density was 30 Ku (Klett Units). A sample of the stock culture was prepared for MPN by serial dilution. 30 mL was inoculated into each of the test vessels described above.

Prior to inoculation, approximately 10 mL was collected from each vessel, including vessel D (which is identical to vessel C but will have the slime forming *Vibrio* introduced as well as the SRB), stabilized with 0.5 mL of concentrated nitric acid, and stored in a polypropylene tube for ICP analysis of iron. This will provide a Time = 0 (pre-inoculation) Fe concentration.

An additional 1 mL was collected from each test vessel for a Time = 0 sulfide concentration. Sulfide was analyzed using the EPA approved Methylene Blue Method (Hach method 8131) and a Hach DR 890 colorimeter. The sulfide concentration in each vessel was zero. Sulfide was also determined for the inoculum transferred into each vessel. The sulfide concentration in the stock culture was over the limit for the Hach method so dilution was required. 0.05 mL was diluted to 25 mL for a 500-fold dilution factor. This yielded a sulfide concentration reading of  $0.46 \text{ mg/L} \times 500 = 230 \text{ mg/L}$  sulfide in the stock cell culture used for inoculation.

*Aspects* technique practiced throughout procedure

10/23/03  
Rogin  
10/22/03

SAMPLE LIST/CHAIN OF CUSTODY		Requested Turnaround:	
		<input checked="" type="checkbox"/> 2 Weeks	<input type="checkbox"/> 3 Weeks
		<input type="checkbox"/> Other:	
Southwest Research Institute Chemistry and Chemical Engineering Division 6220 Culebra Road San Antonio, Texas 78238-5166		SwRI Contact:	
Client Purchase Order/Other ID	Site/Zone ID	Hickman, David C-2483	
Analyses Requested			
REMARKS			
Preservation: a = HCl to pH <2 b = HNO <sub>3</sub> to pH <2 c = H <sub>2</sub> SO <sub>4</sub> to pH <2 d = NaOH to pH >12 e = Cool (4°C<2°C) f = Other (specify)			
Sample ID	Matrix Type	# of Containers	Sample Collection Date (mm/dd/yy)
Vessel A	Water	1	10/20/03
Vessel B	Water	1	10/20/03
Vessel C	Water	1	10/20/03
Vessel D	Water	1	10/20/03
Vessel E	Water	1	10/20/03
Vessel F	Water	1	10/20/03
Vessel G	Water	1	10/20/03
Vessel H	Water	1	10/20/03
Vessel I	Water	1	10/20/03
Vessel J	Water	1	10/20/03
Vessel K	Water	1	10/20/03
Vessel L	Water	1	10/20/03
Vessel M	Water	1	10/20/03
Vessel N	Water	1	10/20/03
Vessel O	Water	1	10/20/03
Vessel P	Water	1	10/20/03
Vessel Q	Water	1	10/20/03
Vessel R	Water	1	10/20/03
Vessel S	Water	1	10/20/03
Vessel T	Water	1	10/20/03
Vessel U	Water	1	10/20/03
Vessel V	Water	1	10/20/03
Vessel W	Water	1	10/20/03
Vessel X	Water	1	10/20/03
Vessel Y	Water	1	10/20/03
Vessel Z	Water	1	10/20/03
Results			
Date	Time	SwRI Project#:	
10/21/03	09:30	20-0003-CV-001	
Date	Time	Received by SwRI Lab:	
10/21/03	09:30	(Signature)	
Date	Time	Relinquished by (Print/Signature)	
10/21/03	09:30	(Signature)	
Date	Time	Received by (Print/Signature)	
10/21/03	09:30	(Signature)	
Comments:			
Comments:	Comments:	Comments:	Comments:





10/22/03  
1020

Removed ~ 1ml from each vessel  
for subseq analysis.

10/24/03  
1020

Removed ~ 1ml from A, B, & C vessels  
for analysis.

10/27/03  
1245

Remove approximately 2 ml solution  
from each of 3 test flasks A, B & C  
for analysis. Replace volume on  
each flask with appropriate media  
for the test cell.

10/27/03  
1310

Added potassium Chloride solution  
to all the Reference probes.

Added 0.5 NaCl to Ingot probes.

Ray NMF  
10/27/03

Date	Time	Medium A	Voltage	Date	Time	Medium A	Voltage
10/27/03	14:00	Control wire to Ref	-0.3250 v8c	10/27/03	14:05	Control wire to Ref	-0.3171 v8c
		Control Coupon to Ref	-0.3522			Control Coupon to Ref	-0.2916
		Test wire to Ref	-0.3335			Test wire to Ref	-0.3122
		Test Coupon to Ref	-0.350			Test Coupon to Ref	-0.3228
		Platinum wire to Ref	-0.366			Platinum wire to Ref	-0.338
Date	Time	Medium A	Voltage	Date	Time	Medium A	Voltage
10/27/03	14:15	Control wire to Ref	-0.351 v8c	10/27/03	14:15	Control wire to Ref	-0.318 v8c
		Control Coupon to Ref	-0.353			Control Coupon to Ref	-0.304
		Test wire to Ref	-0.334			Test wire to Ref	-0.314
		Test Coupon to Ref	-0.341			Test Coupon to Ref	-0.321
		Platinum wire to Ref	-0.365			Platinum wire to Ref	-0.338
Date	Time	Medium A	Voltage	Date	Time	Medium A	Voltage
10/27/03	14:20	Control wire to Ref	-0.325 v8c	10/27/03	14:25	Control wire to Ref	-0.324 v8c
		Control Coupon to Ref	-0.314			Control Coupon to Ref	-0.309
		Test wire to Ref	-0.320			Test wire to Ref	-0.316
		Test Coupon to Ref	-0.341			Test Coupon to Ref	-0.330
		Platinum wire to Ref	-0.350			Platinum wire to Ref	-0.336
Date	Time	Medium A	Voltage	Date	Time	Medium A	Voltage
10/27/03	14:30	Control wire to Ref	-0.325 v8c	10/27/03	14:30	Control wire to Ref	-0.322 v8c
		Control Coupon to Ref	-0.325			Control Coupon to Ref	-0.309
		Test wire to Ref	-0.320			Test wire to Ref	-0.316
		Test Coupon to Ref	-0.341			Test Coupon to Ref	-0.330
		Platinum wire to Ref	-0.350			Platinum wire to Ref	-0.336
Date	Time	Medium A	Voltage	Date	Time	Medium A	Voltage
10/27/03	14:40	Control wire to Ref	-0.325 v8c	10/27/03	14:40	Control wire to Ref	-0.324 v8c
		Control Coupon to Ref	-0.325			Control Coupon to Ref	-0.309
		Test wire to Ref	-0.320			Test wire to Ref	-0.316
		Test Coupon to Ref	-0.341			Test Coupon to Ref	-0.330
		Platinum wire to Ref	-0.350			Platinum wire to Ref	-0.336
Date	Time	Medium A	Voltage	Date	Time	Medium A	Voltage
10/27/03	14:50	Control wire to Ref	-0.340 v8c	10/27/03	14:50	Control wire to Ref	-0.350 v8c
		Control Coupon to Ref	-0.342			Control Coupon to Ref	-0.364
		Test wire to Ref	-0.342			Test wire to Ref	-0.328
		Test Coupon to Ref	-0.351			Test Coupon to Ref	-0.342
		Platinum wire to Ref	-0.356			Platinum wire to Ref	-0.365
Date	Time	Medium A	Voltage	Date	Time	Medium A	Voltage
10/27/03	15:00	Control wire to Ref	-0.345 v8c	10/27/03	15:00	Control wire to Ref	-0.347 v8c
		Control Coupon to Ref	-0.336			Control Coupon to Ref	-0.369
		Test wire to Ref	-0.332			Test wire to Ref	-0.351
		Test Coupon to Ref	-0.351			Test Coupon to Ref	-0.364
		Platinum wire to Ref	-0.358			Platinum wire to Ref	-0.366
Date	Time	Medium A	Voltage	Date	Time	Medium A	Voltage
10/27/03	15:10	Control wire to Ref	-0.346 v8c	10/27/03	15:10	Control wire to Ref	-0.335 v8c
		Control Coupon to Ref	-0.341			Control Coupon to Ref	-0.352
		Test wire to Ref	-0.337			Test wire to Ref	-0.332
		Test Coupon to Ref	-0.333			Test Coupon to Ref	-0.342
		Platinum wire to Ref	-0.357			Platinum wire to Ref	-0.366

Ray NMF  
10/27/03









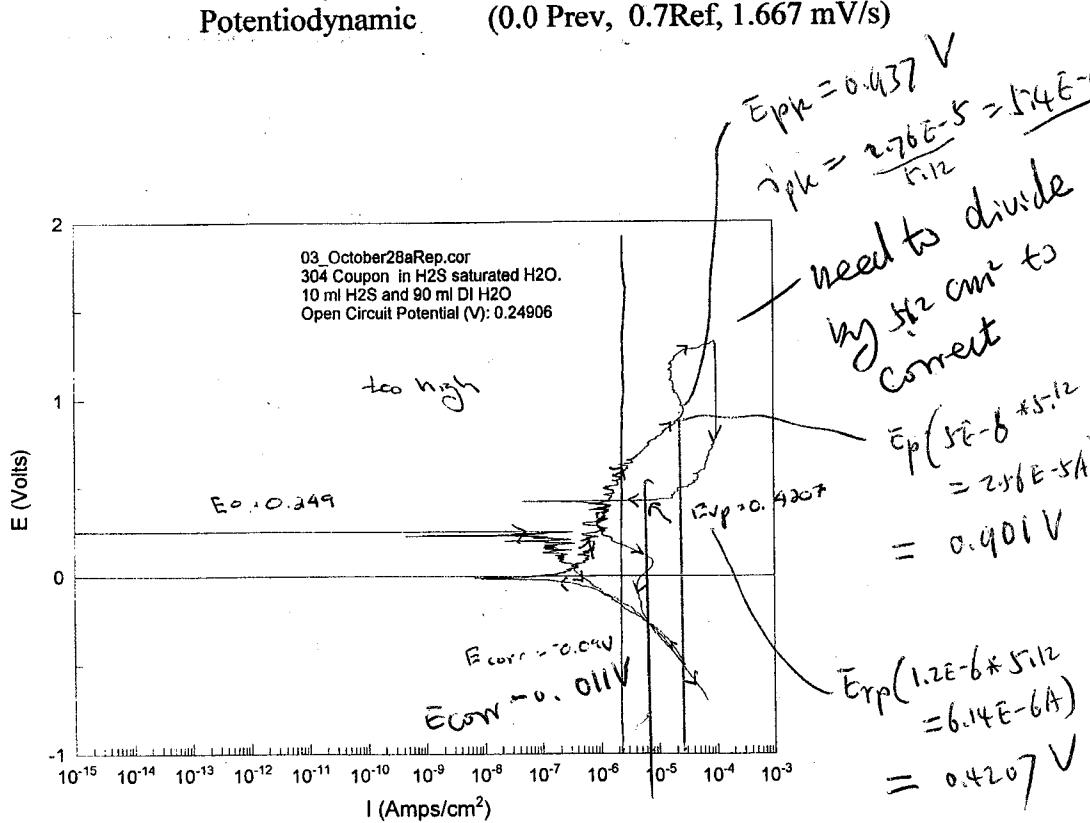
October 28, 2003

- 0715 Started linear polarization test of coupon in solution.  
 Solution is 10mL Saturated H<sub>2</sub>S solution and 90mL of DI H<sub>2</sub>O.  
 Area of coupon = 5.12 cm<sup>2</sup>

File 03\_October28aRep.cor

## Test setup:

- Open Circuit (20 min)  
 Potentiodynamic (0.0C, -0.5Ref, 4.0 Ref, 1.667 mV/s)  
 Galvanostatic (0.0001 A, 2 hours)  
 Potentiodynamic (0.0 Prev, -0.7Ref, 1.667 mV/s)  
 Potentiodynamic (0.0 Prev, 0.7Ref, 1.667 mV/s)

Recorded  
11/3/03

10/31/03

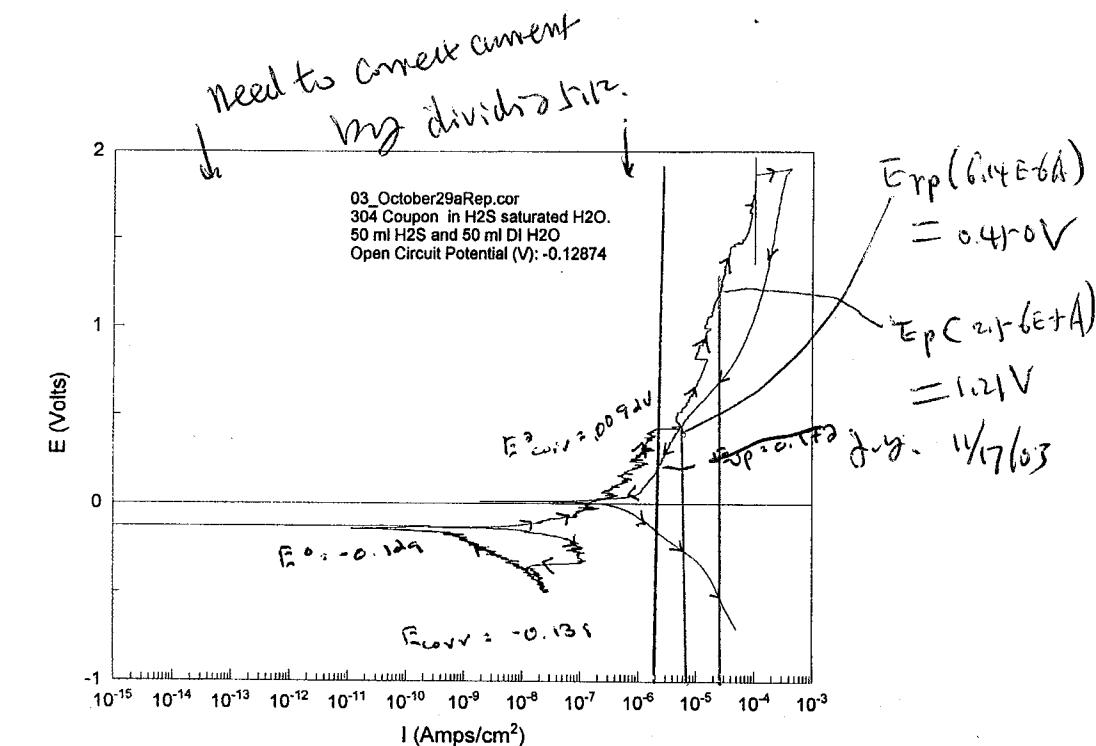
October 29, 2003

- 0715 Started linear polarization test of coupon in solution.  
 Solution is 50mL Saturated H<sub>2</sub>S solution and 50mL of DI H<sub>2</sub>O.  
 Area of coupon = 5.12 cm<sup>2</sup>

File 03\_October29aRep.cor

## Test setup:

- Open Circuit (20 min)  
 Potentiodynamic (0.0C, -0.5Ref, 4.0 Ref, 1.667 mV/s)  
 Galvanostatic (0.0001 A, 2 hours)  
 Potentiodynamic (0.0 Prev, -0.7Ref, 1.667 mV/s)  
 Potentiodynamic (0.0 Prev, 0.7Ref, 1.667 mV/s)

Review  
11/3/03

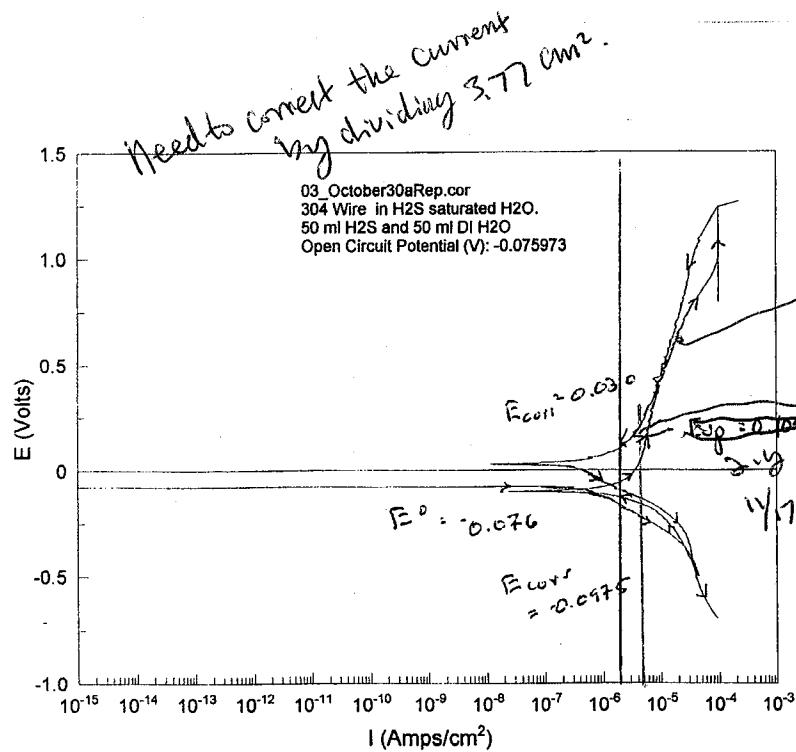
RTP 1/3/03  
October 30, 2003

- 0715 Started linear polarization test of wire in solution.  
Solution is 50mL Saturated H<sub>2</sub>S solution and 50mL of DI H<sub>2</sub>O.  
Area of wire = 3.77 cm<sup>2</sup>

File 03\_October30aRep.cor

Test setup:

- |                 |                                      |
|-----------------|--------------------------------------|
| Open Circuit    | (20 min)                             |
| Potentiodynamic | (0.0C, -0.5Ref, 4.0 Ref, 1.667 mV/s) |
| Galvanostatic   | (0.0001 A, 2 hours)                  |
| Potentiodynamic | (0.0 Prev, -0.7Ref, 1.667 mV/s)      |
| Potentiodynamic | (0.0 Prev, 0.7Ref, 1.667 mV/s)       |



Prepared  
11/03/03

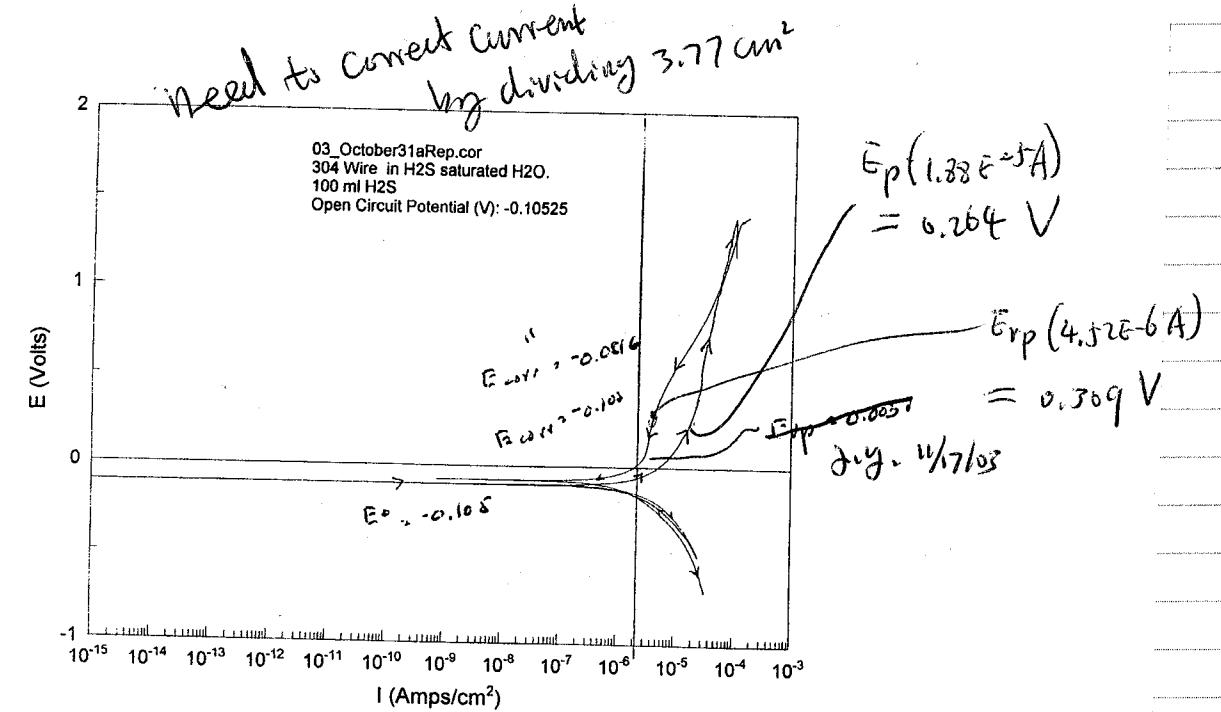
October 31, 2003

- 0715 Started linear polarization test of wire in solution.  
Solution is 100mL Saturated H<sub>2</sub>S solution.  
Area of wire = 3.77 cm<sup>2</sup>

File 03\_October31aRep.cor

Test setup:

- |                 |                                      |
|-----------------|--------------------------------------|
| Open Circuit    | (20 min)                             |
| Potentiodynamic | (0.0C, -0.5Ref, 4.0 Ref, 1.667 mV/s) |
| Galvanostatic   | (0.0001 A, 2 hours)                  |
| Potentiodynamic | (0.0 Prev, -0.7Ref, 1.667 mV/s)      |
| Potentiodynamic | (0.0 Prev, 0.7Ref, 1.667 mV/s)       |



Briefed  
11/3/03

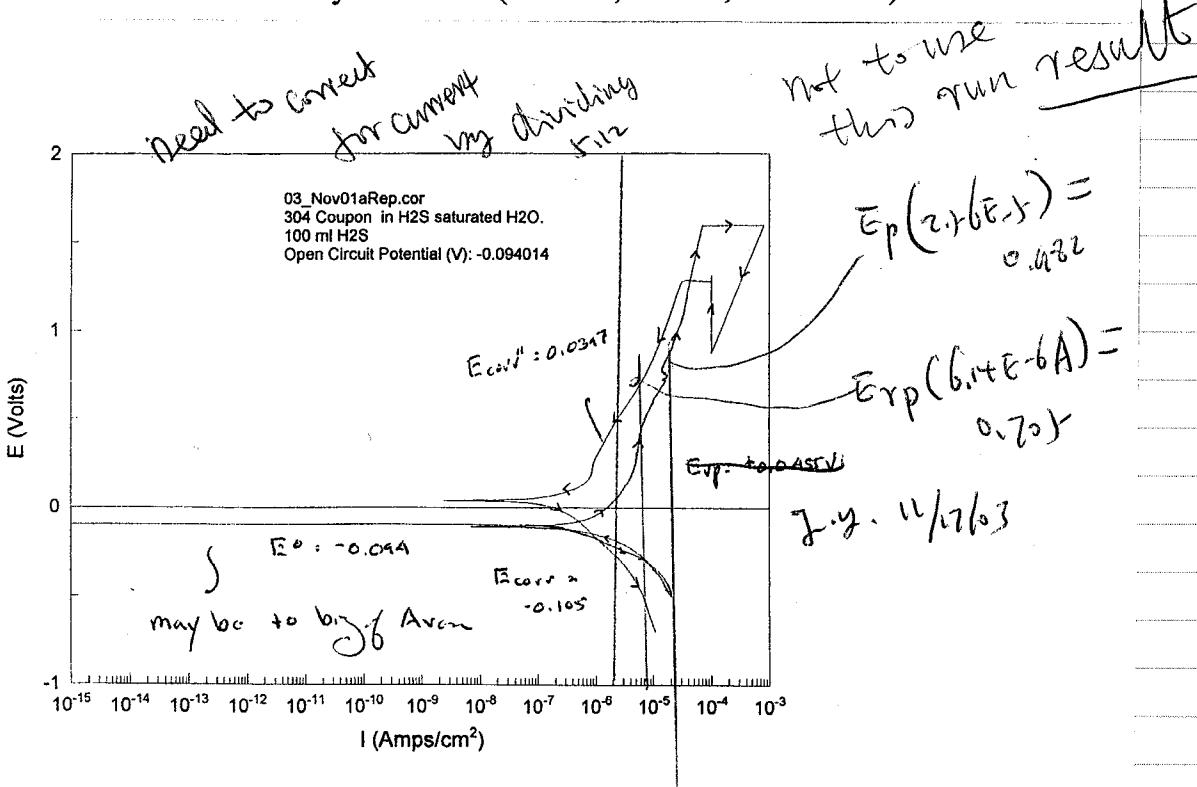
November 1, 2003

0715 Started linear polarization test of coupon in solution.  
Solution is 100mL Saturated H<sub>2</sub>S solution.  
Area of coupon = 5.12 cm<sup>2</sup>

File 03\_November1aRep.cor

Test setup:

Open Circuit	(20 min)
Potentiodynamic	(0.0C, -0.5Ref, 4.0 Ref, 1.667 mV/s)
Galvanostatic	(0.0001 A, 2 hours)
Potentiodynamic	(0.0 Prev, -0.7Ref, 1.667 mV/s)
Potentiodynamic	(0.0 Prev, 0.7Ref, 1.667 mV/s)



11/3/03 1500 increased N<sub>2</sub> rate in all cell's

Medium A 58 bubble/min to 120 bubble/min  
 Postgate C No Cl- 28 bubble/min to 60 bubble/min  
 Postgate C 0.5M Cl- 28 bubble/min to 60 bubble/min  
 Postgate C + Slime form

11/6/03 1545

Postgate C+  $\approx$  60 bubble/min to 120 bubble/min

		Model #
11/7/03 0930 Medium A	Ref electrode	SN 821059
Postgate C No Cl-	Ref electrode	SN 5087405
Postgate C 0.5M Cl-	Ref electrode	SN 8238321
Postgate C + Slime	Ref electrode	SN 9214080

13-G20-52

13-G20-51

13-G20-52

13-G20-51

Verification check of Ref Electrodes vs  
Lab Reference SN 9250074

		RTD 11/7/03
Ref electrode	SN 821059 vs Ref	0.008 mV
Ref electrode	SN 5087405 vs Ref	-0.4 mV
Ref electrode	SN 8238321 vs Ref	-0.3 mV
Ref electrode	SN 9214080 vs Ref	-0.2

RTD  
11/3/03  
11/3/03

RTD  
11/7/03  
11/7/03





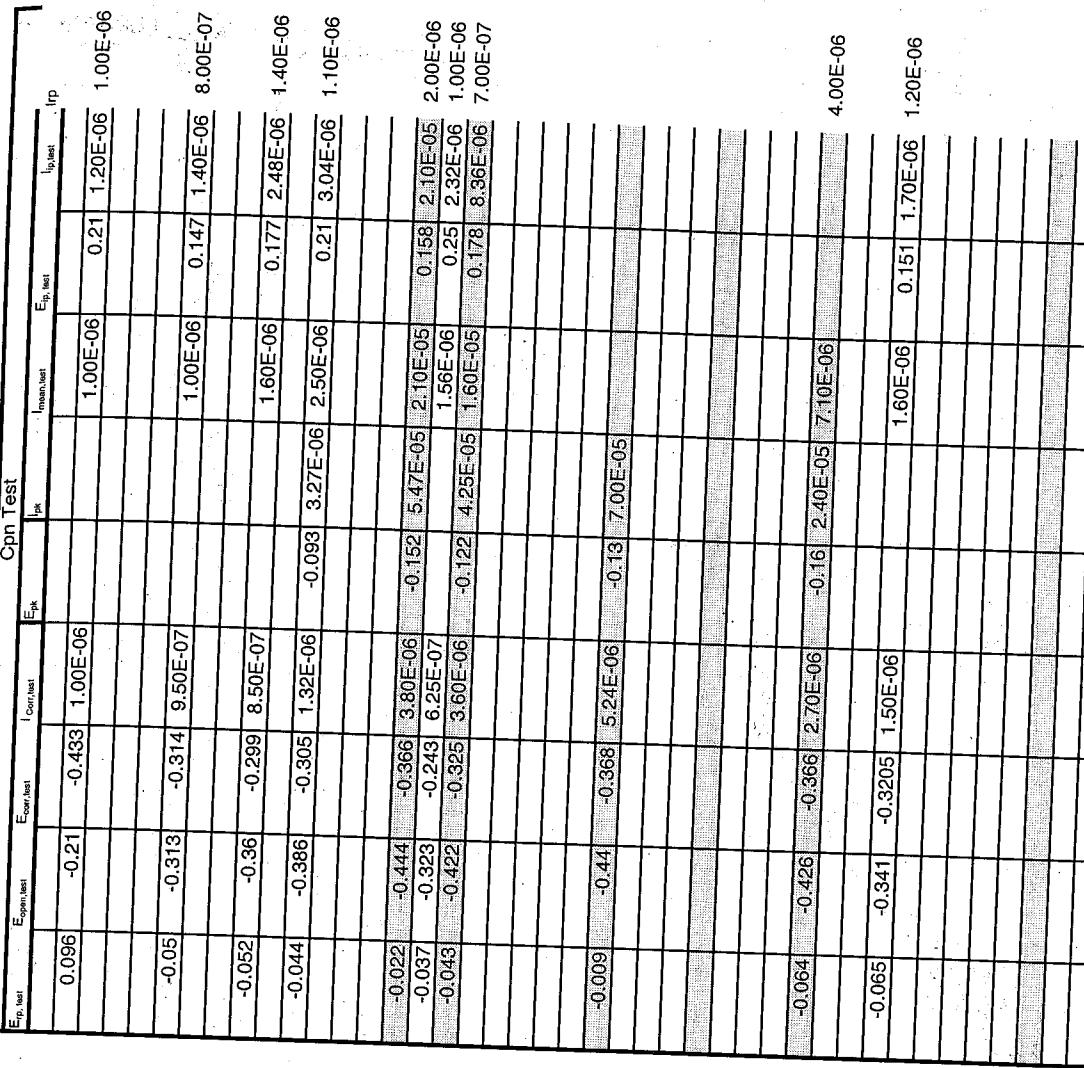
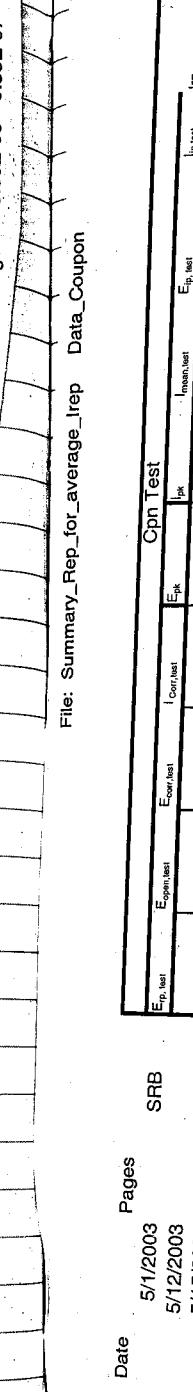
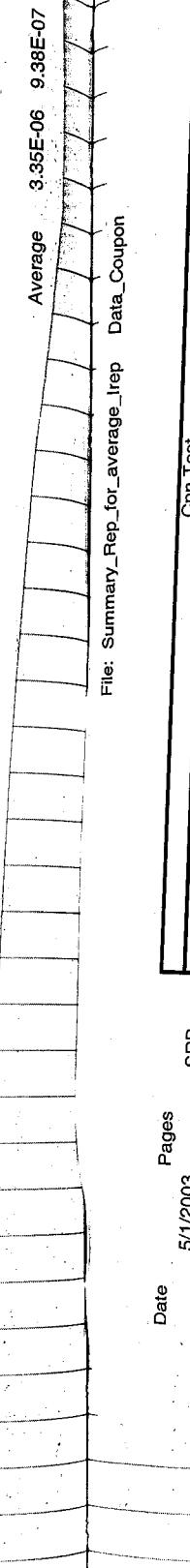


Date	Pages	SRB
<b>N2 bubbling</b>		
5/11/2003		
5/12/2003		
5/15/2003	97, 141	
5/15/2003	98, 175	
5/16/2003		
5/21/2003	102, 142	
5/22/2003	103, 143, 144	
5/23/2003	103, 145	
5/28/2003	108, 147, 148	
5/29/2003	108, 149, 150	
5/30/2003	110, 151	
6/3/2003	130, 152, 153	
6/4/2003	130, 154, 155	
6/5/2003	131, 156, 157	
6/10/2003	134, 158,	
6/11/2003	135, 159, 160	
6/13/2003	136, 161,	
6/18/2003	140, 162, 176	
6/19/2003	140, 178, 180	
6/19/2003	163, 178, 180	
6/20/2003	163, 182	
6/23/2003	163, 184	
6/27/2003	166, 186	
6/30/2003	166, 188	
7/1/2003	167, 190	
7/2/2003	167, 192	
7/7/2003	171, 194	
7/8/2003	171, 196	
nowon direction sweep		
7/18/2003	279, 280	
7/21/2003	280, 281	
7/22/2003	281, 283	
7/25/2003	285, 287	
7/28/2003	285, 288	
7/29/2003	285, 290	
7/31/2003	292, 293	
8/1/2003	293, 295	
8/9/2003	296, 297	Discarded, no repassivation
8/13/2003	17, 22, new	
8/14/2003	17, 23, new	

2-yr

11/12/03

Buy Mem



This color is  
Coupon  
Wire (next page)  
Average  
average of the two:

$1.2 \times 10^{-6}$

Value:  $1.5 \times 10^{-6}$

Dry  
11/12/03  
Rugby Mu  
11/12/03





Monday, October 22, 2003

9:00 AM Start (SB)

Conducted sulfide analysis of sulfide standard water. Got 450 mg/L from Hach test. CRC states 437 mg/L and bottle label states 400 mg/L.

Conducted sulfide analysis of test vessels A, B, C, and D. Used 1 mL from each vessel for the test for a 25-fold dilution factor. Got a reading of zero for each but may have lost sulfide in the time between sample collection and analysis.

Prepared 500 mL each of Ringas A, Postgate C without NaCl, and Postgate C with 0.5 M NaCl. Left SWRI to go to UTSA to autoclave media.

3:00 PM end (SB)

October 27, 2003

12:00 PM start (SB)

Transferred cells into media C to build stock culture for possible inoculation of test vessels on Friday. Tested sulfide concentration of stock. Got reading of 0.16 mg/L with a 250 fold dilution for a final concentration of 40 mg/L.

Extracted approximately 2 mL from each vessel for MPN and sulfide analysis. Conducted sulfide concentration analysis of test vessels A, B, and C using 1 mL from each vessel to yield a 25 X dilution factor. Used 1 mL from each vessel for an MPN test.

Added 5 mL of media back into each vessel (Ringers media A in vessel A, Postgate C no NaCl in vessel B, and Postgate C with 0.5 M NaCl in vessel C).

Sulfide results:

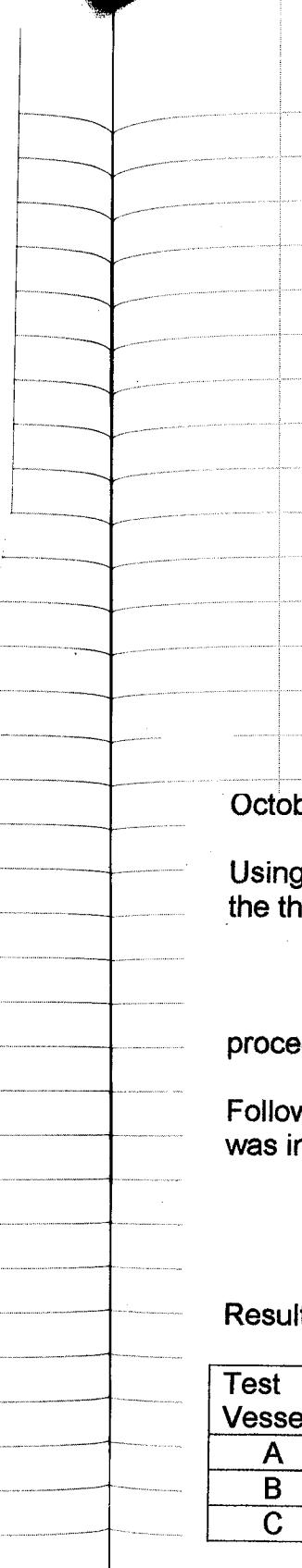
Sample A: 1 mL to 25 mL = 0.0 mg/L

Sample B: 1 mL to 25 mL = 0.01 mg/L x 25 = 0.25 mg/L

Sample C: 1 mL to 25 mL = 0.0 mg/L

Sealed MPN tubes in anaerobe bags.

2:30 PM end (SB)



October 29, 2003

10:00 AM Start (SB)

The three inoculated vessels, A, B, and C, appear to have growth. Vessel A (Ringas A media) is turbid. Vessel B (Postgate C without NaCl) is cloudy. Vessel C (Postgate C with 0.5 M NaCl) is hazy. The sulfide scrubber is blackening, thus indicating sulfide production.

Aseptically collected approximately 2 mL from vessel A, B, and C for sulfide analysis using the Hach colorimeter. Results are in the table below.

Test Vessel	Volume tested	Dilution factor	Hach reading	Sulfide concentration
A	0.1 mL	250	0.41 mg/L	102.5 mg/L
B	1.0 mL	25	0.53 mg/L	13.25 mg/L
C	1.0 mL	25	0.01 mg/L	0.25 mg/L

12:30 PM end (SB)

October 30, 2003 11:00

Using aseptic technique, approximately 7 ml. brine solution was sampled from each of the three inoculated vessels, A, B, and C. Each sample was tested as follows:

Sulfide concentration using the Hach colorimeter

Turbidity using the Klett Spectrophotometer

Culture using serial dilution technique and the MPN (Most Probable Number) procedure to quantitate growth of SRB (Sulfur reducing bacteria, *Desulfovibrio vulgaris*).

Following the harvest, approximately 10 ml of the corresponding sterile broth medium was inoculated into each of the three test vessels to replace the volume harvested.

A: sterile Ringas A broth medium

B: sterile Postgate C without NaCl broth medium

C: sterile Postgate C with 0.5 M NaCl broth medium

Results are in the table below:

Test Vessel	Volume tested	Dilution Factor	Hach Reading	Sulfide Concentration	Klett Reading	*MPN Quantitation
A	0.1 ml	250	0.62 mg/L	155.0 mg/L	2 KU	$5 \times 10^8$
B	0.1 ml	250	0.55 mg/L	137.5 mg/L	12 KU	$1.6 \times 10^9$
C	1.0 ml	25	0.0 mg/L	0.0 mg/L	5 KU	N/G

\*MPN cultures should incubate at least 7 days prior to data entry. Cultures should be kept and reviewed for 30 days for final result verification.

11/4/03

Reg J/M

November 5, 2003

~~10:00 AM~~

Using aseptic technique, approximately 6 ml. brine solution was sampled from each of the three inoculated vessels, A, B, and C. Each sample was tested as follows:

Sulfide concentration using the Hach colorimeter

Turbidity using the Klett Spectrophotometer

Following the harvest, approximately 6 ml of the corresponding sterile broth medium was inoculated into each of the three test vessels to replace the volume harvested.

A: sterile Ringas A broth medium

B: sterile Postgate C without NaCl broth medium

C: sterile Postgate C with 0.5 M NaCl broth medium

Because Vessel C did not appear to contain viable organisms, approximately 10 ml aliquot of a recent (7day) subculture of SRB (10 ml subculture placed into 30 ml of sterile Postgate C broth without NaCl) was inoculated into the test Vessel.

Results of analyses are in the table below:

Test Vessel	Volume tested	Dilution Factor	Hach Reading	Sulfide Concentration	Klett Reading	*MPN Quantitation
A	0.05 ml	500	0.3 mg/L	150.0 mg/L	18 KU	N/A
B	0.01 ml	2500	0.11 mg/L	275.0 mg/L	51 KU	N/A
C	1.0 ml	25	0.01 mg/L	0.25 mg/L	6 KU	N/A

\*MPN cultures should incubate at least 7 days prior to data entry. Cultures should be kept and reviewed for 30 days for final result verification.

N/A = MPN determination not performed

By: J. m.  
11/5/03

November 6, 2003

~~10:00 AM~~

Using aseptic technique, Vessel D was inoculated with approximately 10 ml aliquot of a recent (7day) subculture of slime former, (1 ml subculture *Vibrio natriegens* placed into 9 ml of sterile Nutrient Broth with 0.1% NaCl). Serial dilution with MPN quantitation technique was performed on this stock culture. 250 11/6/03

Using aseptic technique approximately 1 ml of brine solution was harvested from each of the three test Vessels A, B and C. Cultures were performed using serial dilution with MPN quantitation technique.

Results are as follows:

Test Vessel	*MPN
A Ringas A	
B Postgate C without NaCl	
C Postgate C with 0.5 M NaCl	
D Postgate C with 0.5 M NaCl Slime Former	

\*MPN cultures should incubate at least 7 days prior to data entry. Cultures should be kept and reviewed for 30 days for final result verification.

November 7, 2003

~~start 8:00 AM SB~~

Checked MPN from Oct 30<sup>th</sup> (collected by Geri).

Vessel A:  $10^6 = 5, 10^7 = 5, 10^8 = 2$  for a 5-5-2 MPN reading =  $5.4 \times 10^8$  cells/mL.

Vessel B:  $10^6 = 5, 10^7 = 5, 10^8 = 4$  for a 5-5-4 MPN reading =  $1.6 \times 10^9$  cells/mL.

Vessel C:  $10^2 = 0, 10^3 = 0, 10^4 = 0$  for a 0-0-0 MPN reading =  $<1.8 \times 10^2$  cells/mL.

Visual inspection of the vessels reveal vessel A is turbid, creamy yellow in color; vessel B is turbid, greenish black in color; and vessel C is slightly cloudy. Geri re-inoculated vessel C with SRB on November 5<sup>th</sup>.

Discussed progress with Lietai and planned possible changes.

end 9:30 AM SB

Progress  
11/6/03







November 18, 2003

0730 Started linear polarization of 304 coupon in solution  
of 304 coupon in solution  
Solution is 100% ml saturated H<sub>2</sub>S solution  
Area of coupon = 5.12 cm<sup>2</sup>  
Initial pH = 3.28 Final pH = 3.08  
Hydrogen sulfide counter not # 3209-08  
File 03-Nov 18a Rep.cor

## Test setup

Open CKT (20min)

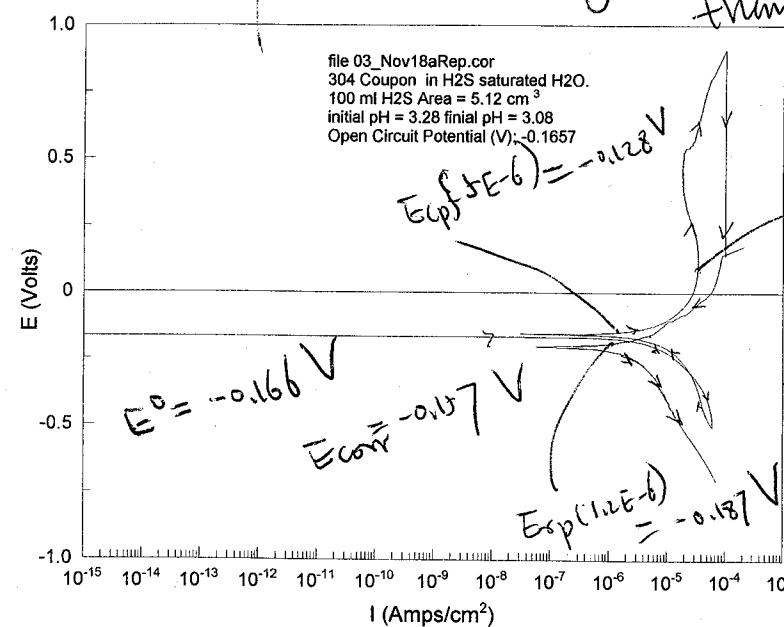
Potentiodynamic (-0.0L, -0.5 Ref, 4.0 Ref, 1.667 mV/s)

Galvanostatic (0.001 A, 2hr)

Potentiodynamic (0.0 Pro, -0.7 Ref, 1.667 mV/s)

Potentiodynamic (0.0 Pro, 0.7 Ref, 1.667 mV/s)

New Surface Area  
May be bigger  
than 5.12 cm<sup>2</sup>!

Rajesh  
11/19/03

1045

11/19/03 Remove ≈ 7 ml from each cell. Replace with appropriate media.

11/21/03

Remove ≈ 7 ml from each cell. Replace with appropriate media. Hold sample tube in cell A to bottom and remove ≈ 7 ml more.

1230

Remove ≈ 7 ml from cell's A, B & C. Replace with appropriate media.

1330

From discontinued test series, removed approximately 10<sup>4</sup> ml solution from test, control and oxygen cell using aseptic technique. Replaced volume with approximately 30 ml sterile Baar's Broth inoculated into each vessel. Cultures taken <sup>sent</sup> 11-24-03 performed using serial dilution technique. (D)

11/26/03

Remove ≈ 7 ml from cells A, B, C, D. Replace with appropriate media (D). Did a nitrogen burst of cells A & B, before and after harvest and inoculation of replacement media (D).

Test	SRB
Dummy	10 <sup>7</sup>
QC	10 <sup>6</sup>

11/00.	Vibrio
10 <sup>7</sup>	10 <sup>7</sup>
10 <sup>6</sup>	10 <sup>6</sup>
—	—
10 <sup>7</sup>	10 <sup>7</sup>
10 <sup>6</sup>	10 <sup>6</sup>

11/00.  
10<sup>7</sup>  
10<sup>6</sup>  
—  
10<sup>7</sup>  
10<sup>6</sup>Rajesh  
11/19/03

20 November 03

0730 Started linear polarization test of platinum wire in 0.1 Na<sub>2</sub>S solution  
Area of wire = 3.77 cm<sup>2</sup>

File 03-Nov20a Rep

Test setup:

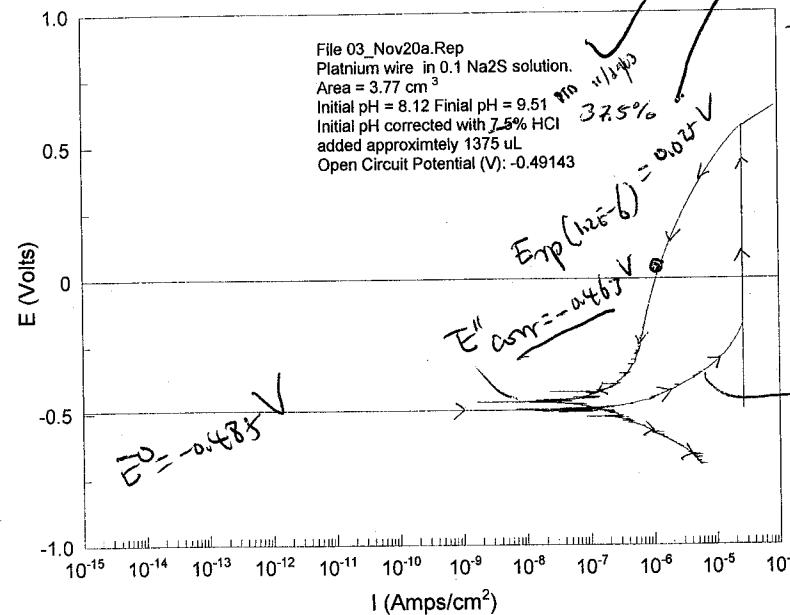
Open CK2 (20 min)

Potentiodynamic (0.0C, -0.5R<sub>L</sub>, 4.0R<sub>L</sub>, 1.667 mV/s)

Galvanostatic (0.001A, 2 hours)

Potentiodynamic (0.0Prw, 0.7R<sub>L</sub>, 1.667 mV/s)Potentiodynamic (0.0Prw, 0.7R<sub>L</sub>, 1.667 mV/s)  
Na<sub>2</sub>S + HCl — Aug. 12/8/03 mw=240.18 ✓4.8 g Na<sub>2</sub>S with balance of DI for 200 mL  
Lot # 033454

Initial pH = 12.3 used ≈ 1375 uL of 37.5% HCl to lower pH to 8.12

Roger T. Ross  
11/24/03

21 Nov 03

0720 Started linear polarization test of platinum wire in 0.1 Na<sub>2</sub>S + 0.5M NaCl solution  
Area of wire = 3.77 cm<sup>2</sup>

File 03\_Nov21a Rep

Test setup:

Open CK2 (20 min)

Potentiodynamic (0.0C, -0.5R<sub>L</sub>, 4.0R<sub>L</sub>, 1.667 mV/s)

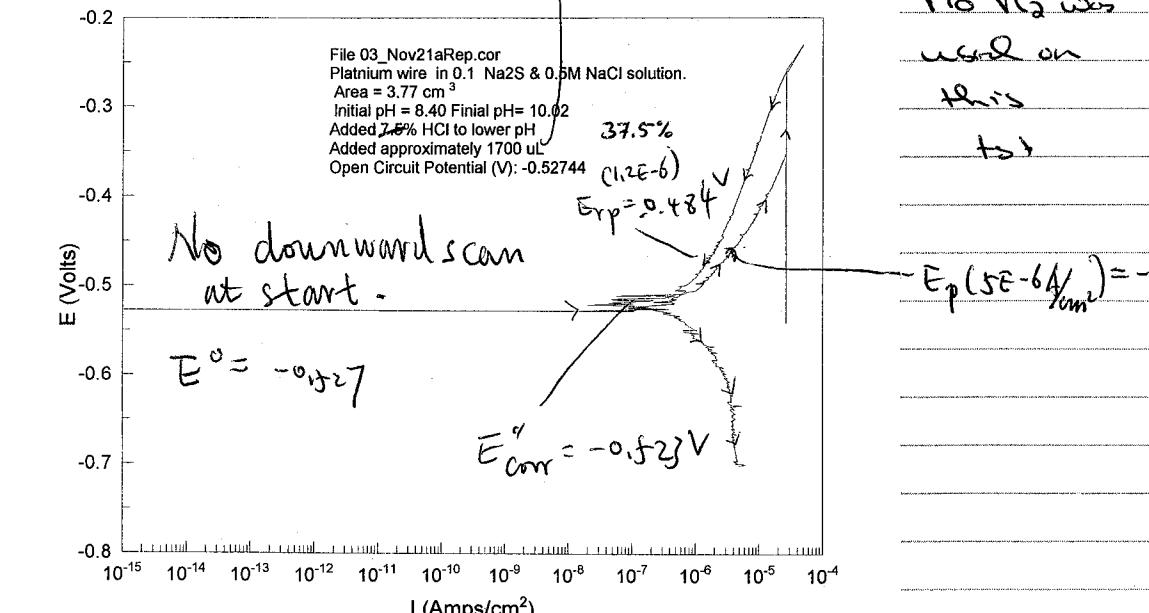
Galvanostatic (0.001A, 2 hrs)

Potentiodynamic (0.0Prw, 0.7R<sub>L</sub>, 1.667 mV/s)Potentiodynamic (0.0Prw, 0.7R<sub>L</sub>, 1.667 mV/s)4.78 g Na<sub>2</sub>S + 5.8 g NaCl with Balance of DI for 200 mL  
Lot # 033454 : lot # 034103 37.5 HCl 11/24/03

Initial pH = 12.2 used ≈ 1700 uL of 37.5% HCl to lower pH to 8.40

$1.7 \times 1.19 \times 0.375 \approx 0.164 \text{ M}$   
 $\text{HCl} = 0.164 + 0.5$   
 $= 0.6$

No Na<sub>2</sub>S used on this test

Roger T. Ross  
11/24/03

24 Nov 03

0730 Started linear polarization test of platinum wire in 0.1M Na<sub>2</sub>S + 0.5M NaCl  
Area = 3.77 cm<sup>3</sup>

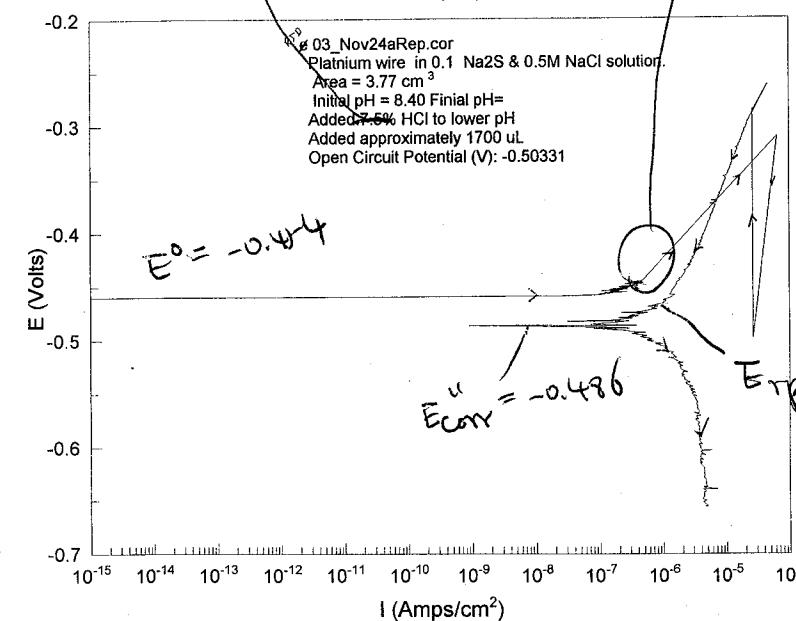
File 03-Nov24aRep.cor

Test set up:

open (K)	(20 min)
Potentiodynamic	(0.0c, -0.5Rcf, 4.0Rcf, 1.667 mV/s)
Galvanostatic	(0.001A, 2hrs)
Potentiodynamic	(0.0Rico, -0.7Rcf, 1.667 mV/s)
Potentiodynamic	(0.0Rraw, +0.7Rcf, 1.667 mV/s)

used solution that was made for 21 Nov 03  
test see page 189.

Bubbled na during test.

Roy J Flynn  
12/1/03

25 Nov 03

0725 Started linear polarization test of platinum wire in 0.1M Na<sub>2</sub>S solution  
Area = 3.77 cm<sup>3</sup>

File 03-Nov25aRep.cor

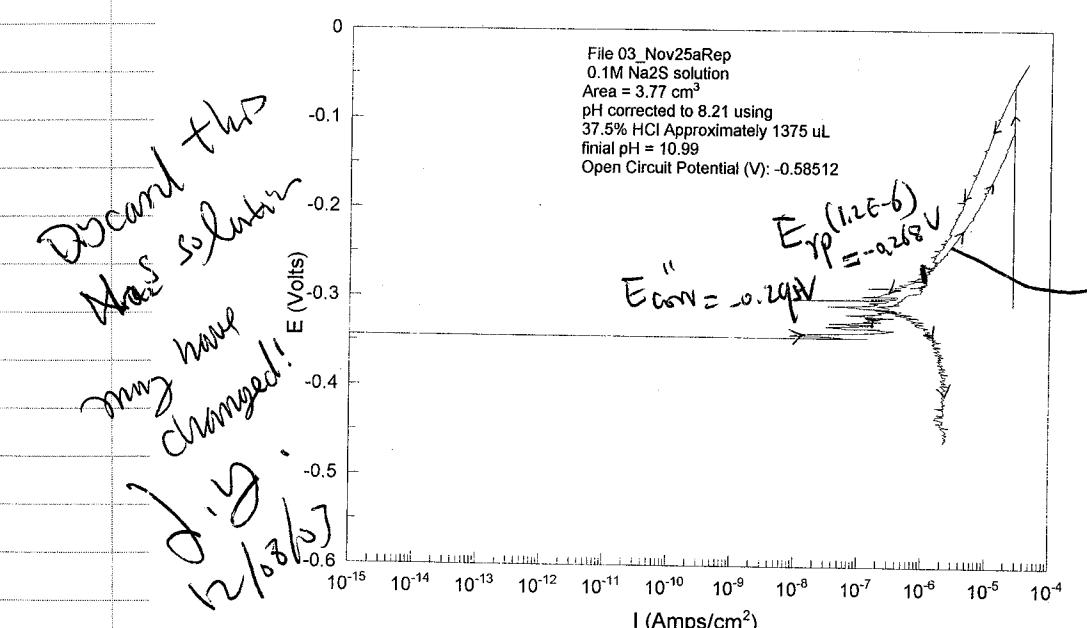
Test set up:

open (K)	(20 min)
Potentiodynamic	(0.0c, -0.5Rcf, 4.0Rcf, 1.667 mV/s)
Galvanostatic	(0.001A, 2hrs)
Potentiodynamic	(0.0Rico, -0.7Rcf, 1.667 mV/s)
Potentiodynamic	(0.0Rraw, +0.7Rcf, 1.667 mV/s)

used solution that was made for 20 Nov 03  
test see page 188.

Bubbled na during test.

$$\text{Cl}^- = 0.084 \text{ M}$$

Roy J Flynn  
12/1/03

1115

1 Dec 03

Removed  $\approx$  7 ml of solution from cell  
cell. Replenished with appropriate media

1215

3 Dec 03

Removed  $\approx$  7 ml of solution from cell  
cell. Replenished with appropriate media

12:30

November 12, 2003

Using aseptic technique, approximately 7 ml sample is harvested from test vessels A, B, C, and D. Determinations are made for sulfide content using the Hach procedure and for turbidity using the Klett spectrophotometer.

Results are in the table below:

Test Vessel	Volume tested	Dilution Factor	Hach Reading	Sulfide Concentration	Klett Reading	*MPN Quantitation
A	0.01 ml	2500	0.07 mg/L	175 mg/L	6 KU	
B	0.01 ml	2500	0.11 mg/L	300 mg/L	86 KU	
C	1.0 ml	25	0.02 mg/L	0.50 mg/L	12 KU	
D	1.0 ml	25	0.02 mg/L	0.50 mg/L	10 KU	

\*MPN cultures should incubate at least 7 days prior to data entry. Cultures should be kept and reviewed for 30 days for final result verification.

Vessel D should have no sulfide present. Positive reading could be instrument error as it relates to sensitivity at the low end.

6730

2/Dec/03

Started linear polarization test of platinum wire in 0.1 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution  
Area of wire = 3.77 cm<sup>2</sup>

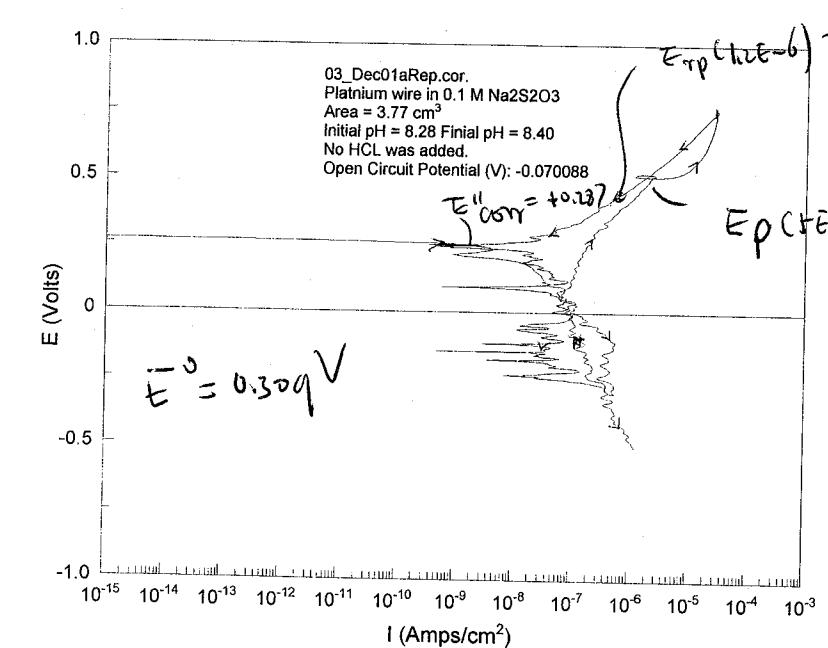
file 03 Dec1aRep.cor

Test Set up:

open ckt (20 min)  
Potentiodynamic (0.0C, -0.5 Ref, 1.0 Ref, 1.667 mV/s)  
Galvanostatic (0.001 A, 2 hrs)  
Potentiodynamic (0.0 Ref, -0.7 Ref, 1.667 mV/s)  
Potentiodynamic (0.0 Ref, +0.7 Ref, 1.667 mV/s)

3.25g Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> with balance of DI water for 200ml  
hot # 923931A

Initial pH = 8.28 Final pH = 8.40  
Bubbled N<sub>2</sub> during test.



Rogfflns  
12/1/03

Rogfflns  
12/1/03

2 Dec 03

6730

Started test of wire in 0.1M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>  
(platinum wire). Repeat of 01 Dec 03 test

File 03-Dec02aRep.cor

Test Setup:

Open circuit

(20 min)

Potentiodynamic

(0.0C, -0.5Rcf, 1.0Rcf, 1.667 mV/s)

galvanostatic

(0.001A, 2mV)

Potentiodynamic

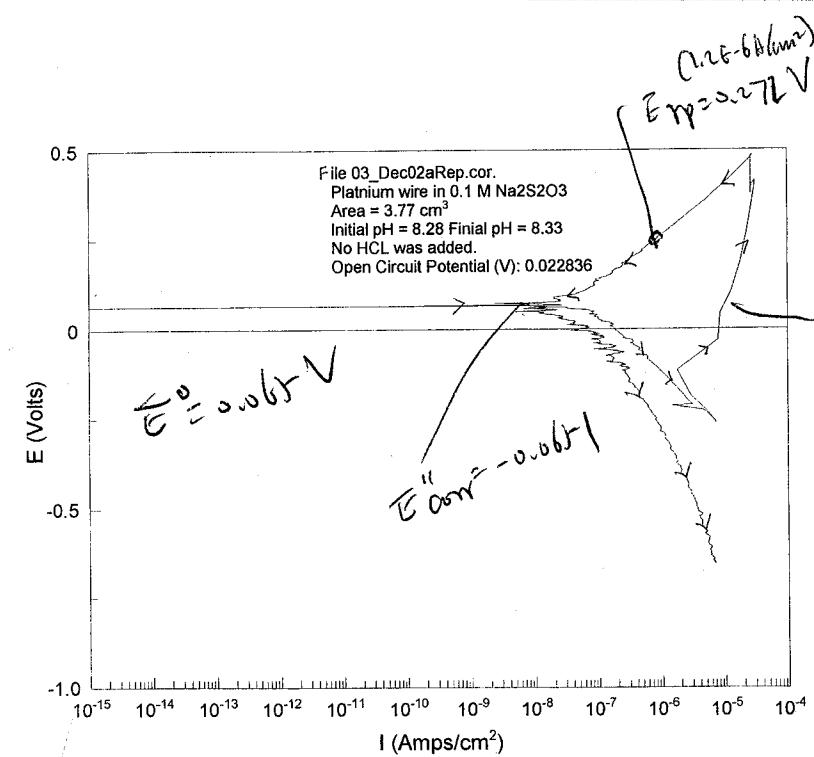
(0.0 Pro, -0.7 Rcf, 1.667 mV/s)

potentiodynamic

(0.0 Pro, +0.7 Rcf, 1.667 mV/s)

Initial pH = 8.28 Final pH = 8.33

Same solution as per 03Dec01aRep cor page 193

Bubbled N<sub>2</sub> during test.Roger J. Blane  
12/2/03

December 1, 2003 12:00

Using aseptic technique, approximately 7 ml. Sample is harvested from test vessels A, B, C, and D. Determinations are made for sulfide content using the Hach procedure and for turbidity using the Klett spectrophotometer.

Results are shown in the table below:

Test Vessel	Volume Tested	Dilution Factor	Hach Reading	Sulfide Concentration	Klett Reading	MPN Quantitation
A	0.01 ml	2500	0.12 mg/L	300 mg/L	17	N/A
B	0.01 ml	2500	0.04 mg/L	100 mg/L	51	N/A
C	1.0 ml	25	0.02 mg/L	0.50 mg/L	20	N/A
D	N/A	N/A	N/A	N/A	26	N/A

Nitrogen burst administered to vessels A and B following harvest.

SMB  
12/2/03

12/1/03 2:00 PM

Harvest ~7 ml. solution from each vessel A, B, C, D. Replenish with appropriate media. N<sub>2</sub> blast delivered to vessel A, B, C prior to harvest. 1500.  
Perform pH on samples at 3:00 PM.

Roger J. Blane  
12/2/03

3 Dec 03

0740

Started test of platinum wire in  
0.1 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>; 0.5 M NaCl

File 03-Dec03aRep.cor

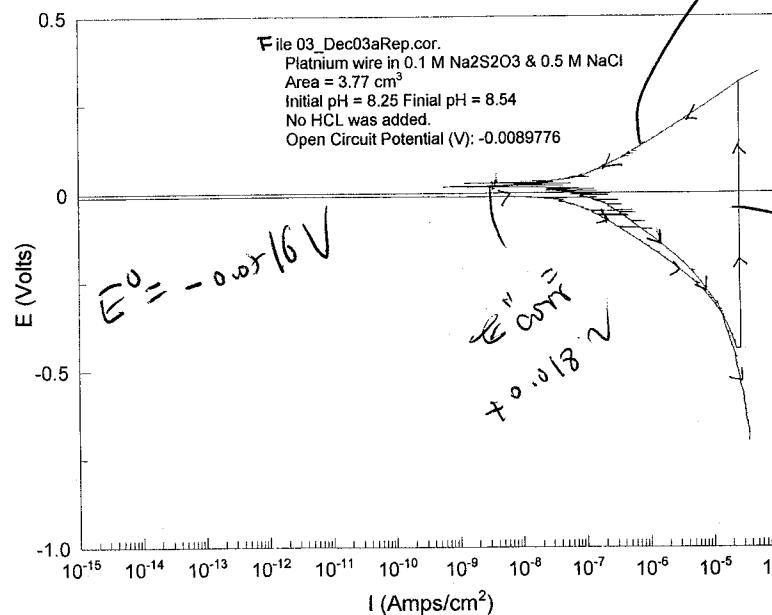
Test setup:

open (K<sub>E</sub>)  
potentiodynamic  
galvanostatic  
potentiodynamic  
potentiodynamic

(20 min)  
(0.0 C, -0.5 R<sub>Ref</sub>, 4.0 R<sub>Ref</sub>, 1.667 mV/s)  
(0.001 A, 2 hr)  
(0.0 P<sub>Ref</sub>, -0.7 R<sub>Ref</sub>, 1.667 mV/s)  
(0.0 P<sub>Ref</sub>, +0.7 R<sub>Ref</sub>, 1.667 mV/s)

Initial pH = 8.25, Fin. pH = 8.54

Solution 3.16 g Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> lot # 923931A  
5.71 g NaCl lot # 034103  
200 mL DI H<sub>2</sub>O

Rigout J.D.  
12/6/03

4 Dec 03

0730

Started test of platinum wire in 0.1  
M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>; 0.5 M NaCl

File 03-Dec04aRep.cor

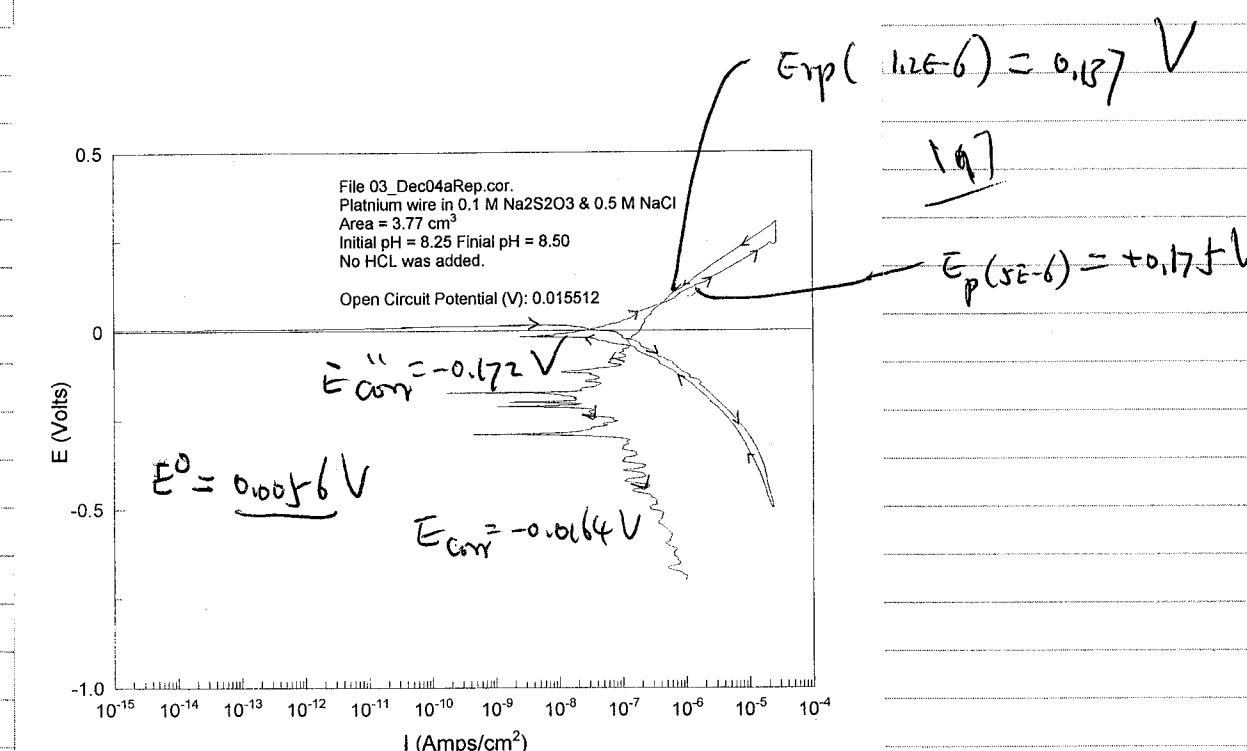
Test setup:

open (K<sub>E</sub>)  
potentiodynamic  
galvanostatic  
potentiodynamic  
potentiodynamic

(20 min)  
(0.0 C, -0.5 R<sub>Ref</sub>, 4.0 R<sub>Ref</sub>, 1.667 mV/s)  
(0.001 A, 2 hr)  
(0.0 P<sub>Ref</sub>, -0.7 R<sub>Ref</sub>, 1.667 mV/s)  
(0.0 P<sub>Ref</sub>, +0.7 R<sub>Ref</sub>, 1.667 mV/s)

Initial pH = 8.25, Fin. pH = 8.54

Solution 3.16 g Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> lot # 923931A  
5.71 g NaCl lot # 034103  
200 mL DI H<sub>2</sub>O

Rigout J.D.  
12/6/03

12/8/03  
1245 Started test of platinum wire in  
0.5M NaCl : 0.5M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> : 100ml H<sub>2</sub>S

File 03 Dec 08a Rep.cor

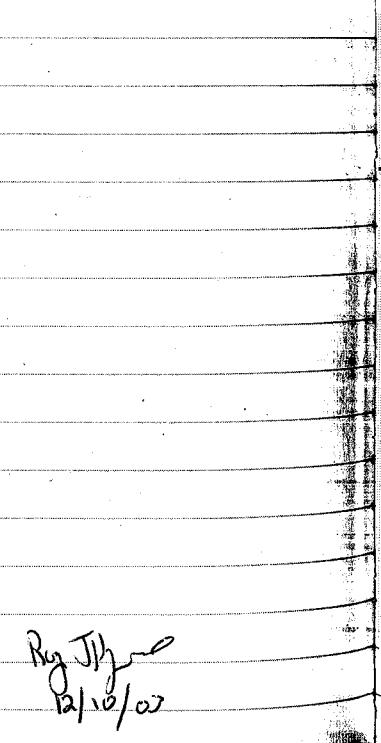
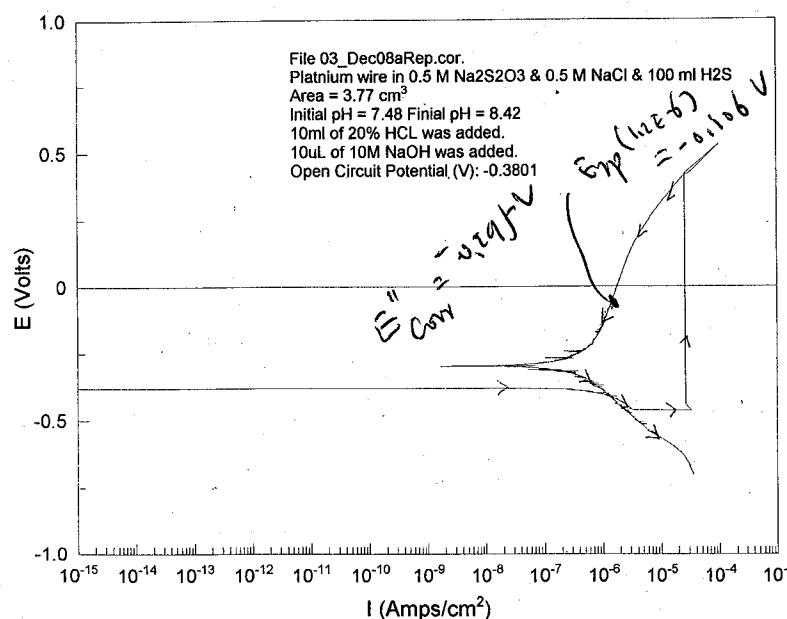
Test setup:

Open circuit	(20 min)	RSD 12/10/03
Potentiodynamic	(0.0C, 0.5Rcf, 1.0Rcf, 0.1667 mV/s)	
Galvanostatic	(0.001A, 2hr)	
Potentiodynamic	(0.0Pro, -0.7Rcf, 0.1667 mV/s)	RSD 12/10/03
Potentiodynamic	(0.0Pro, +0.7Rcf, 0.1667 mV/s)	RSD 12/10/03

Initial pH = 7.48, Final pH = 8.42

10ml of 20% HCl was added to adjust pH  
10ml of 10M NaOH was added to adjust pH

Solution 1.471g of NaCl lot # 034103  
3.996g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> lot # 023931A  
50ml of H<sub>2</sub>S water lot # 3209-08



12/9/03 Started test of platinum wire in  
0.5M NaCl : 0.5M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> : 100ml H<sub>2</sub>S

File 03 Dec 08a Rep.cor  
Test set-up:

Open circuit	(20 min)
Potentiodynamic	(0.0C, -0.5C, 0.1667 mV/s)
Galvanostatic	(0.0Pro, 1.5Rcf, 0.1667 mV/s)
Potentiodynamic	(0 Pro, 1.5Rcf, 0.1667 mV/s)
Galvanostatic	(0.0001A, 2hr)
Potentiodynamic	(0 Pro, -0.7Rcf, 0.1667 mV/s)
Potentiodynamic	(0 Pro, +0.7Rcf, 0.1667 mV/s)

Initial pH = 8.42 Final pH = 8.77

Solution same as 12/8/03

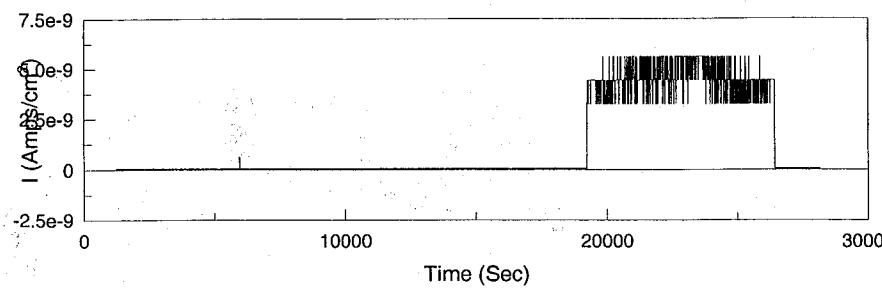
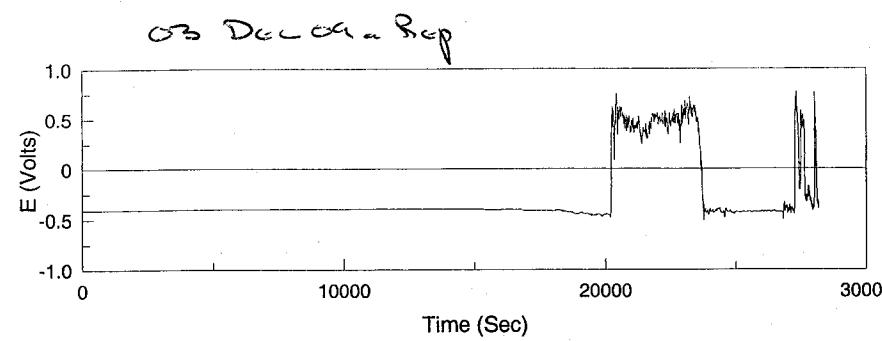
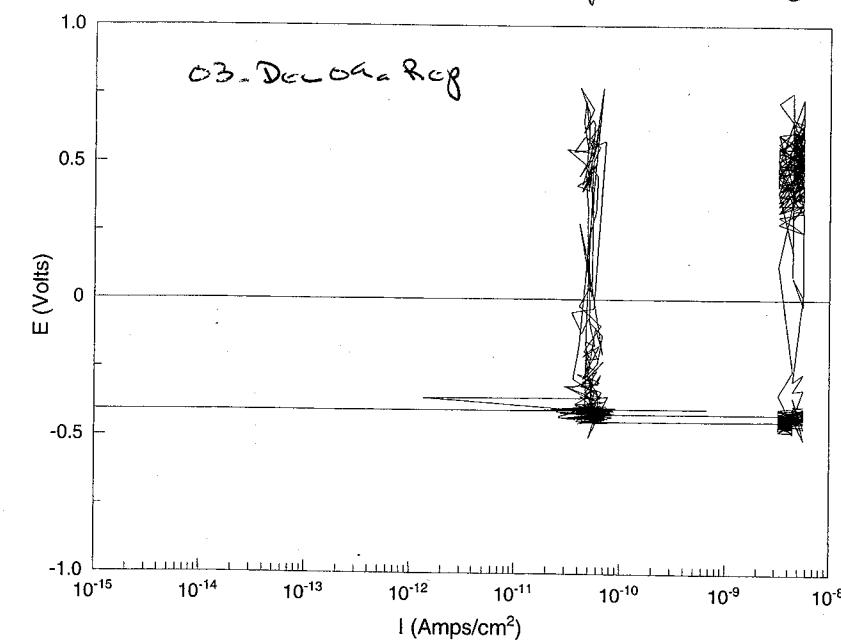
See page 200 for graphs.

Test to be done, 12/08/2003				
CF	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	Na <sub>2</sub> S	H <sub>2</sub> S (mmole/L)	S
to be done Dec 8				
0.5	0.5		100 ml	Pt, No N2 bubbling
0.5			100 ml	SS304 W, coupon, and Pt, No N2 bubbling
1			100 ml	SS304 W, coupon, and Pt, No N2 bubbling
			100 ml	Pt, no N2 bubbling
done, Nov 14				
Done, Nov 20				
Done, Nov 21, 24				
Done, Dec 1, 2		0.1		Pt, N2 bubbling
Done, Dec 3, 4	0.5	0.1		Pt, N2 bubbling
	0.001			Pt, N2 bubbling
	0.001			Pt, No N2 bubbling
	0.5			Pt, N2 bubbling
	1			Pt, N2 bubbling
	0.001			0.3M W, N2 bubbling
	0.001			0.3M W, No bubbling
	0.5			0.3M W
	1			0.3 W

J. Yang  
5/25/04

Supplied  
water is  
0.3M  
see if  
different

Ry Jho  
12/10/03

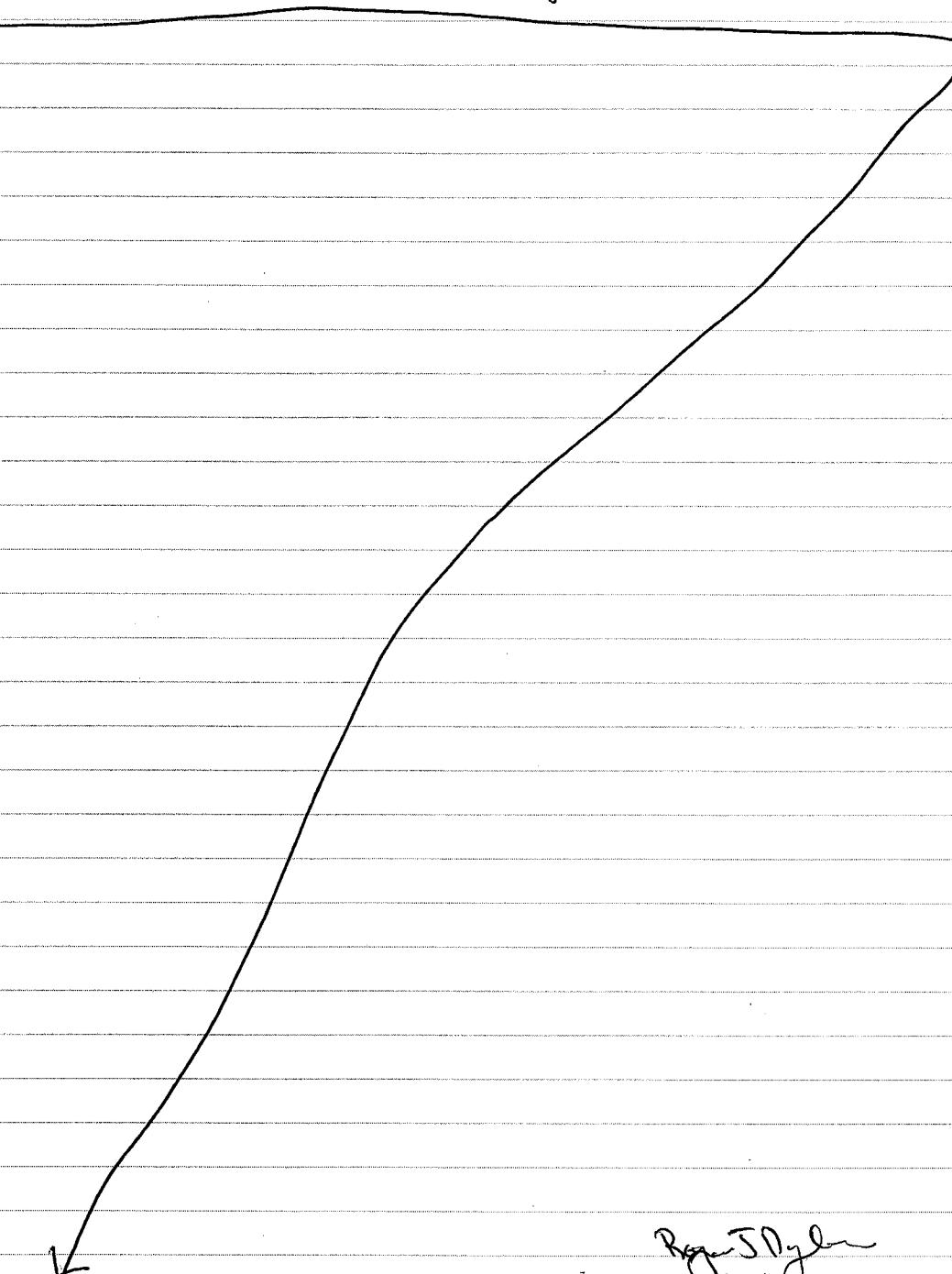


RJD  
Rep 11/11/03

J. Y.  
10/31/03

10/10/03 Run off  $\approx$  50 ml from each cell. Replaced with appropriate media. Cell A had some of the bubbles  $H_2O$  drawn into it. It was sterilized when introduced to bladder  $\rightarrow$  should not cause a problem.

With draw  $\approx$  small for pH test.



RJD  
Rep 11/11/03

12/11/03  
6730  
Starch test on Platinum wire in  
0.5 M NaCl & 100 ml H<sub>2</sub>S

file name 03 Dec 11aRep.cor

Test setup:

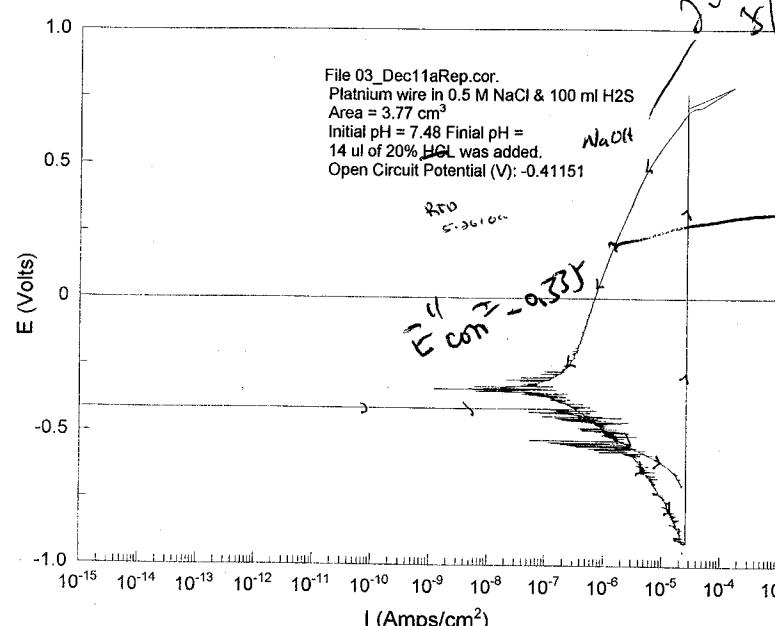
Open Circuit Potential (20 min)  
Potentiodynamic (-0.2 OCP, 0.1 OCP (no tcv))  
Galvanostatic (0.0001 A, 2 hr)  
Potentiodynamic (0.0 PRO, -0.7 RDE, 0.1667 mV/s)  
Potentiodynamic (0.0 PRO, -0.7 RDE, 0.1667 mV/s)

Solution 29.52 g NaCl

100 ml of H<sub>2</sub>S water

Add ~14 ul NaOH to ↑ pH to 7.48 (10m NaOH)

Final pH = 8.81 (pH taken on 12/16/03 after  
starch test)



Rogofsky  
12/12/03

12/12/03  
10:13  
Starch test on Platinum wire in  
0.5 M NaCl & 100 ml H<sub>2</sub>S

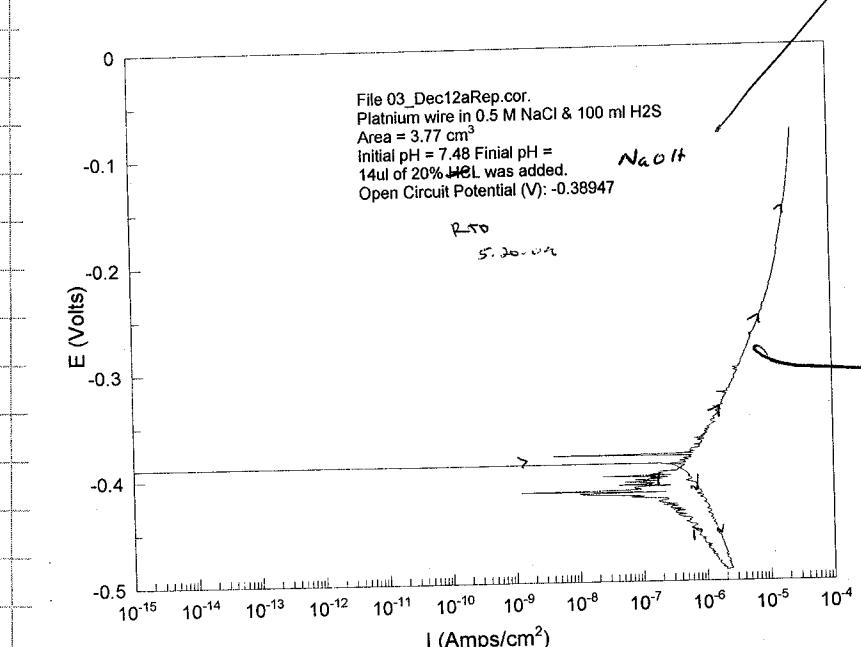
file name 03 Dec 12aRep.cor

Open Circuit Potential (20 min)  
Potentiodynamic

(0.0 OCP, 0.2 OCP )  
(-0.2 OCP, 0.1 OCP (no tcv))  
(0.0 PRO, 0.0001 A tcv)

Same as 205

2mV/s scan for all steps



Rogofsky  
12/12/03

No back sweep.

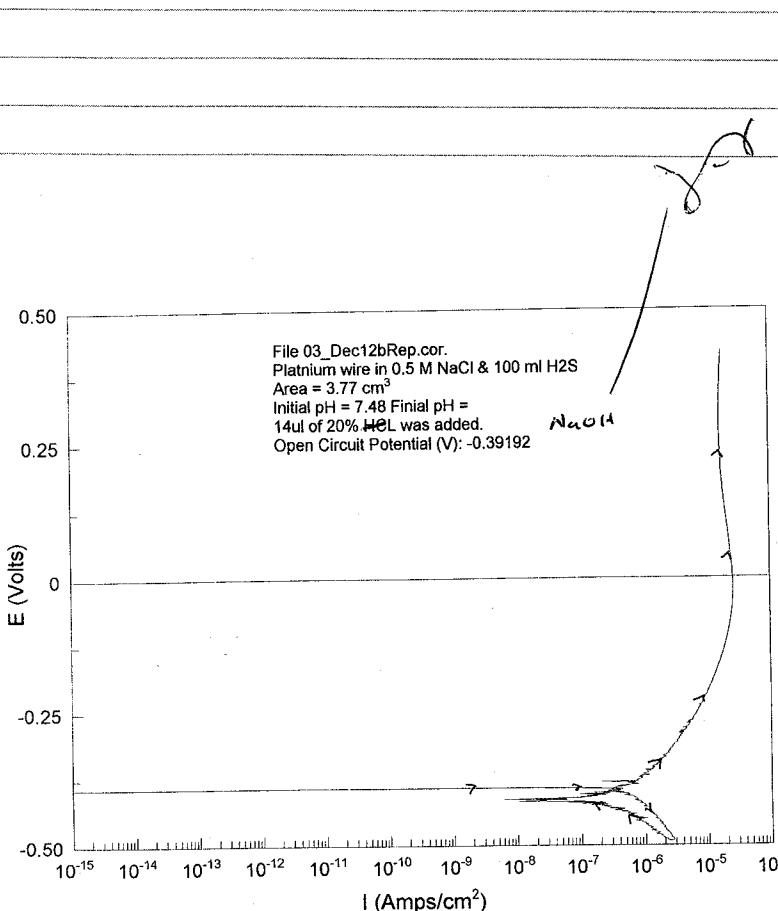
12/12/03 Starved test on platinum wire in  
0.5 M NaCl & 100 ml H<sub>2</sub>S

File num 03 Dec 12b Rep.cor

Open (K<sub>T</sub>) (20 min)  
Potentiodynamic RSD 12/11/03

Replot test of 03 Dec 11a @ scan rate  
of 2 mV/s, turn voltage

Results of 03 Dec 11a test ok.



Ry Phm  
12/12/03

12/12/03  
1200

Starved test on platinum wire in  
0.5 M NaCl & 100 ml H<sub>2</sub>S

File num 03 Dec 12c Rep.cor

RSD 12/11/03

Open (K<sub>T</sub>) (20 min)

Potentiodynamic (-0.0C, -0.5C, 0.1667 mV/s)

Potentiodynamic (-0.5C, 0.1C, 0.1667 mV/s)

Potentiodynamic (0 Proc, 1.5 Rcf, 0.1667 mV/s)

Galvanostatic (0.0001A, 2hr)

Potentiodynamic (0 Proc, -0.7 Rcf, 0.1667 mV/s)

Potentiodynamic (0 Proc, -0.7 Rcf, 0.1667 mV/s)

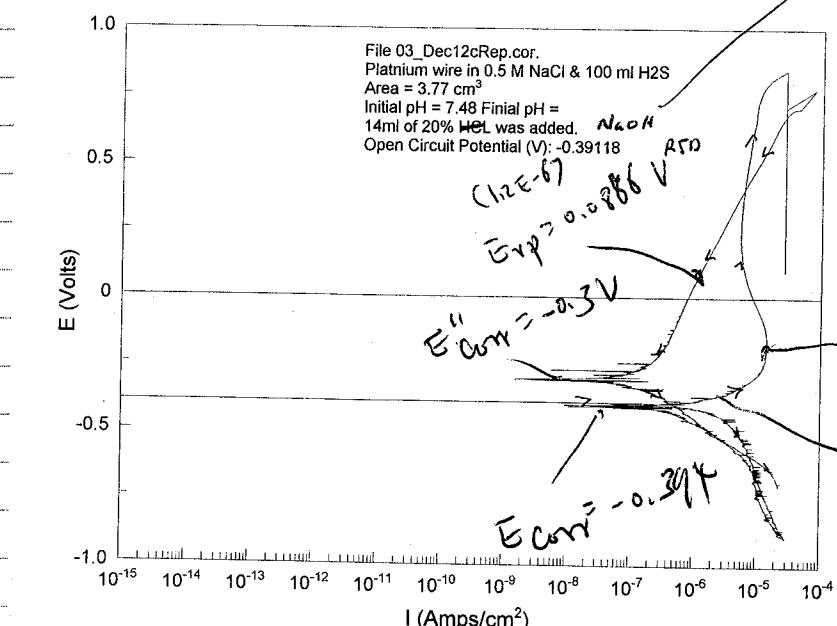
Solution 2.954g NaCl

100ml of H<sub>2</sub>S water

Added 14ul NaOH to ↑ pH to 7.48 (same solution

Final pH = 8.81 (pH taken on 12/16/03 on 0/11/03)

RSD 5.30.0% after final test



Ry Phm  
12/12/03

$$\begin{aligned} E_{ph} &= -0.1842 \\ I_{ph} &= 1.42 \text{ A}^{-1} \\ E_p(5E-6) &= -0.333 \end{aligned}$$

12/15/03  
10:45

Removed ~ 8ml of solution from cell  
cell. Replaced with appropriate media.

Roy J. Rhine  
12/15/03

12/15/03 Started test on 304 coupon in  
0.5M NaCl & 100ml H<sub>2</sub>S

SL name 03 Dec15aRep.cor

Open circuit

(20 min)

(0.0C, -0.5C, 0.1667 mV/s)

(-0.5C, 0.1C, 0.1667 mV/s)

(0.0C, 1.5R<sub>Ref</sub>, 0.1667 mV/s)

(0.0001 A, 2hr)

(0.0R<sub>Ref</sub>, -0.7R<sub>Ref</sub>, 0.1667 mV/s)

(0.0R<sub>Ref</sub>, -0.7 R<sub>Ref</sub>, 0.1667 mV/s)

Some solution as 12/11/03

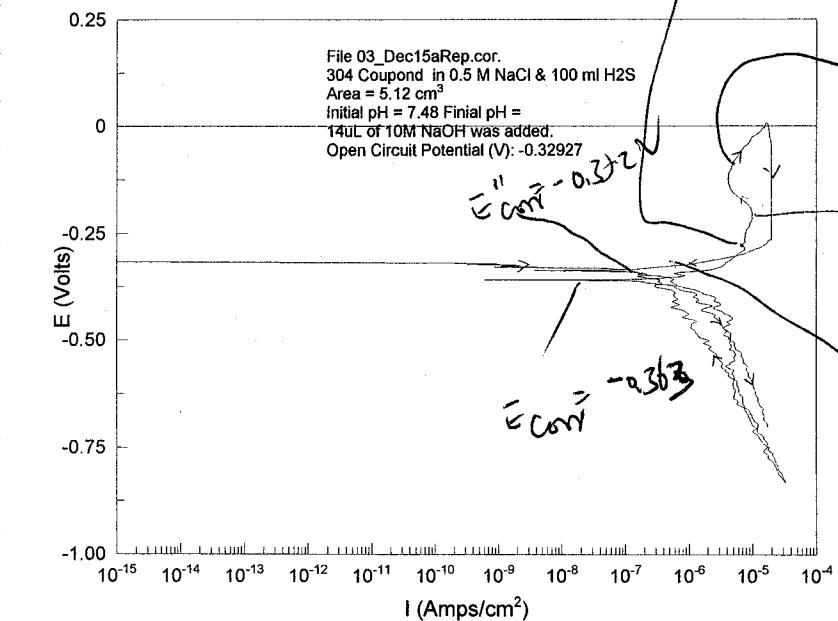
Final pH = 8.81

(pH taken on 12/16/03

after final test)

RDP

5.20.04



Roy J. Rhine  
12/15/03

12/16/03 Started test on 304 wire in 0.5M NaCl and 100ml H<sub>2</sub>S water

File name 03 Dec 16a Rep.cor

Set up

Open CKT

Potentiodynamic

Potentiodynamic

Potentiodynamic

Galvanostatic

Potentiodynamic

Potentiodynamic

(20 min)

(0.0c, -0.5c, 0.1667 mV/s)

(6.8E-05, 0.1 c, 0.1667 mV/s)

(0 Pre, 1.5 Rf, 0.1667 mV/s)

(0.0001A, 2hr)

(0 Pre, -0.7 Rf, 0.1667 mV/s)

(0 Pre, -0.7 Rf, 0.1667 mV/s)

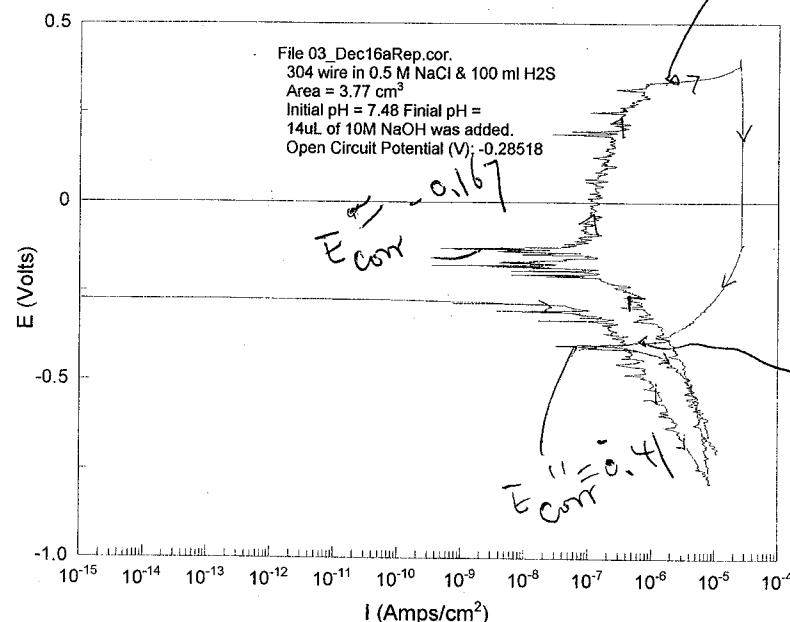
Solution same as 12/11/02

Final pH 6.98 (This plt was recorded

for final pH for test from

12/11/03 to 12/16/03)

$$\begin{aligned} E &= E_{corr} + \frac{RT}{4F} \ln \left( \frac{I_{corr}}{I_p} \right) \\ &= -0.28518 + \frac{(8.314 \times 298)}{4 \times 96485} \ln \left( \frac{0.167}{1.28 \times 10^{-6}} \right) \\ &= -0.28518 + 0.03076 \\ &= -0.25442 \end{aligned}$$



Re JMO  
12/19/03

12/17/03 Started N<sub>2</sub> in cell B (Postgate C  
1310 No (1') per phone instruction with  
Terri Becker.

Started test on platinum wire in  
1.0M NaCl and 100ml H<sub>2</sub>S water  
File name 03 Dec 17a Rep.cor

Set up

Open CKT

Potentiodynamic

Potentiodynamic

Potentiodynamic

Galvanostatic

Potentiodynamic

Potentiodynamic

(20 min)

(0.0c, -0.5c, 0.1667 mV/s)

(-0.5c, 0.1c, 0.1667 mV/s)

(0 Pre, 1.5 Rf, 0.1667 mV/s)

(0.0001A, 2hr)

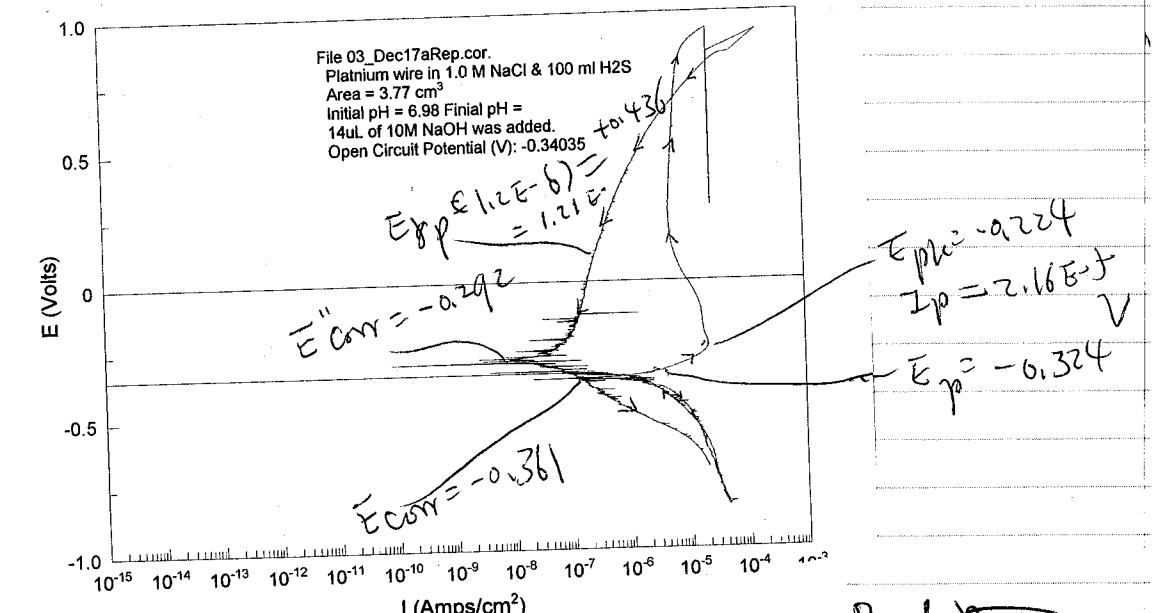
(0 Pre, -0.7 Rf, 0.1667 mV/s)

(0 Pre, -0.7 Rf, 0.1667 mV/s)

Solution 3.73g NaCl in 15ml of H<sub>2</sub>S solution  
initial pH = 3.61 adjusted ↑ by adding 14μL of 10M NaOH

Final pH 6.98

NaCl lot # 034103 H<sub>2</sub>S lot # 923931A



Re JMO  
12/19/03

12/18/03

Started test of 304 wire in 1.0m NaCl  
and 100 ml of H<sub>2</sub>S water.

File name 03 Dec 18a Rep.cor

Open circuit (20 min)

Potentiodynamic (-0.0C, -0.5C, 0.1667 mV/s)

Potentiodynamic (-0.5C, 0.1C, 0.1667 mV/s)

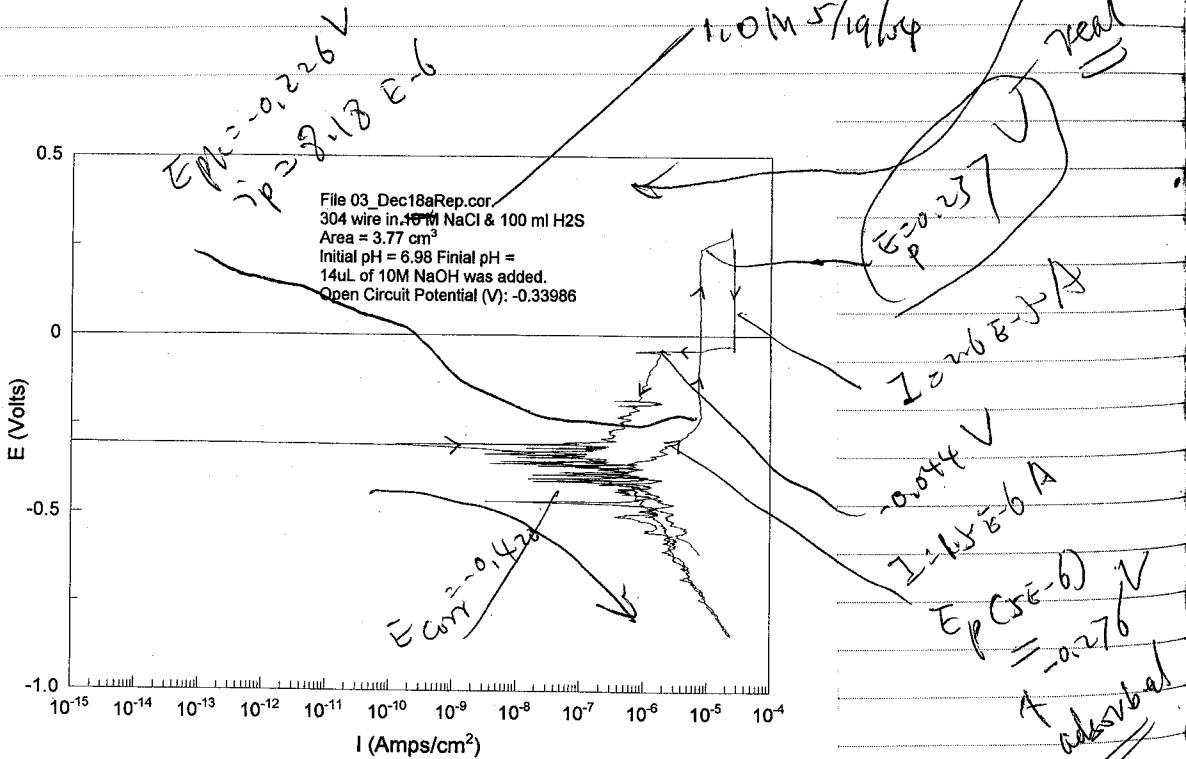
Potentiodynamic (0.0Rho, 1.5Rho, 0.1667 mV/s)

Galvanostatic (0.0001A, 2hr)

Potentiodynamic (0.0Rho, -0.7Rho, 0.1667 mV/s)

Potentiodynamic (0.0Rho, -0.7Rho, 0.1667 mV/s)

Solution see page 209

Rep. 1hr  
12/18/03

1045

12/19/03

Removed ~ 20ml of solution from vessel B for pH test. Added 20ml of appropriate media.

Started test of 304 coupon in 1.0m NaCl and 100ml of H<sub>2</sub>S water

File name 03-Dec19a Rep.cor

Test setup:

Open circuit (20 min)

Potentiodynamic (-0.0C, -0.5C, 0.1667 mV/s)

Potentiodynamic (-0.5C, 0.1C, 0.1667 mV/s)

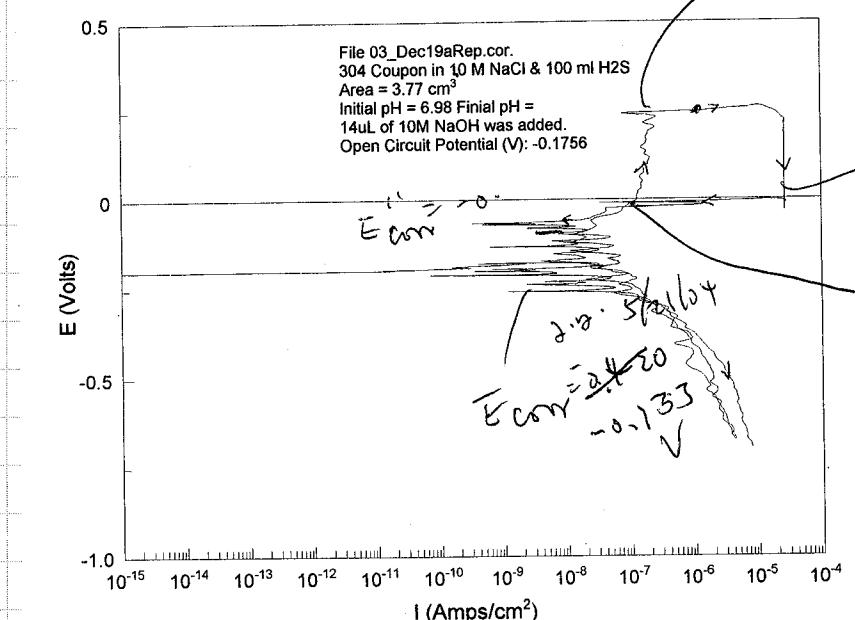
Potentiodynamic (0.0Rho, 1.5Rho, 0.1667 mV/s)

Galvanostatic (0.0001A, 2hr)

Potentiodynamic (0.0Rho, -0.7Rho, 0.1667 mV/s)

Potentiodynamic (0.0Rho, -0.7Rho, 0.1667 mV/s)

Solution see page 209

Rep 5 hr  
12/22/03

**SOUTHWEST RESEARCH INSTITUTE**  
SAMPLE ANALYSIS DATA SHEET

010000

Lab Name: Southwest Research Institute

Lab Code: SwRI

Matrix: Liquid

SRR: 25126

Client: Division 20

Date Received: 10/21/03

Project No.: 20.06002.01.081

TO: 031021-4

Sample ID	Lab System ID	Iron Results (mg/L)
Prep Blank	----	<0.025
Lab Control	----	1.04
True Value	----	1.00
Recovery	----	104%
Vessel A	236555	0.140
Vessel B	236556	1.13
Vessel C	236557	1.29
Duplicate result	236557	1.11
RPD	236557	15.0%
Vessel D	236558	1.17
Spike result	236558	5.98
Spike added	236558	5.00
Recovery	236558	96.2%

Reporting Limit:

0.025 mg/L

Page 1 of 1

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Rogers  
12/20/03

1030	8/22/03 Removed ~ 10ml from cell cell. Added ~ 3ml of 20% HCl to cell B. Added 20ml to cell D. Removed ~ 7ml from B for pH test.
1115	
1120	
1025	8/23/03 Removed ~ 18ml from cell B, replaced with media. adjust pH: add 3ml 20% HCl. RPD.
1000	12/29/03 Removed ~ 12ml from all cell's : replaced with app. media.
1330	1/5/04 Removed ~ 10ml from <del>all</del> cell's : replaced with app. media
1030	1/7/04 Removed ~ 10ml from all cell's , replaced with app. media.

Rogers 1-7-04

12/29/03

0930

Started test on platinum wire in solution 1.0M NaCl + 100ml of H<sub>2</sub>S water. The wire was in test solution for six days prior to test.

Site name 03-Dec29aRep.cor

Open ckt

(20 min)

Potentiodynamic

(-0.0C, -0.5C, 0.1667 mV/s)

Potentiodynamic

(-0.5C, 0.1C, 0.1667 mV/s)

Potentiodynamic

(0.0Pc0, 1.5Rb8, 0.1667 mV/s)

Galvano static

(0.0001A, 2hr)

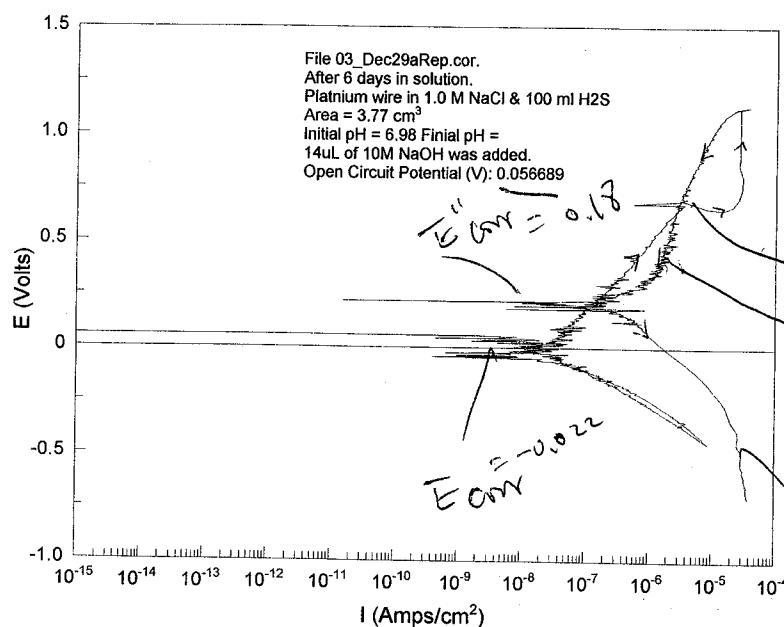
Potentiodynamic

(0.0Prc0, -0.7Rb8, 0.1667 mV/s)

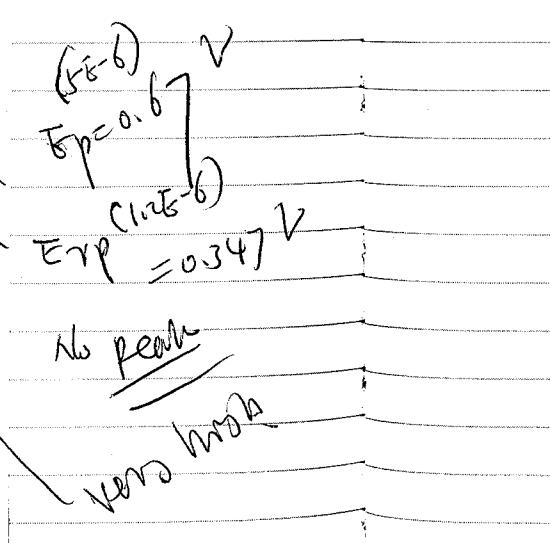
Potentiodynamic

(0.0Prc0, -0.7Rb8, 0.1667 mV/s)

Solution see page 209



No N<sub>2</sub> purge.  
5/20/04



Roger T. Price  
1/7/04

1/5/04

Started test on platinum wire in solution 0.001M NaCl in 0.3M sulfur H<sub>2</sub>O. No N<sub>2</sub> Purge. Site name 04-Jan05aRep.cor

test setup

Open ckt

(20 min)

Potentiodynamic

(-0.0C, -0.5C, 0.1667 mV/s)

Potentiodynamic

(-0.5C, 0.1C, 0.1667 mV/s)

Potentiodynamic

(0.0Prc0, 1.5Rb8, 0.1667 mV/s)

Galvano static

(0.0001A, 2hr)

Potentiodynamic

(0.0Prc0, -0.7Rb8, 0.1667 mV/s)

Potentiodynamic

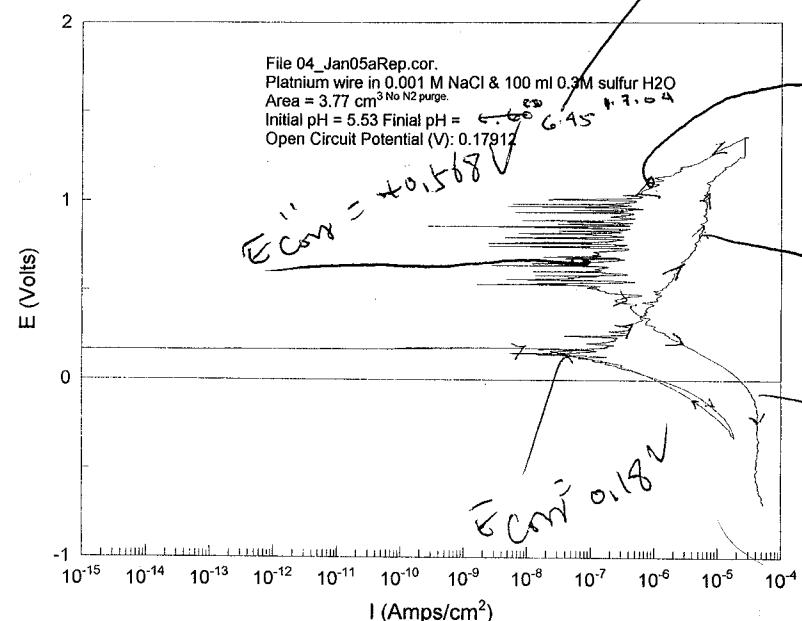
(0.0Prc0, -0.7Rb8, 0.1667 mV/s)

solution 0.006g of NaCl in 100 mL 0.3M sulfur H<sub>2</sub>O

NaCl lot # 035421

sulfur H<sub>2</sub>O lot # R8-1345

5/20/04



E(1.2E-6) = 1.13 V

Ep(0.6) = 0.347

Ep(1.2E-6) = 0.568

Very high.

Roger T. Price  
1/7/04

1/6/04

0800 Started test of 304 wire in solution

0.001 M NaCl and 0.3 M sulfur H<sub>2</sub>ONo N<sub>2</sub> Purge

File name 04-Jan06aRep.cor

test setup

Open ckt

Potentiodynamic

( -0.0C, -0.5C, 0.1667 mV/s )

Potentiodynamic

( -0.5C, 0.1C, 0.1667 mV/s )

Potentiodynamic

( 0.0 Rev, 1.5 Rev, 0.1667 mV/s )

Galvanostatic

( 0.0001 A, 2 hr )

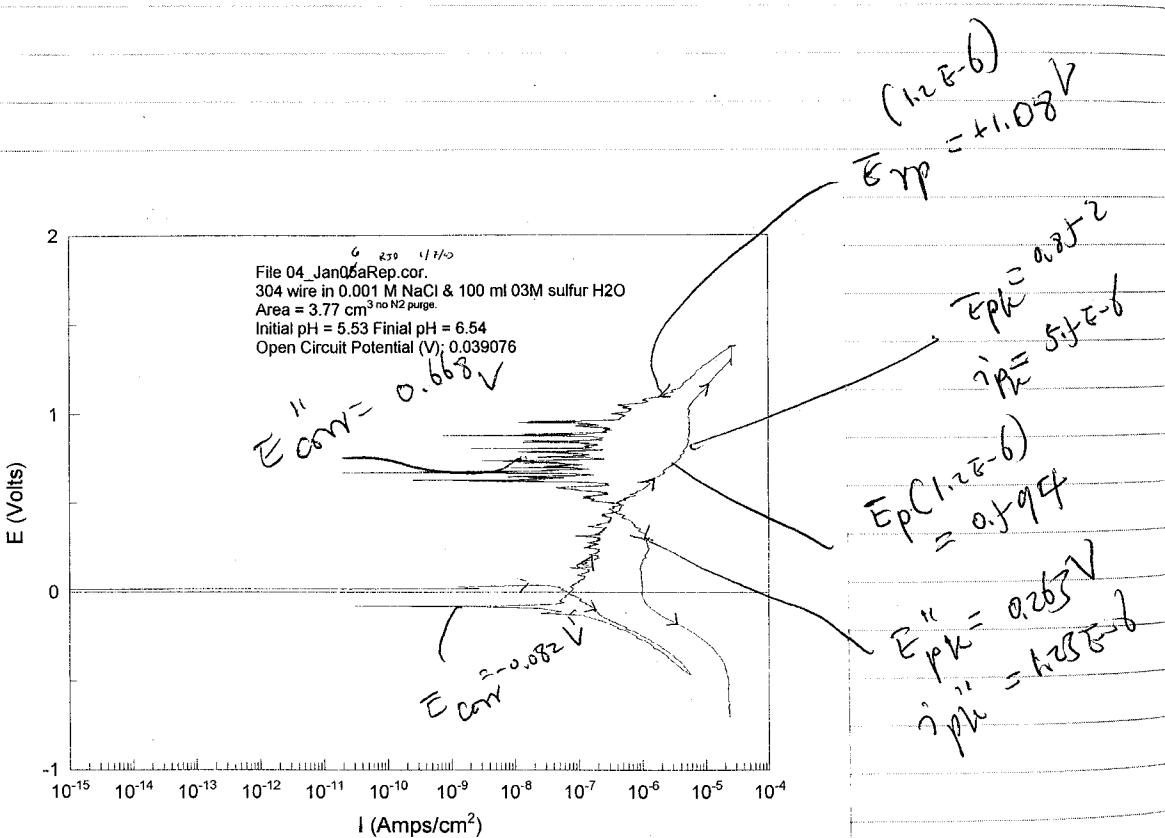
Potentiodynamic

( 0.0 Rev, -0.7 Rev, 0.1667 mV/s )

Potentiodynamic

( 0.0 Rev, -0.7 Rev, 0.1667 mV/s )

see page 215 for solution

Roger J. Price  
1/7/04

November 12, 2003

Started at SWRI at 8:30.

Measured sulfide concentration for all vessels using the Hach procedure and measured the Klett optical density for all vessels.

Vessel	Sulfide Concentration	Klett optical density	MPN**
A	175 mg/L	6	$1.3 \times 10^7$
B	300 mg/L	86	$8.0 \times 10^4$
C	0.5 mg/L	12	$2.0 \times 10^4$
D	0.5 mg/L*	10	MPN not conducted

\* Vessel D has the *Vibrio* slime former and should not produce sulfide. This is probably instrument error. This also suggests that vessel C has no sulfide too.

\*\* MPN read on November 21, 2003.

Ended 11:30

November 17, 2003

Started at SWRI at 12:00

Read MPN from November 7<sup>th</sup>.Vessel A  $10^{-7} = 2, 10^{-8} = 0, 10^{-9} = 0$  yields  $4 \times 10^5$  cells per mL.Vessel B  $10^{-8} = 0, 10^{-9} = 0, 10^{-10} = 0$  yields no useable data. Assumed high cell density and began MPN at too great a dilution.Vessel C  $10^{-3} = 5, 10^{-4} = 5, 10^{-5} = 3$  yields  $9 \times 10^5$  cells per mL.

Vessel	Sulfide Concentration	Klett optical density
A	150 mg/L	5
B	200 mg/L	60
C	0.25 mg/L	12
D	Didn't measure	8
Stock culture*	525	Didn't record

\* reinoculated vessel C – measured [S<sup>-</sup>] of inoculum.

Ended 14:00

Roger J. Price  
1/8/04

**November 21, 2003**

Started at SWRI at 12:00

Discussed experiments with Lietai and Geri.

Measured sulfide and klett.

Vessel	Sulfide Concentration	Klett optical density
A	200 mg/L	11
A*	200 mg/L	15
B	175 mg/L	58
C	1.0 mg/L	42
D	0.25 mg/L	9

\* After Nitrogen bursts to agitate sediment.

Read MPN from November 12<sup>th</sup>

Vessel A  $10^{-6} = 5, 10^{-7} = 4, 10^{-8} = 0$  yields  $1.3 \times 10^7$  cells per mL.

Vessel B  $10^{-6} = 3, 10^{-7} = 0, 10^{-8} = 0$  yields  $8 \times 10^4$  cells per mL

Vessel C  $10^{-5} = 0, 10^{-6} = 1, 10^{-7} = 0$  yields  $2 \times 10^4$  cells per mL.

Ended 14:00.

**November 24, 2003**

Started at SWRI at 11:30.

Visual inspection of vessels suggests that vessel C is becoming turbid. Measured sulfide and klett.

Vessel	Sulfide Concentration	Klett optical density
A	350 mg/L*	24*
B	175 mg/L	50
C	0.75 mg/L	35

\* Note significant increase after the nitrogen burst of November 21<sup>st</sup>.

Ended 14:00

Roger J. Rymer  
11/8/04

**November 26, 2003**

Started at SWRI at 9:00.

Measured sulfide and klett.

Vessel	Sulfide Concentration	Klett optical density
A*	325 mg/L	21
B*	150 mg/L	53
C	0.75 mg/L	30
D	0.25 mg/L	9

\* Nitrogen burst to mix samples A and B before and after sampling.

Ended 11:00

**December 1, 2003**

Using aseptic technique, approximately 7 mL. Sample is harvested from test vessels A, B, C, and D. Determinations are made for sulfide content using the Hach procedure and for turbidity using the Klett spectrophotometer.

Results are shown in the table below:

Test Vessel	Volume Tested	Dilution Factor	Hach Reading	Sulfide Concentration	Klett Reading	MPN Quantitation
A	0.01 ml	2500	0.12 mg/L	300 mg/L	17	N/A
B	0.01 ml	2500	0.04 mg/L	100 mg/L	51	N/A
C	1.0 ml	25	0.02 mg/L	0.50 mg/L	20	N/A
D	N/A	N/A	N/A	N/A	26	N/A

Nitrogen burst administered to vessels A and B following harvest.

**December 3, 2003**

Started at SWRI at 11:30.

Visual inspection of vessels:

A - cream colored and turbid; cream colored sediment at the bottom of the vessel.

B - green colored and turbid - black biofilm appears as threads on coupons and wires.

C - green and clear.

D - pale cream and cloudy - mucous biofilm floating.

Roger J. Rymer  
11/10/04

Measured sulfide and klett.

Vessel	Sulfide Concentration	Klett optical density
A	300 mg/L	14
B	75 mg/L	47
C	0.5 mg/L	12
D*	0.5 mg/L	21

\* Vessel D has the *Vibrio* slime former and should not produce sulfide. This is probably instrument error. This also suggests that vessel C has no sulfide too.

Vessel C - lowered gas tube and gave nitrogen burst for approximately 1 minute.

Ended 13:30

**December 5, 2003**

Started at SWRI at 13:30

Measured sulfide and klett. Started measuring pH as well as of this date.

Vessel	Sulfide Concentration	Klett optical density	pH
A	275 mg/L	17	8.11
B	25 mg/L*	48	9.47
C	0.25 mg/L	10	8.38
D	Didn't measure	18	7.90

\* The reading is too low to be reliable ( $0.01 \times 2500$ ) so will change to 25 fold dilutions for vessel B. The high pH is probably suppressing growth.

Ended 15:30.

**December 8, 2003**

Started at SWRI at 8:00.

Media for the MPN has still not arrived. Measured sulfide, klett and pH.

Vessel	Sulfide Concentration	Klett optical density	pH
A	325 mg/L	12	8.03
B	12.5 mg/L*	45	9.37
C	0.25 mg/L	7	8.29
D	0.5 mg/L	16	8.01

\*  $0.05 \times 250$

Ron J Mac  
1/8/04

NOTE: vessel D should not yield any sulfide. This calls into question the reading for vessel C as well.

Ended 11:00

**December 9, 2003**

Started at SWRI at 7:00.

Prepared for MPN by labeling dilution tubes. Prepared media C with 0.5 M NaCl. Prepared one liter with a pH = 7.43.

Measured sulfide, klett and pH.

Vessel	Sulfide Concentration	Klett optical density	pH	MPN*
A	300 mg/L	14	7.95	$2 \times 10^9$
B	4 mg/L*	45	9.33	NG
C	0.25 mg/L	8	8.25	NG
D	0.25 mg/L	15	8.03	$4.3 \times 10^6$ <sup>®</sup>

\* MPN read on Dec 15, 2003

⊗ 3-tube MPN owing to the lack of media.

Finished at SWRI and took media to UTSA to autoclave. Ended at 14:30.

**December 10, 2003**

Using aseptic technique, approximately 50 ml solution is withdrawn from each of the 4 vessels. Volume is replaced with appropriate sterile media for each vessel. Following a nitrogen burst, another 7 ml fluid is harvested from each vessel for pH determination. Sulfide testing is performed on vessels B, C, and D using a 25ml sample volume. Results are as follows:

VESSEL	SULFIDE	PH
A	N/A	7.88
B	0.33 mg/L	9.33
C	0.09 mg/L	8.26
D	0.17 mg/L	7.54

25ml samples; no dilution factor

Ph standards: 7.0 reads 6.99

10.0 reads 9.94

**December 12, 2003**

Using aseptic technique, approximately 7 ml solution is harvested from each of the four test vessels: A, B, C and D. All samples are tested for sulfide concentration using the

Ron J Mac  
1/8/04

Hach colorimeter. Turbidity is assessed using the Klett spectrophotometer, and pH is also measured.

Results are as follows:

VESSEL	SULFIDE MG/L	KLETT TURBIDITY	PH
A (2500x) (.14) = 350	17	7.87	
B (25x) (.06) = 1.5	26	9.34	
C (25x) (.01) = 0.25	8	8.27	
D (25x) (.01) = 0.25	15	8.08	

#### December 15, 2003

Using aseptic technique, approximately 8 ml solution is harvested from each of the four test vessels: A, B, C and D. All samples are tested for sulfide concentration using the Hach colorimeter. Turbidity is assessed using the Klett spectrophotometer, and pH is also measured. Cultures are set up using serial dilution technique and MPN determinations are performed on samples taken from A, B, C.

Results are as follows:

VESSEL	SULFIDE MG/L	KLETT TURBIDITY	PH	MPN
A (2500x) (.12) = 300	13	7.87		$2 \times 10^9$
B (25x) (.02) = 0.50	22	9.31		NG
C (25x) (.01) = 0.25	9	8.25		NG
D (25x) (.01) = 0.25	19	8.23		$9.3 \times 10^6$

PH meter standards: 7.0 reads 6.97  
10.0 reads 9.98

Cultures are held for 30 days before discard

#### MPN

Results from 12/9/03

VESSEL	10(5)	10(6)	10(7)	10(8)	10(9)	10(10)	10(11)	10(12)	MPN
A	5	5	5	5	0	0	1	0	$2 \times 10^9$
B	N/G								NG
C	N/G								NG
D	4	4	4	2 OF 3	0	0	0	0	$9.3 \times 10^6$ *

\* This was a 3-tube MPN owing to the lack of media.

This is the preliminary read for these cultures taken on 12/9. There is absolutely no growth in ANY tubes taken from vessels B and C.

#### December 19, 2003

Started at SWRI at 9:00.

Discussed test vessels with Lietai and Geri. Vessel B has no indication of cells and no sulfide. Decided it is likely the pH that is causing the problems with growth and determined that we should adjust the pH and reinoculate.

1. We will extract about 20 mL from vessel B and titrate with 20% (v/v) HCl to determine the volume to use to adjust the pH to approximately 8.3.
2. We will add the appropriate amount of HCl to vessel B and measure the pH.
3. Will leave vessel B until Monday, December 22 and measure pH again.
4. Need to inoculate vessel B again. Must grow stock culture in preparation for inoculation. Perhaps use a 100 mL inoculum.
5. Need to prepare glutaraldehyde/cacodylic acid buffer solution for sample preparation for SEM.

Collected 22 mL from vessel B; initial pH = 9.44. 125 µL of 20% (v/v) HCl reduced the pH to 8.34. This is a 176 fold dilution factor ( $22/0.125 = 176$ ). Assuming 750 mL volume in the vessel, then  $750/176 = 4.26$  mL of 20% (v/v) HCl required.

Added 4.3 mL of the HCl and the final pH = 8.0.

Ended 12:00.

#### December 22, 2003

Started at 8:00. Picked up reagents and media at UTSA; arrived at SWRI at 9:00.

- Photocopied section from Rick Klar's thesis describing the SEM preparation protocols.

Rick Klar  
1/8/04

Rick Klar  
1/8/04

- Prepared glutaraldehyde/cacodylic acid solution for SEM sample preparation as follows:
  - Need 0.1 M cacodylic acid in 2% glutaraldehyde
    - Glutaraldehyde is 25% in water – used 40 mL into 500 mL of water
    - Cacodylic acid FW = 138.00, so 0.1 M = 13.8 g/L or 6.9 g/500 mL
    - Adjusted pH to 7.3 with 10M NaOH solution

Prepared specimens for the SEM by using a syringe and a swinney filter. Approximately 1 mL was passed onto a 0.22  $\mu\text{m}$  filter using a syringe. Approximately 3 mL of the glutaraldehyde/cacodylic acid solution was passed through the filter and allowed to sit in contact with the filter for 10 minutes. This was rinsed again with the glutaraldehyde/cacodylic acid solution. The filter was removed from the swinney filter holder and placed in a 10 mL disposable beaker filled with the glutaraldehyde/cacodylic acid solution. This is stored in the refrigerator awaiting further preparation.

Measured the sulfide, klett and pH as follows.

Vessel	Sulfide Concentration	Klett optical density	pH
A	250 mg/L	12	7.94
B	0.75 mg/L*	25	8.99*
C	0.0 mg/L	9	8.25
D	0.0 mg/L	21	8.37

\* Added 4 mL of 20% (v/v) HCl, burst nitrogen 5 minutes to mix. Repeat pH = 5.29.  
Ended 13:00.

#### December 23, 2003

Started at SWRI at 8:30.

Visual inspection of vessels: vessel B appears turbid, but the color is cream rather than greenish. Vessel C appears clear with subtle green tint.

Prepared one liter of media C without NaCl; pH = 7.54

Transferred cells to prepare for reinoculation of vessels B and C. The stock culture had a klett of 58 and a pH of 6.91. Inoculated from 48 hours old media C into each of the following:

- Media B
- Media C without NaCl
- Media C with NaCl

Measured pH of vessel B = 8.47; klett = 25. Added 3 mL of 20% (v/v) HCl. Adjusted pH = 5.39.

Took media to UTSA to autoclave. Ended 13:00.

#### December 29, 2003

Started at 8:30. Went to UTSA to pick up media and cells. The cells were transferred into a 500 mL bottle of media C on December 26<sup>th</sup> in preparation to inoculate vessels B and C. Bottle is darkening but is not yet turbid. Will allow to grow until December 31.

Measured sulfide, klett and pH for all vessels.

Vessel	Sulfide Concentration	Klett optical density	pH
A	250 mg/L	12	7.97
B	0.75 mg/L	23	8.67
C	0.25 mg/L	5	8.28
D	0.25 mg/L	19	8.43

Decided to leave vessel B with current pH.

Ended 11:00.

#### December 31, 2003

Started at SWRI at 9:00.

Inspected bottles inoculated on December 23, 2003:

- Postgate C with 0.5 M NaCl is cloudy and has a strong sulfide odor.
- Postgate B is dark green to black and has a strong sulfide odor.

Inspected bottle inoculated on December 26, 2003:

- Postgate C without NaCl is cloudy with a mild sulfide odor.

Measured sulfide, Klett and pH for four bottles as follows:

bottle	Sulfide Concentration	Klett optical density	pH
PGB from 12/23/03	100 mg/L	325*	6.90
PGC w/NaCl from 12/23/03	87.5 mg/L	46	6.88
PGC w/NaCl from 10/27/03 <sup>†</sup>	12.5 mg/L	28	6.92
PGC no NaCl from 12/26/03	10 mg/L	22	7.09

\* High Klett count owing to the black iron sulfide precipitate.

+ This bottle represents the original inoculum.

Also plated each of the above on media B plates and blood agar plates; incubated anaerobically.

Measured sulfide, Klett and pH for each of the test vessels.

Vessel	Sulfide Concentration	Klett optical density	pH
A	250 mg/L	10	7.88
B	0.75 mg/L	25	8.67
C	0.50 mg/L	7	8.16
D	0.50 mg/L	21	8.36

Ended at 12:30.

Ron Thru  
1/8/04

1/7/04

0800 Started test of platinum wire in solution (0.001 M NaCl : 0.3 M sulfur H<sub>2</sub>O) N<sub>2</sub> purge

File name 04\_Jan07aRep.cor

test setup

Open (k2)

Potentiodynamic

Potentiodynamic

Potentiodynamic

Galvanostatic

Potentiodynamic

Potentiodynamic

(20 min)

(-0.0C, -0.5C, 0.1667 mV/s)

(0.5C, 0.1C, 0.1667 mV/s)

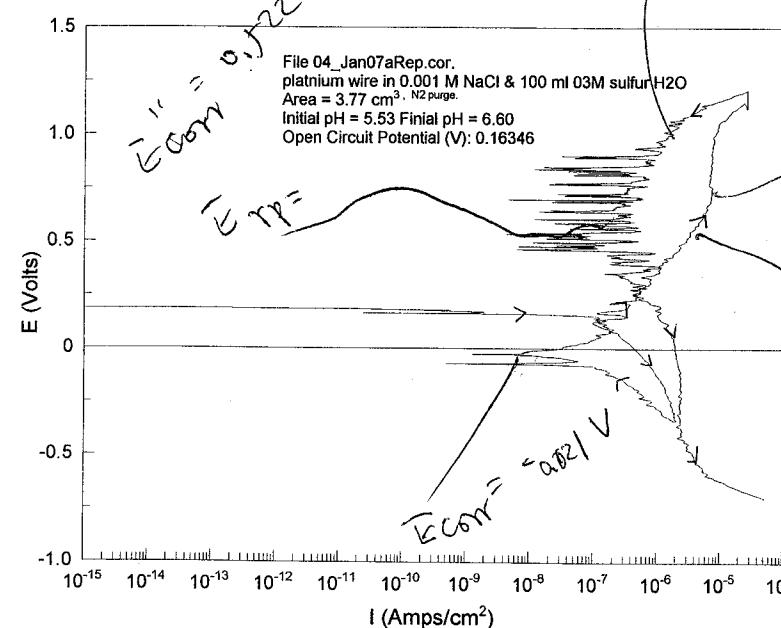
(0.0 Proc, 1.5 Rcf, 0.1667 mV/s)

(0.0001A, 2 hr)

(0.0 Proc, -0.7 Rcf, 0.1667 mV/s)

(0.0 Proc, -0.7 Rcf, 0.1667 mV/s)

See page 215 for solution.



Roger Pyne  
1/15/04

1/8/04

0800 Started test of 304 wire in solution (0.001 M NaCl : 0.3 M sulfur H<sub>2</sub>O) N<sub>2</sub> purge

File name 04\_Jan08aRep.cor

test setup

Open (k2)

Potentiodynamic

Potentiodynamic

Potentiodynamic

Galvanostatic

Potentiodynamic

Potentiodynamic

(20 min)

(-0.0C, -0.5C, 0.1667 mV/s)

(0.5C, 0.1C, 0.1667 mV/s)

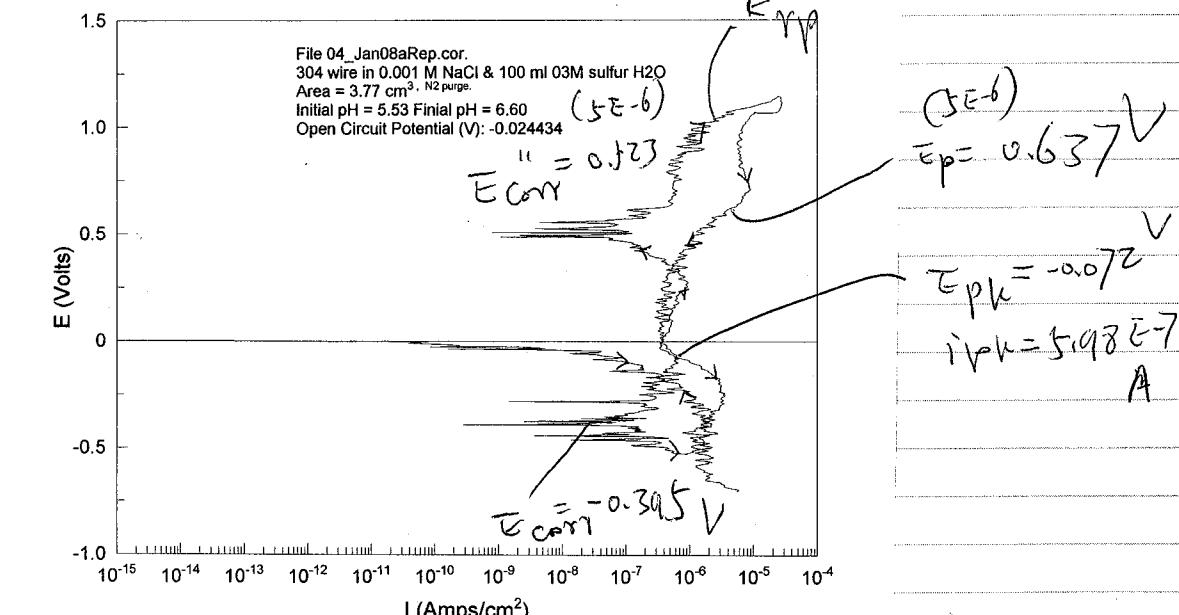
(0.0 Proc, 1.5 Rcf, 0.1667 mV/s)

(0.0001A, 2 hr)

(0.0 Proc, -0.7 Rcf, 0.1667 mV/s)

(0.0 Proc, -0.7 Rcf, 0.1667 mV/s)

See page 215 for solution.



Roger S Pyne  
1/15/04

1/16/04 14:20. 229

$$E_{\text{cont. comp}} - E_{\text{ref}} = -22 \text{ mV}_{\text{SCE}} = -14 \text{ mV} (\text{Flake})$$

$$E_{\text{test-comp}} - E_{\text{ref}} = -330 \text{ mV} (\#704936) = -170 \text{ mV}$$

$$E_{\text{test-comp}} - E_{\text{cont comp}} = -247 \text{ mV} (\text{Flake})$$

1/16/04  
14:27

Vessel A opened.  
Strong H<sub>2</sub>S smell.

Vessel B. Not much smell.

Vessel C. No smell

Vessel D. control coupon.

No smell.

No apparent pitting found.

1/16/04 all vessels closed, no bubbles

resumed, & shortly after they were opened for examination.

1/19/04

Started test of platinum wire in solution  
solution (0.5M NaCl : 0.3M sulfur water)  
No N<sub>2</sub> Purge

File name 04-Jan09aRep.cor

test setup

Open (K)

Potentiodynamic

Potentiodynamic

Potentiodynamic

Galvanostatic

Potentiodynamic

Potentiodynamic

(20min)

(-0.0C, -0.5C, 0.1667 mV/s)

(-0.5C, 0.1C, 0.1667 mV/s)

(0.0P<sub>Ref</sub>, 1.5R<sub>Ref</sub>, 0.1667 mV/s)

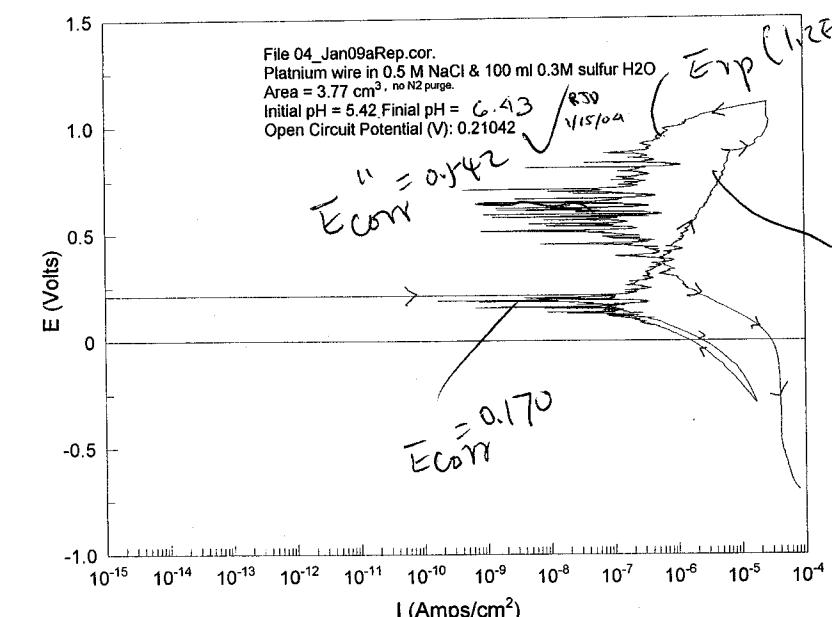
(0.00DIA, 2hr)

(0.0P<sub>Ref</sub>, -0.7R<sub>Ref</sub>, 0.1667 mV/s)

(0.0P<sub>Ref</sub>, -0.7R<sub>Ref</sub>, 0.1667 mV/s)

Solution 2.948g NaCl hot # 635427

100ml 0.3M sulfur hot # 28-1345



Roger Pino  
1/15/04

Roger Pino  
1/15/04

1/12/04

0800

Started test of 304 wire in solution  
Solution (0.5M NaCl in 0.3M sulfur H<sub>2</sub>O)  
No N<sub>2</sub> purge

File name 04-Jan12aRep.cor

test setup:

Open (K)

Potentiodynamic

Potentiodynamic

Potentiodynamic

Galvanostatic

Potentiodynamic

Potentiodynamic

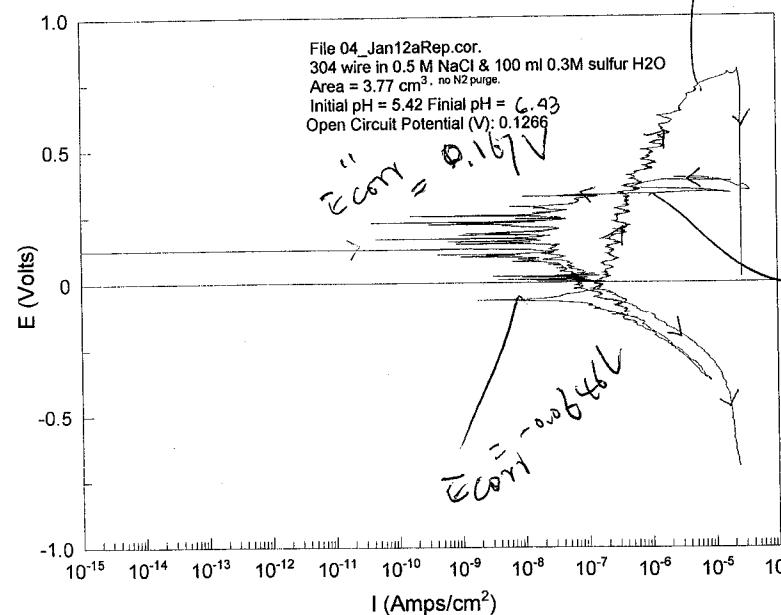
(20 min)

(0.0C, 0.5C, 0.1667 mV/s)

(-0.5C, 0.1C, 0.1667 mV/s)

(0.0P<sub>100</sub>, 1.5R<sub>0.5</sub>, 0.1667 mV/s)

(0.0001A, 2hr)

(0.0P<sub>100</sub>, -0.7R<sub>0.5</sub>, 0.1667 mV/s)(0.0P<sub>100</sub>, 0.7R<sub>0.5</sub>, 0.1667 mV/s)scr pg. 229 Rev 1.15.04  
231 Sur solutionRajan Jha  
1/15/04

1/12/04

0800

Started test of platinum wire in solution  
Solution (0.5M NaCl in 0.3M sulfur water)  
N<sub>2</sub> Purge

File name 04-Jan13aRep.cor

test setup:

Open (K)

Potentiodynamic

Potentiodynamic

Potentiodynamic

Galvanostatic

Potentiodynamic

Potentiodynamic

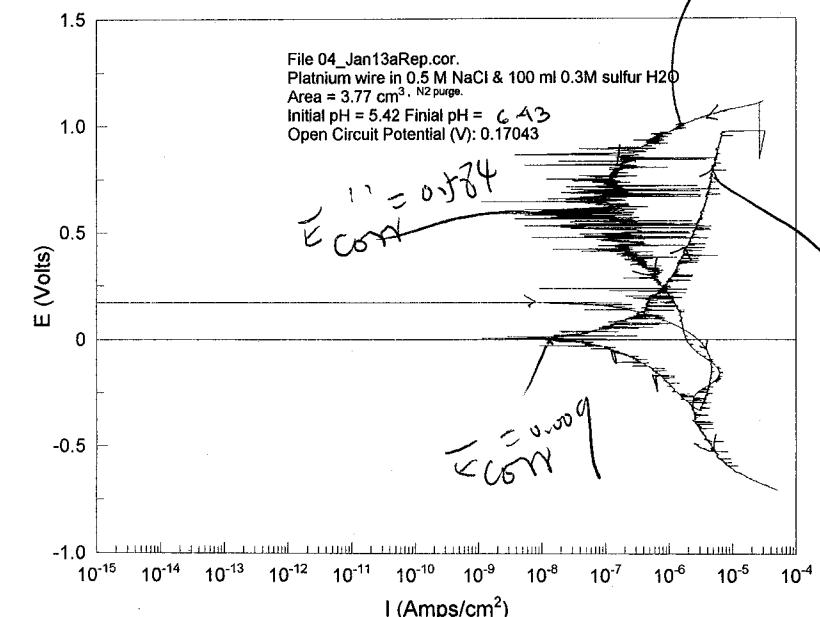
(20 min)

(0.0C, 0.5C, 0.1667 mV/s)

(-0.5C, 0.1C, 0.1667 mV/s)

(0.0P<sub>100</sub>, 1.5R<sub>0.5</sub>, 0.1667 mV/s)

(0.0001A, 2hrs)

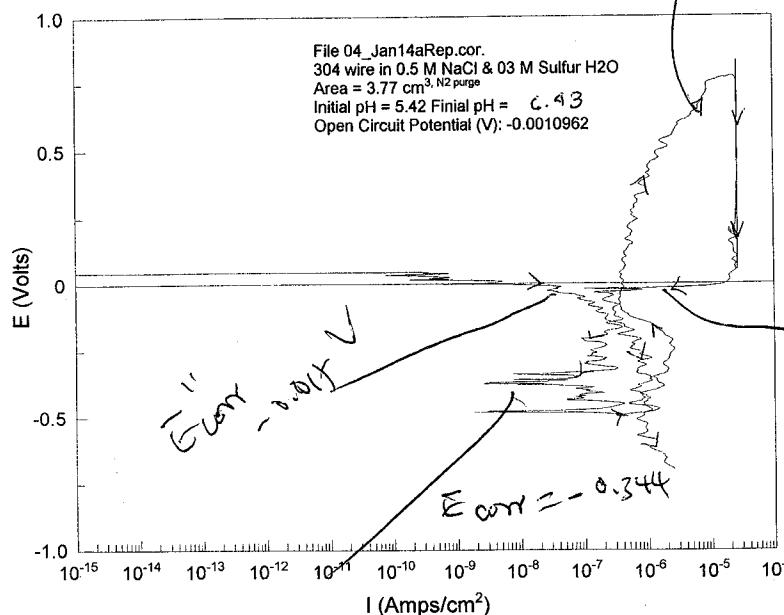
(0.0P<sub>100</sub>, -0.7R<sub>0.5</sub>, 0.1667 mV/s)(0.0P<sub>100</sub>, 0.7R<sub>0.5</sub>, 0.1667 mV/s)scr pg. 229 Rev 1.15.04  
231 Sur solutionRajan Jha  
1/15/04

0900 1/19/08A RJD 1/19/08  
 Started test on all 4 cells.  
 Stand test of 304 A wire in solution  
 Solution (0.5M NaCl & 0.3M sulfur H<sub>2</sub>O)  
 File name 04\_Jan14aRep.cor

## Test setup:

Open (k<sub>2</sub>) (20 min)  
 Potentiodynamic (-0.0C, 0.5C, 0.1667 mV/s)  
 Potentiodynamic (-0.5C, 0.1C, 0.1667 mV/s)  
 Potentiodynamic (0.0P<sub>Ref</sub>, 1.5R<sub>Ref</sub>, 0.1667 mV/s)  
 Galvanostatic (0.0001A, 2hr)  
 Potentiodynamic (0.0P<sub>Ref</sub>, -0.7R<sub>Ref</sub>, 0.1667 mV/s)  
 Potentiodynamic (0.0P<sub>Ref</sub>, -0.7R<sub>Ref</sub>, 0.1667 mV/s)

See page 229 for solution

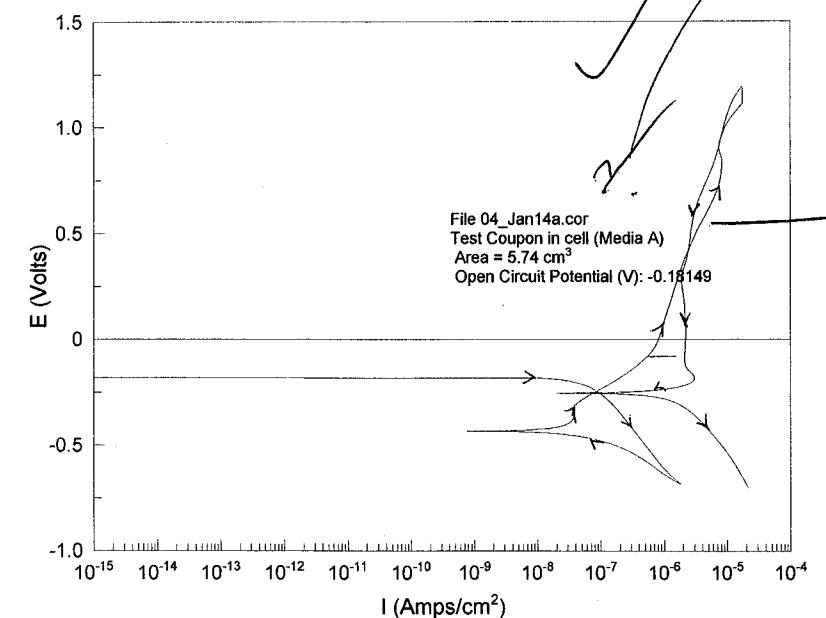


RJH  
 1/15/08

1/19/08A RJD 1/19/08  
 Test of Test coupon in cell A (Media A)  
 file name 04\_Jan14a.cor  
 Test set up?

Open (k<sub>2</sub>) (20 min)  
 Potentiodynamic (-0.0C, 0.5C, 0.1667 mV/s)  
 Potentiodynamic (-0.5C, 0.1C, 0.1667 mV/s)  
 Potentiodynamic (-0.0P<sub>Ref</sub>, 1.5R<sub>Ref</sub>, 0.1667 mV/s)  
 Galvanostatic (0.0001A, 2hr)  
 Potentiodynamic (0.0P<sub>Ref</sub>, -0.7R<sub>Ref</sub>, 0.1667 mV/s)  
 Potentiodynamic (0.0P<sub>Ref</sub>, -0.7R<sub>Ref</sub>, 0.1667 mV/s)

J. Yud  
 J. Yud  
 same name as  
 the one in  
 page 230



RJH  
 1/15/08

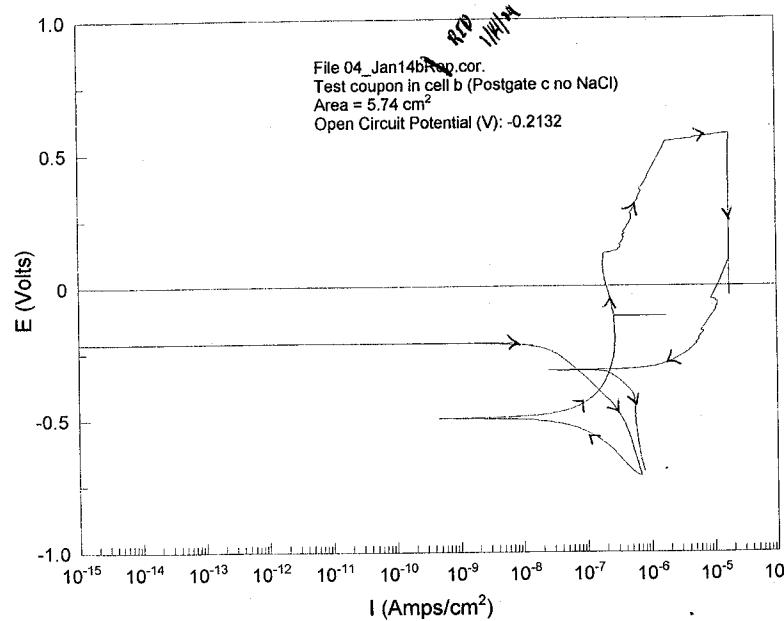
1/14/09A RJD 1/14/09

Test of test coupon in cell B (Postgate c, no NaCl)

File name 04-Jan14b.cor

Test setup °

open circuit (20 min)  
 Potentiodynamic (-0.5C, 0.1C, 0.1667 mV/s)  
 Potentiodynamic (-0.5C, 0.1C, 0.1667 mV/s)  
 Potentiodynamic (0.0Preo, 1.5R&f, 0.1667 mV/s)  
 Galvanostatic (0.0001A, 2hr)  
 Potentiodynamic (0.0Preo, -0.7R&f, 0.1667 mV/s)  
 Potentiodynamic (0.0Preo, -0.7R&f, 0.1667 mV/s)



RJD Rep 1/15/09

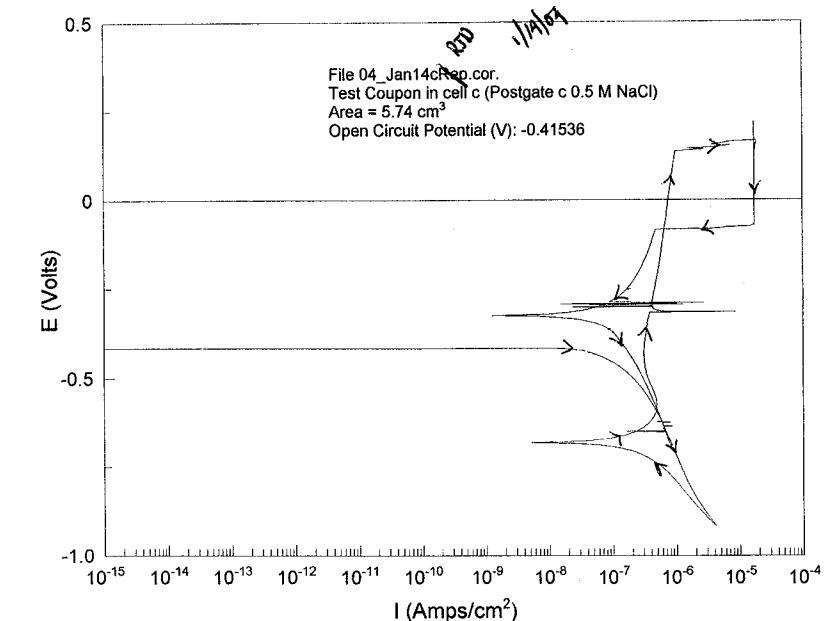
1/14/09A RJD 1/14/09

Test of test coupon in cell c (Postgate c, 0.5M NaCl)

File name 04-Jan14c.cor

Test setup °

open circuit (20 min)  
 Potentiodynamic (-0.5C, 0.1C, 0.1667 mV/s)  
 Potentiodynamic (-0.5C, 0.1C, 0.1667 mV/s)  
 Potentiodynamic (0.0Preo, 1.5R&f, 0.1667 mV/s)  
 Galvanostatic (0.0001A, 2hr)  
 Potentiodynamic (0.0Preo, -0.7R&f, 0.1667 mV/s)  
 Potentiodynamic (0.0Preo, -0.7R&f, 0.1667 mV/s)



RJD Rep 1/15/09

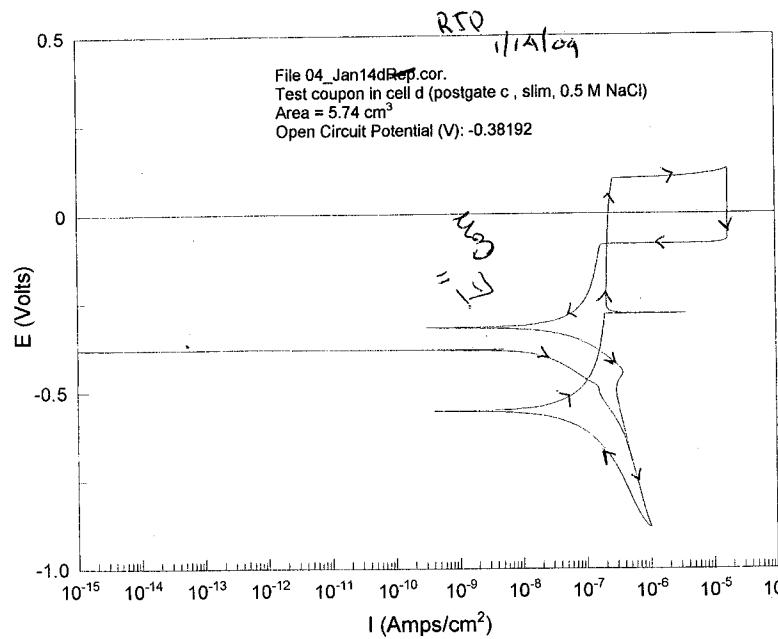
1/14/04

Test of test coupon in cell D (postgate c, slim, NaCl)

File name 04-Jan14d.cor

Test setup:

Open circuit  
 Potentiodynamic (20 min)  
 $(0.0C, -0.5C, 0.1667 \text{ mV/s})$   
 Potentiodynamic  
 $(0.0C, 0.1C, 0.1667 \text{ mV/s})$   
 Potentiodynamic  
 $(0.0Pro, 1.5C, 0.1667 \text{ mV/s})$   
 Galvanostatic  
 $(0.0001A, 2\text{hr})$   
 Potentiodynamic  
 $(0.0Pro, 0.7Ref, 0.1667 \text{ mV/s})$   
 Potentiodynamic



Roughness  
1/15/04

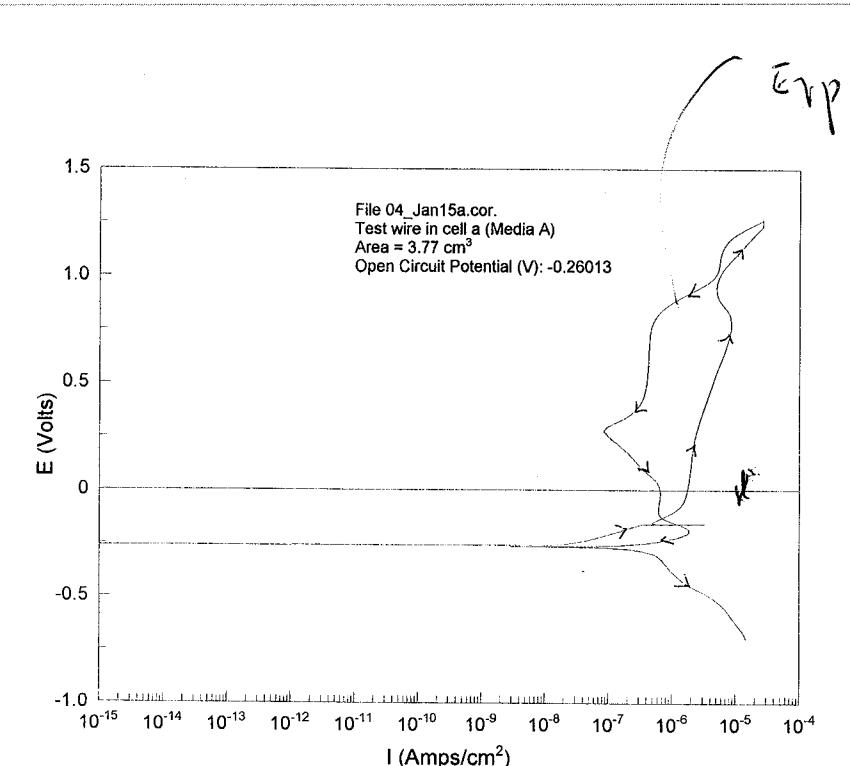
1/15/04

Test of wire in cell A (media a)

File name 04-Jan15a.cor

Test setup:

Open circuit (20 min)  
 Potentiodynamic  
 $(0.0C, 0.1C, 0.1667 \text{ mV/s})$   
 Potentiodynamic  
 $(0Pro, 1.5Ref, 0.1667 \text{ mV/s})$   
 Galvanostatic  
 $(0.0001A, 2\text{hr})$   
 Potentiodynamic  
 $(0Pro, -0.7Ref, 0.1667 \text{ mV/s})$   
 Potentiodynamic  
 $(0Pro, -0.7Ref, 0.1667 \text{ mV/s})$



Roughness  
1/16/04

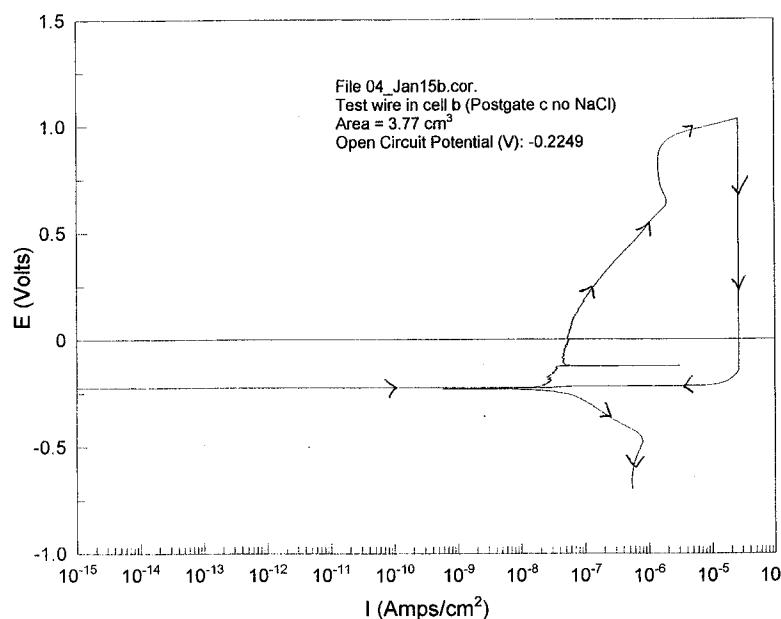
1/15/04

Test of test wire in cell b (Postgate c, no Cl)

filename: 04\_Jan15b.cor

## Test setup

Open Ckt	(20 min)
Potentiodynamic	(0.0C, 0.1C, 0.1667 mV/s)
Potentiodynamic	(0.0Pre, 1.5Rcf, 0.1667 mV/s)
Galvanostatic	(0.0001 A, 2hr)
Potentiodynamic	(0 Pre0, -0.7Rcf, 0.1667 mV/s)
Potentiodynamic	(0 Pre0, -0.7Rcf, 0.1667 mV/s)

Rajesh  
1/24/04

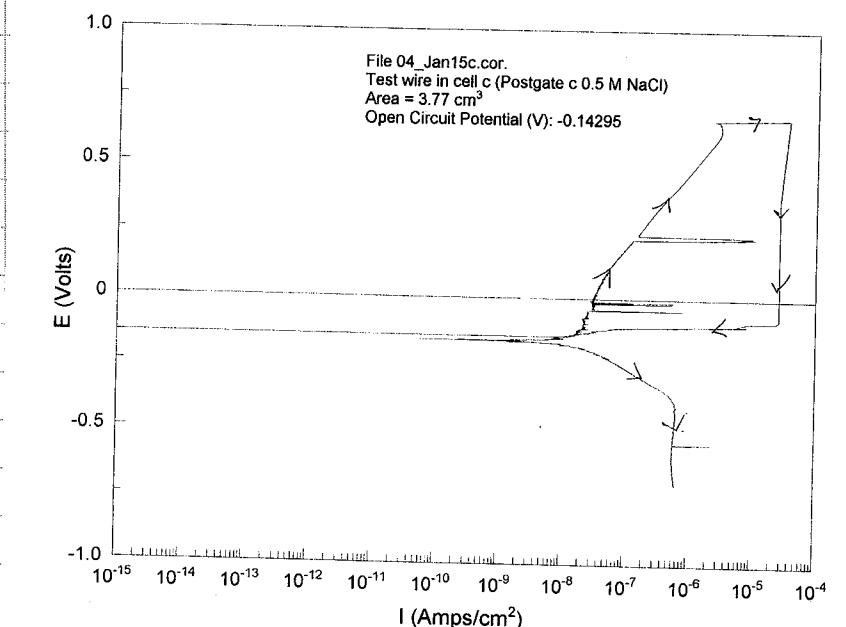
1/15/04

Test of test wire in cell c (Postgate c, 0.5M NaCl)

file name: 04\_Jan15c.cor

## Test set-up

Open Ckt	(20 min)
Potentiodynamic	(0.0C, 0.1C, 0.1667 mV/s)
Potentiodynamic	(0.0Pre, 1.5Rcf, 0.1667 mV/s)
Galvanostatic	R <sub>TG</sub> <sup>00</sup> (0.001 A, 2hr)
Potentiodynamic	(0 Pre0, -0.7Rcf, 0.1667 mV/s)
Potentiodynamic	(0 Pre0, -0.7Rcf, 0.1667 mV/s)

Rajesh  
1/24/04

1/15/04

Test of test wire in cell d (postgate c, slim, NaCl)

file name: 04\_Jan15d.cor

Test setup:

Open circuit

(20 min)

(0.0C, 0.1C, 0.1667 mV/s)

Potentiodynamic

(0.0Pre, 1.5R<sub>L</sub>, 0.1667 mV/s)

Galvanostatic

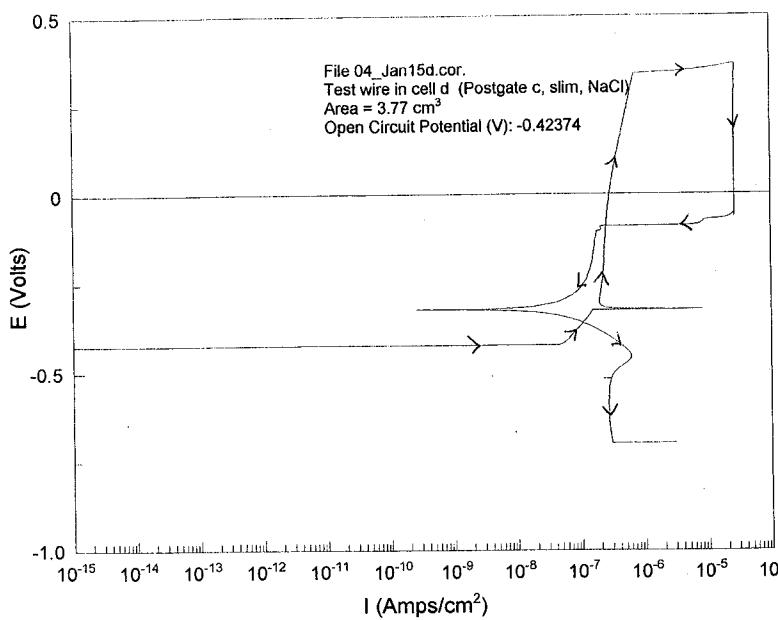
(0.0001A, 2hr)

Potentiodynamic

(0.0Pre, -0.7R<sub>L</sub>, 0.1667 mV/s)

Potentiodynamic

(0.0Pre, -0.7R<sub>L</sub>, 0.1667 mV/s)



Boyle  
1/26/04

1/16/04

1415 Sampled from each cell.

Started test on all cells (test coupon)

Test of Platinum wire in 1.0m NaCl : 0.3m sulfur H<sub>2</sub>O

file name: 04\_Jan16aRep.cor

Test setup:

Open circuit

(20 min)

(0.0C, 0.1C, 0.1667 mV/s)

Potentiodynamic

(0.0Pre, 1.5R<sub>L</sub>, 0.1667 mV/s)

Galvanostatic

(0.0001A, 2hr)

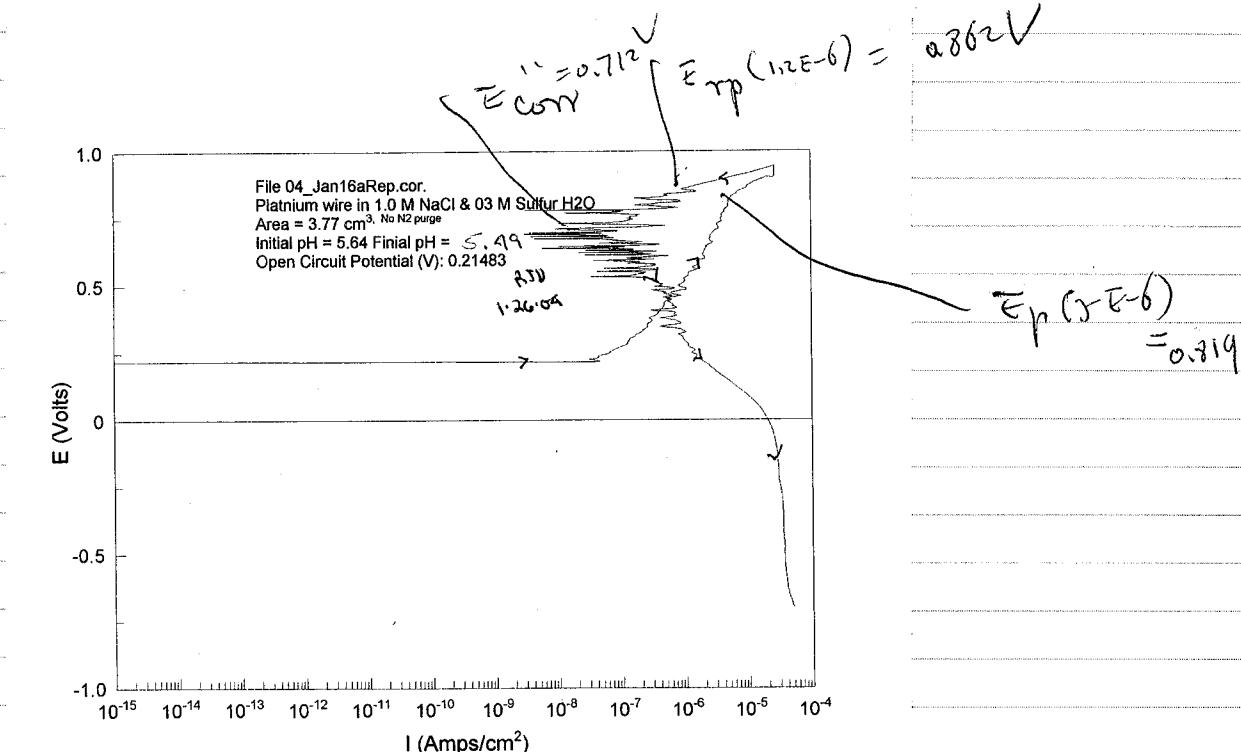
Potentiodynamic

(0.0Pre, -0.7R<sub>L</sub>, 0.1667 mV/s)

Potentiodynamic

(0.0Pre, -0.7R<sub>L</sub>, 0.1667 mV/s)

Solution 5.844 g NaCl  
100 mL 0.3 m sulfur H<sub>2</sub>O hot # 035-421  
hot # 28-1345



Miret  
1/26/04

1/26/04

1530

Removed cells A, B, C from scrvice.  
Removed cells (3) from 1st test from  
scrvice. Removed test specimens from  
cell D.

1/2

RJM  
1/26/04RJM  
1/26/04

1/19/04

Test of test coupon in cell A (Media A)

File name 04-Jan16a.cor

Test set up:

open ckt (20 min)

Potentiodynamic (-0.5C, -0.5L, 0.1667 mV/s)

Potentiodynamic (0.0P<sub>NaCl</sub>, 0.5C, 0.1667 mV/s)

Galvanostatic (0.0001A, 2hr)

Potentiodynamic (0.0P<sub>NaCl</sub>, -0.7Rcf, 0.1667 mV/s)Potentiodynamic (0.0P<sub>NaCl</sub>, -0.7Rcf, 0.1667 mV/s)

1/19/04

Test of test coupon in cell B (Postgate, No Cl<sup>-</sup>)

File name 04-Jan16b.cor

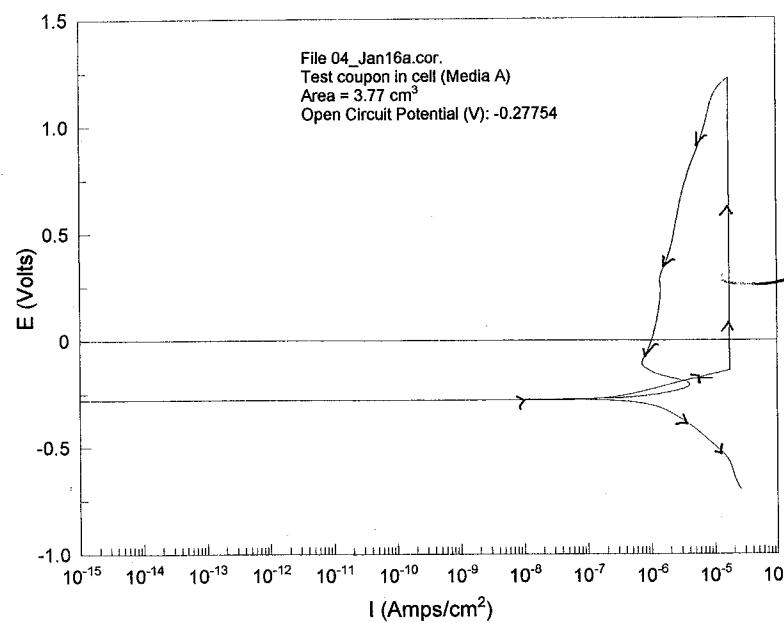
Test set up:

open ckt (20 min)

Potentiodynamic (-0.5C, -0.5L, 0.1667 mV/s)

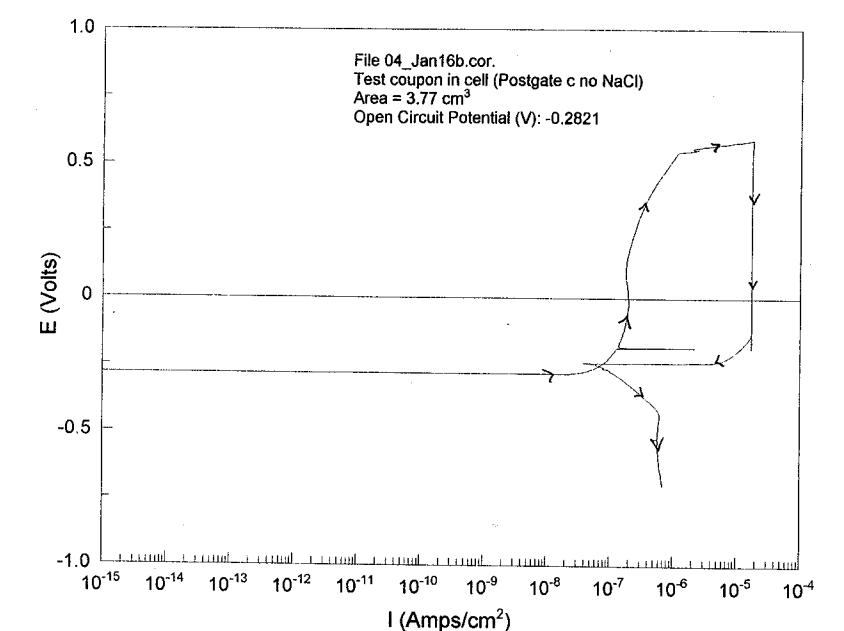
Potentiodynamic (0.0P<sub>NaCl</sub>, 0.5C, 0.1667 mV/s)

Galvanostatic (0.0001A, 2hr)

Potentiodynamic (0.0P<sub>NaCl</sub>, -0.7Rcf, 0.1667 mV/s)Potentiodynamic (0.0P<sub>NaCl</sub>, -0.7Rcf, 0.1667 mV/s)

due to  
intermediate  
product

Ryan  
1/26/04



Ryan  
1/26/04

1/19/04

Test of test coupon in cell C (Postgate c 0.5 M NaCl)

File name 04\_Jan16c.cor

Test setup:

Open (K1)

(20 min)

Potentiodynamic

(0.0L, -0.5L, 0.1667 mV/s)

Potentiodynamic

RTO (0.0<sup>RTD</sup>, 0.5<sup>RTD</sup>, 0.1667 mV/s)

Galvanostatic

(0.0001A, 2hr)

Potentiodynamic

(0.0<sup>RTD</sup>, -0.7<sup>RTD</sup>, 0.1667 mV/s)

Potentiodynamic

(0.0<sup>RTD</sup>, -0.7<sup>RTD</sup>, 0.1667 mV/s)

1/19/04

Test of test coupon in cell D (Postgate c, slim, NaCl)

File name 04\_Jan16d.cor

Test setup:

Open (K1)

(20 min)

Potentiodynamic

(0.0L, -0.5L, 0.1667 mV/s)

Potentiodynamic

RTO (0.0<sup>RTD</sup>, 0.5<sup>RTD</sup>, 0.1667 mV/s)

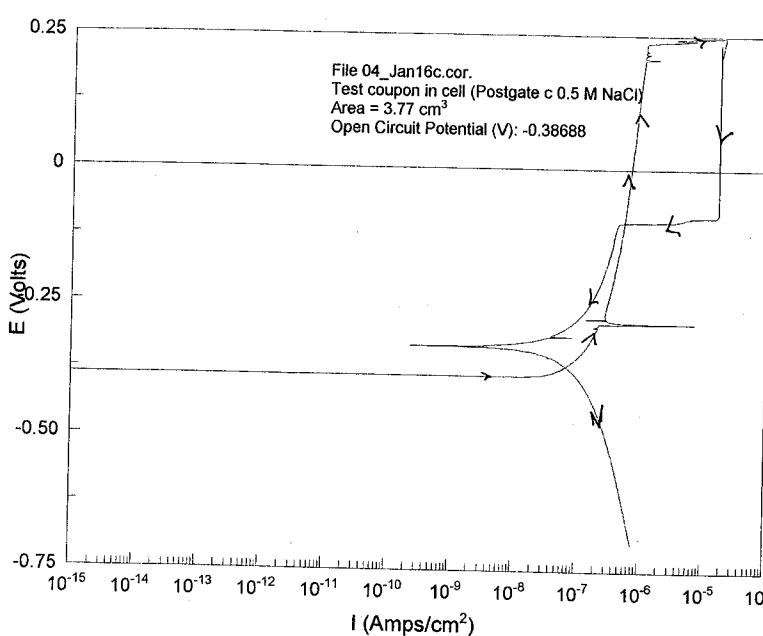
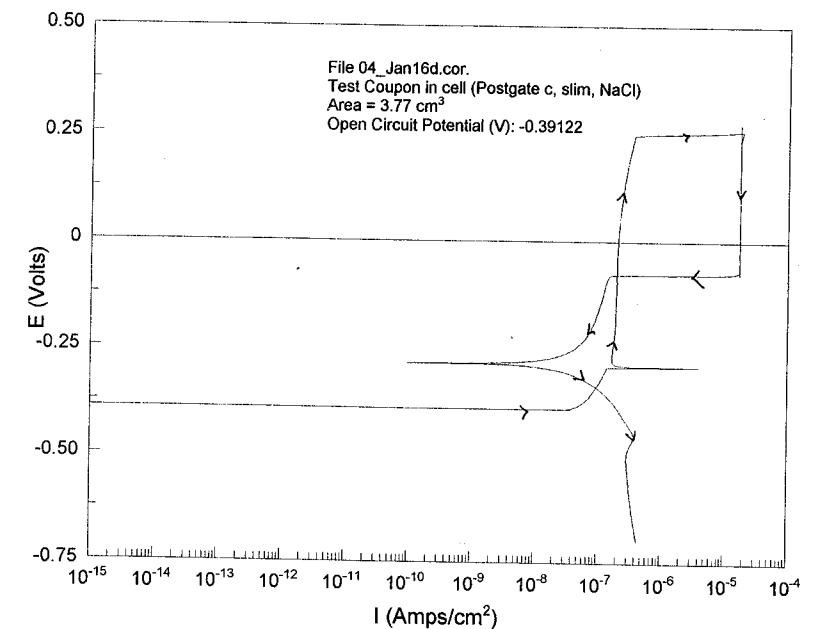
Galvanostatic

(0.0001A, 2hr)

Potentiodynamic

(0.0<sup>RTD</sup>, -0.7<sup>RTD</sup>, 0.1667 mV/s)

Potentiodynamic

(0.0<sup>RTD</sup>, -0.7<sup>RTD</sup>, 0.1667 mV/s)RogJPhu  
1/26/04RogJPhu  
1/26/04

1/19/04

Test of 304 wire in solution  
Solution 1.0 M NaCl : 0.3 M sulfur water

test file : 04-Jan19aRep.cor

Test of wire in all A cells.

test files : 04-Jan19a(b,c,d).cor

Test set-up's

Open CKT

Potentiodynamic

Potentiodynamic

Galvanostatic

Potentiodynamic

Potentiodynamic

see page 241 for solution

(20min)

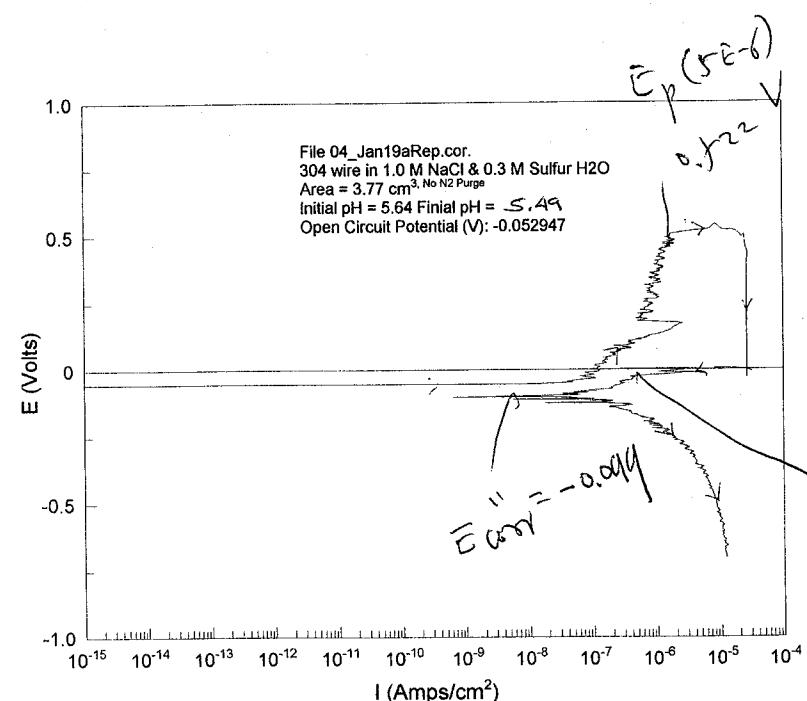
(0.0L, -0.5C, 0.1667 mV/s)

(0.0L0, 1.5R<sub>Ref</sub>, 0.1667 mV/s)

(0.0001A, 2hr)

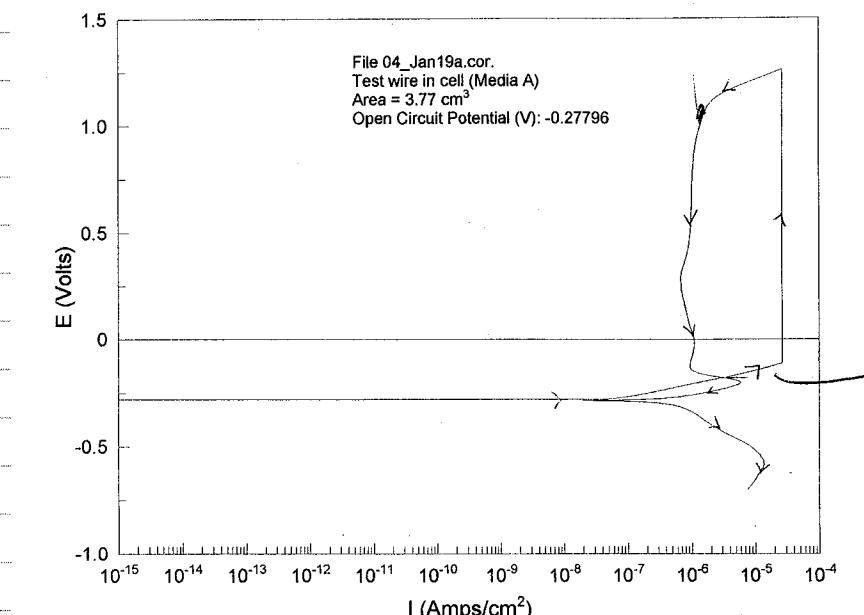
(0.0P<sub>Ref</sub>, -0.7R<sub>Ref</sub>, 0.1667 mV/s)

(0.0P<sub>Ref</sub>, -0.7R<sub>Ref</sub>, 0.1667 mV/s)

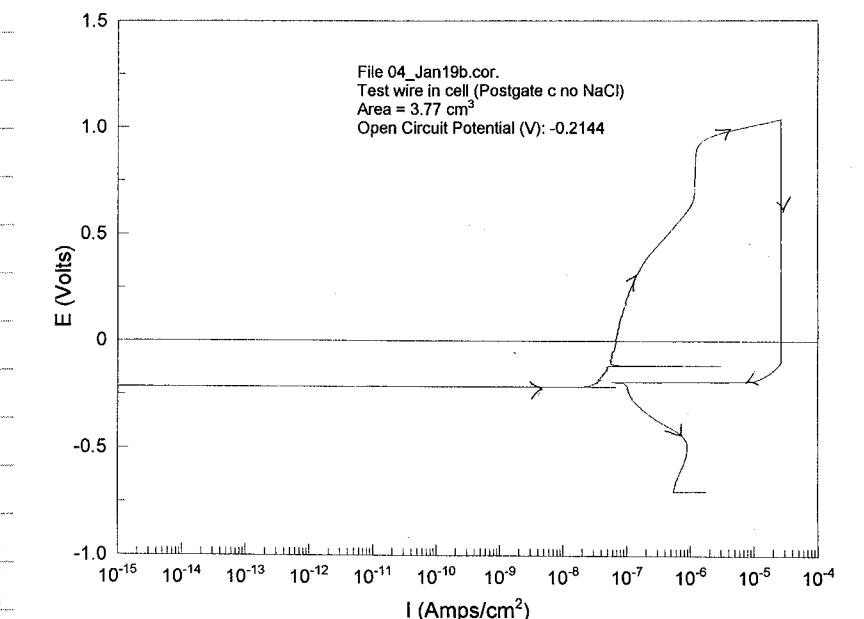


Rgms  
1/26/04

1/19/04

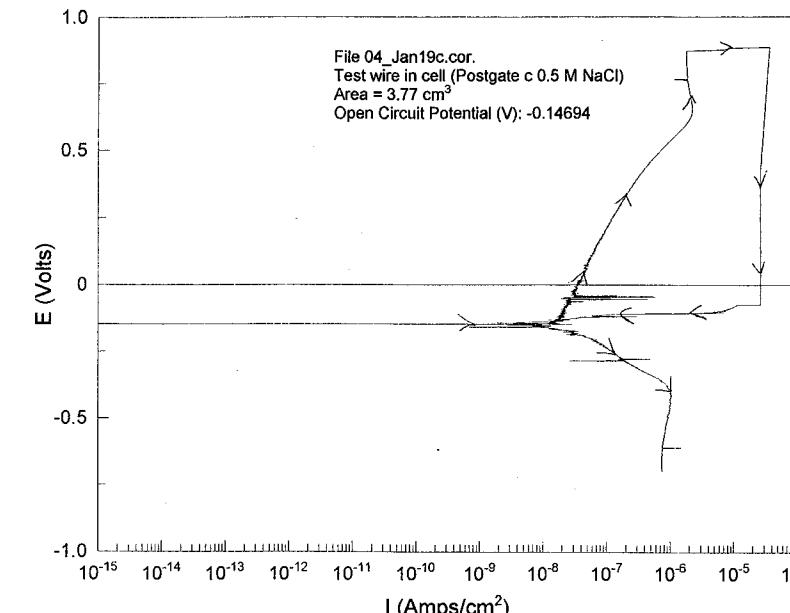


due to  
adsorbed  
species.

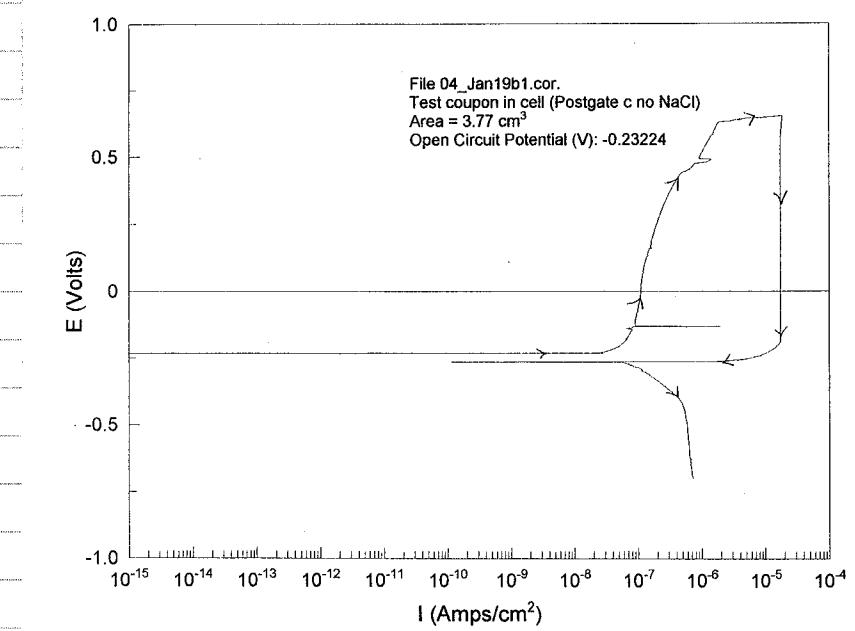
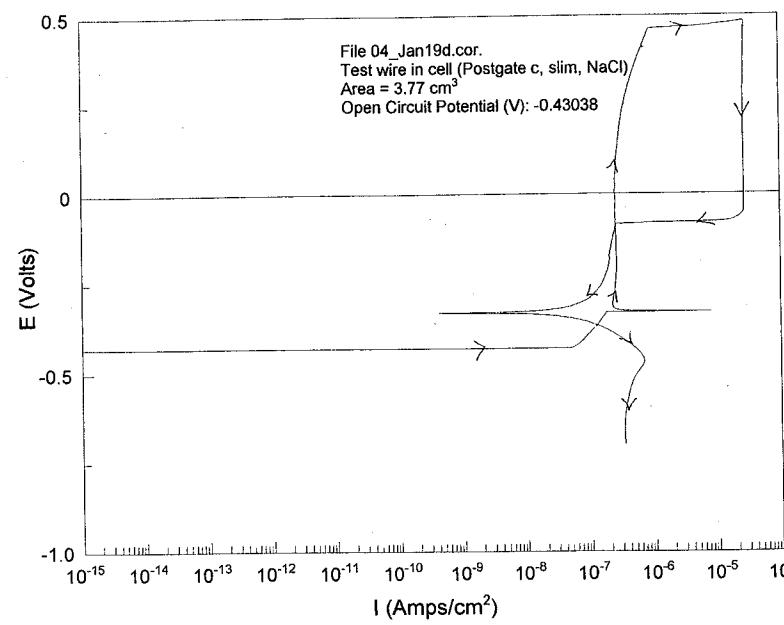
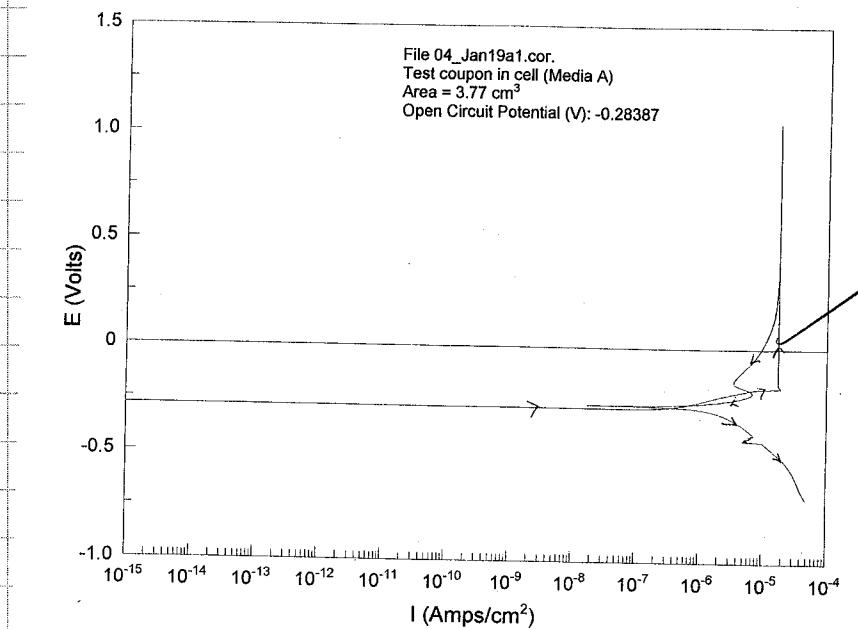


Rgms  
1/26/04

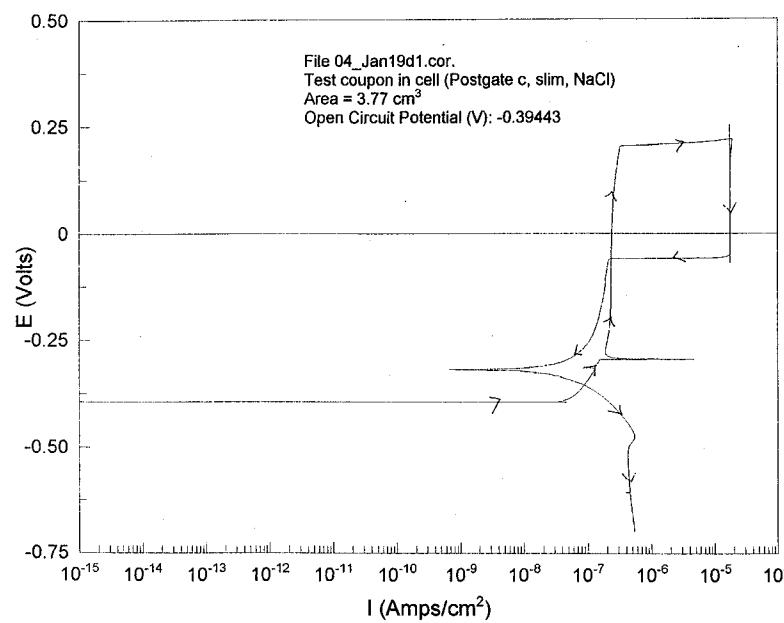
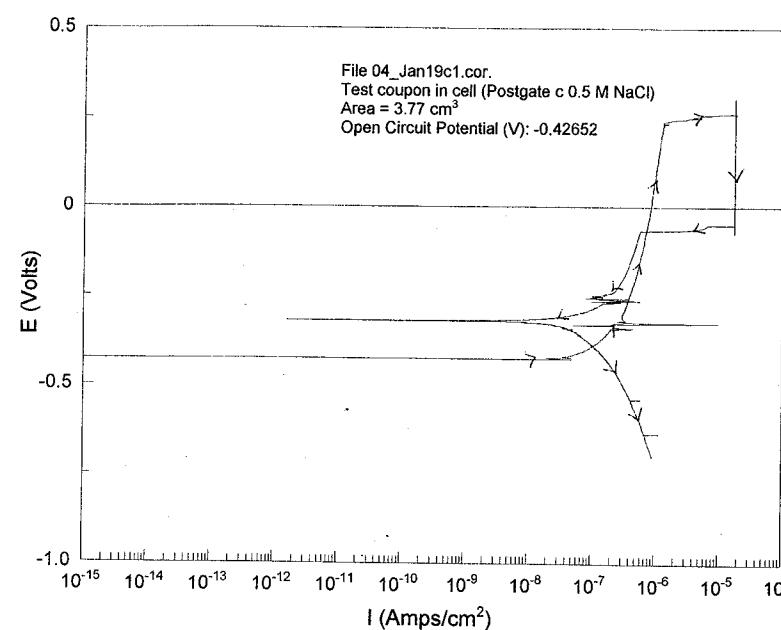
1/19/04



1/19/04

Reg. No.  
1/26/04Reg. No.  
1/26/04

1/19/04

Rg/mm  
1/21/04

1/22/04

Test of 304 wire in Solution  
Solution 1.0 NaCl & 0.3 M sulfur H<sub>2</sub>O

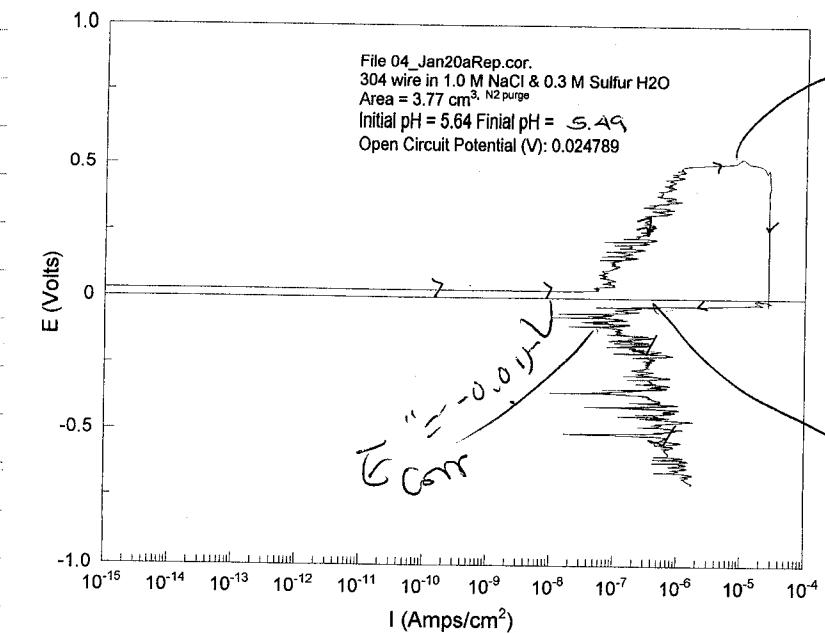
Test file: 04\_Jan20aRep.cor

Test setup:

Open (kt)  
Potentiodynamic  
Potentiodynamic  
Galvanostatic  
Potentiodynamic  
Potentiodynamic

(20 min)  
(0.0 C, 0.1C, 0.1667 mV/s)  
(0.0 Proc, 1.5 Ref, 0.1667 mV/s)  
(0.0001 A, 2 hr)  
(0.0 Proc, -0.7 Ref, 0.1667 mV/s)  
(0.0 Proc, 0.7 Ref, 0.1667 mV/s)

See page 241 for solution

Rg/mm  
1/24/04

1/23/04

Test of platinum wire in solution  
Solution 1.0 NaCl + 0.3 M Sulfur H<sub>2</sub>O

Test Site 04-Jan21a.Rep.cor

Test Setup:

Open (±2) (20min)

Potentiodynamic

(0.0C, 0.1C, 0.1667 mV/s)

Potentiodynamic

(0.0 Proc, 1.5 Ref, 0.1667 mV/s)

Galvanostatic

(0.0001 A, 2hr)

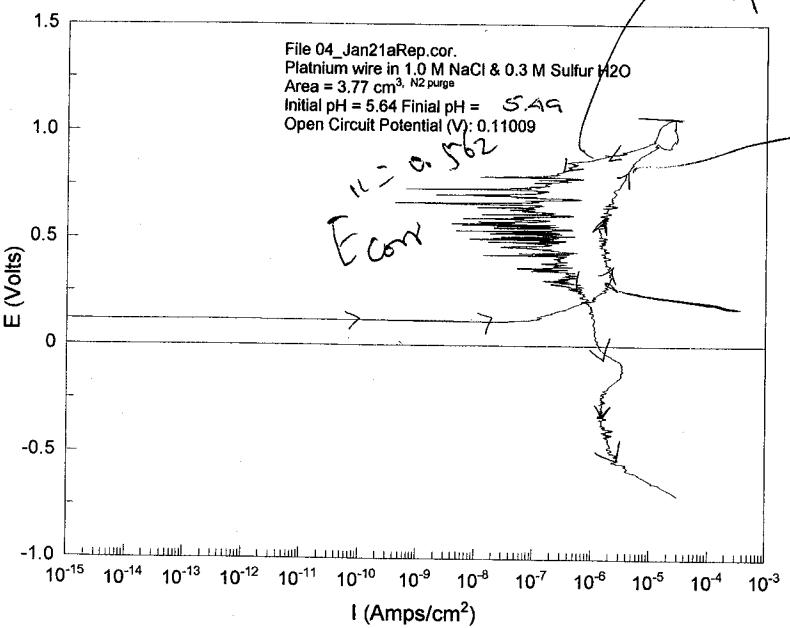
Potentiodynamic

(0.0 Proc, 0.7 Ref, 0.1667 mV/s)

Potentiodynamic

(0.0 Proc, 0.7 Ref, 0.1667 mV/s)

See page 241 for solution



$E_{corr} = -0.562$

$E_{pt} = 0.311$

$i_{pc} = 2.198 \text{ A}$

Riggs  
1/20/04

SAMPLE LIST/CHAIN OF CUSTODY									
Southwest Research Institute Chemistry and Chemical Engineering Division 8220 Culebra Road San Antonio, Texas 78238-5166									
Client	Shipper Name/Address	Client Purchase Order/Other ID	Site/Zone ID	Analyses Requested	Sample ID	Matrix Type	Sample Collection Date (mmddyy)	Sample Collection Time	# of Containers
A	✓	012004	L	D	✓	HC P Analysis	..	..	1
B	✓	..	L	D	✓	..	..	..	1
C	✓	..	L	D	✓	..	..	..	1
D	✓	..	L	D	✓	..	..	..	1
A Filtered	✓	..	L	D	✓	..	..	..	1
B Filtered	✓	..	L	D	✓	..	..	..	1
A Filtered	✓	..	L	D	✓	..	..	..	1
D Filtered	✓	..	L	D	✓	..	..	..	1
DUM	✓	..	L	D	✓	..	..	..	1
DUM 11/16/04	✓	..	L	D	✓	..	..	..	1
Relinquished by (Print/Signature)									
Date Time SWR Project# 20040104									
Received by (Print/Signature)									
Date Time Received by SWR Lab: (Signature)									
Relinquished by (Print/Signature)									
Date Time									
Received by (Print/Signature)									
Date Time									
Relinquished by (Print/Signature)									
Comments: Therm #: 027 Temp: 27.0°C									

Riggs  
1/20/04

1/16/04. All cells opened, coupons take out. 1/16/04

0930

1/26/04  
Test coupon A may have some pits on it. 1/16/04  
RSD 1/26/04

No evidence of pitting on A test wire

Test coupon B has  $\approx$  5 pits  
No evidence of pitting on wire

10/10/03  
J.Y.  
6/9/04

→ 1/16/04  
98 days.

Test coupon C has  $\approx$  3 pits ✓  
wire has 1 pit's near liquid/air interface

Test coupon D has  $\approx$  1 pit  
wire has no pits

These specimens were polarized during the test. whether pitted or not are not important. Pictures not collected in this note book. However they were saved in 4/20/04 folder. "Post-test-surfaces" saved

Test wire B, there is one pit

near liquid/air interface. J.Y. 1/29/04

Test wire D has one pit. J.Y.

1/29/04

Microstop paint on all coupon surfaces

peeled off easily!! electrical leads also came off easily

— continued Page 264

## SOUTHWEST RESEARCH INSTITUTE SAMPLE ANALYSIS DATA SHEET

Lab Name: Southwest Research Institute

Client: Division 20

Lab Code: SwRI

Date Received: 01/09/04

Matrix: Liquid

Project No.: 20.06002.01.081

SRR: 25389

Sample ID	Lab System ID	Iron Results (mg/L)
Prep Blank - A19E2	---	<0.025
Lab Control - A19E2	---	0.963
True Value	---	1.00
Recovery	---	96.3%
Prep Blank - A21E2	---	<0.025
Lab Control - A21E2	---	0.983
True Value	---	1.00
Recovery	---	98.3%
Vessel A	239636	<0.250
Vessel B	239637	0.874
Spike result	239637	20.9
Spike added	239637	20.0
Recovery	239637	100%
Vessel C	239638	1.56
Vessel D	239639	0.857

Reporting Limit:

0.025 mg/L

Page 1 of 1

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Reg S Pgs  
04/07/04

**SOUTHWEST RESEARCH INSTITUTE**  
SAMPLE ANALYSIS DATA SHEET

Lab Name: Southwest Research Institute

Lab Code: SwRI

Matrix: Liquid

SRR: 25435

Client: Division 20

Date Received: 01/21/04

Project No.: 06002.01.081

TO: 040121-5

Sample ID	Lab System ID	Cadmium Results (mg/L)	Chromium Results (mg/L)	Copper Results (mg/L)	Iron Results (mg/L)	Lead Results (mg/L)	Nickel Results (mg/L)
Prep Blank - A22E1	----	<0.005	<0.005	<0.005	<0.025	<0.005	<0.005
Lab Control - A22E1	----	0.044	0.185	0.222	0.875	0.450	0.463
True Value	----	0.050	0.200	0.250	1.00	0.500	0.500
Recovery	----	88.8%	92.7%	88.9%	87.5%	90.0%	92.5%
A	239979	<0.100	<0.100	<0.100	<0.500	<0.100	<0.100
Duplicate result	239979	<0.100	<0.100	<0.100	<0.500	<0.100	<0.100
RPD	239979	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
A Filtered	239980	<0.100	<0.100	<0.100	<0.500	<0.100	<0.100
Spike result	239980	0.911	3.81	4.72	18.3	9.28	9.50
Spike added	239980	1.00	4.00	5.00	20.0	10.0	10.0
Recovery	239980	91.1%	95.3%	94.4%	91.5%	92.8%	95.0%
B	239981	<0.050	<0.050	<0.050	1.40	<0.050	0.105
B Filtered	239982	<0.050	<0.050	0.061	1.39	<0.050	0.096
C	239983	<0.050	0.087	<0.050	2.15	<0.050	0.136
C Filtered	239984	<0.050	<0.050	<0.050	2.08	<0.050	0.138
D	239985	<0.050	0.076	<0.050	1.59	<0.050	0.132
D Filtered	239986	<0.050	0.058	0.108	1.39	<0.050	0.123
Dum	239987	<0.050	<0.050	<0.050	2.08	<0.050	<0.050
Dum Filtered	239988	<0.050	<0.050	0.997	<0.050	<0.050	<0.050

Reporting Limit: 0.005 mg/L 0.005 mg/L 0.005 mg/L 0.025 mg/L 0.005 mg/L 0.005 mg/L

*Page 1 of 1*

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*Roger T. Hsu  
04/07/04*

**SOUTHWEST RESEARCH INSTITUTE**  
SAMPLE ANALYSIS DATA SHEET

Lab Name: Southwest Research Institute

Client: Division 20

Date Received: 01/15/04

Project No.: 20.06002.01.081

TO: 040115-4

Sample ID	Lab System ID	Iron Results (mg/L)
Prep Blank - A19E2	----	<0.025
Lab Control - A19E2	----	0.963
True Value	----	1.00
Recovery	----	96.3%
Prep Blank - A21E2	----	<0.025
Lab Control - A21E2	----	0.983
True Value	----	1.00
Recovery	----	98.3%
E	239746	1.86
Duplicate result	239746	1.87
RPD	239746	0.54%

Reporting Limit: 0.025 mg/L

*Page 1 of 1*

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*Roger T. Hsu  
04/07/04*

3.23.04 Repolished D test coupon with  
1A00 600 grit sand paper

3.29.04 Material C-22 Heat # 2277-8.3175  
Polished 12 specimens to 240 grit finish.

Heat treated @ 870°C for 5 min then  
rapid H<sub>2</sub>O Quench.

<sup>RSD 4.7.04</sup>  
4.7.04 Made following sensors for up coming  
test

SS316 (1100°C e 10min, H<sub>2</sub>O Quench) # 040704-1

SS 316 (1100°C e 10min, H<sub>2</sub>O Quench) # 040704-2  
made from wire PO 1844395

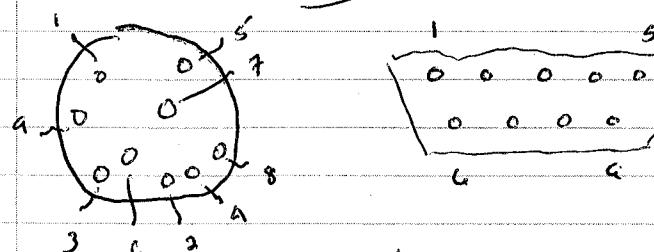
Alloy 22 (1121°C e 10min, H<sub>2</sub>O Quench) # 040704-3 <sup>A7.04</sup>

Alloy 22 (1121°C e 10min, H<sub>2</sub>O Quench) # 040704-4 <sup>A7.04</sup>  
Made from wire Heat XX 1977 BG-11

Alloy 22 (870°C e 5min, Air cooled) # 040704-5

Alloy 22 (870°C e 5min, Air cooled) # 040704-6  
Made from wire Heat XX 1977 BG-11

040704-1 SS316

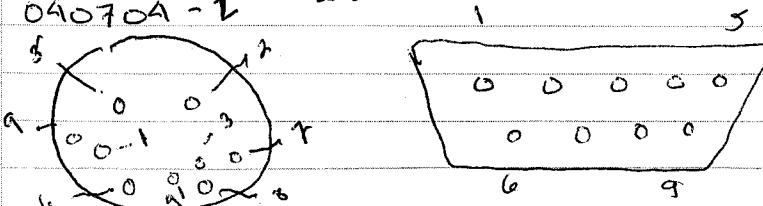


All probes polished

to 600 grit paper

05/11/04

040704-2 SS316

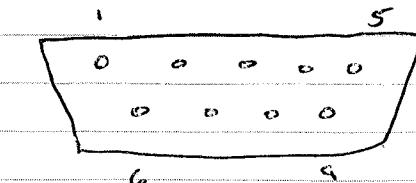
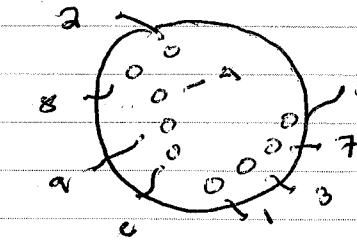


RTD probe  
04/07/04

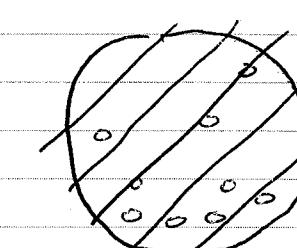
4.7.04

# 040704-3

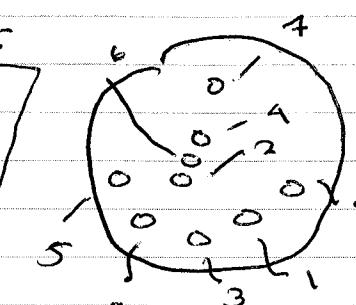
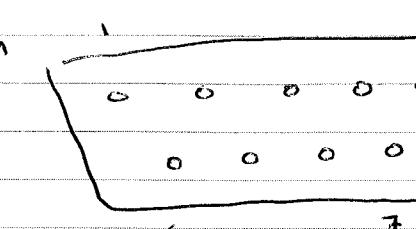
Alloy -22, 1121°C



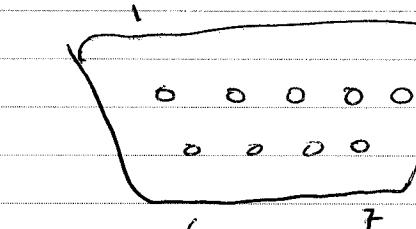
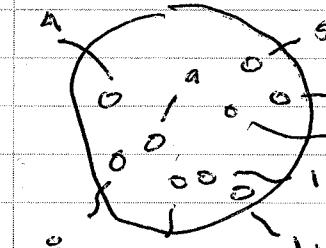
040704-4 <sup>RSD 4.7.04</sup>



Alloy -22, 1121°C

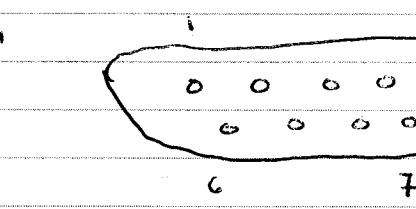
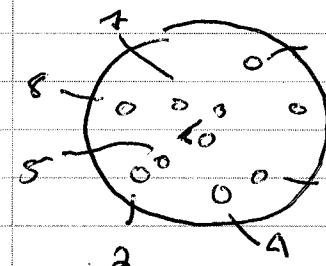


040704-5 Alloy -22, 870°C



RSD 4.7.04

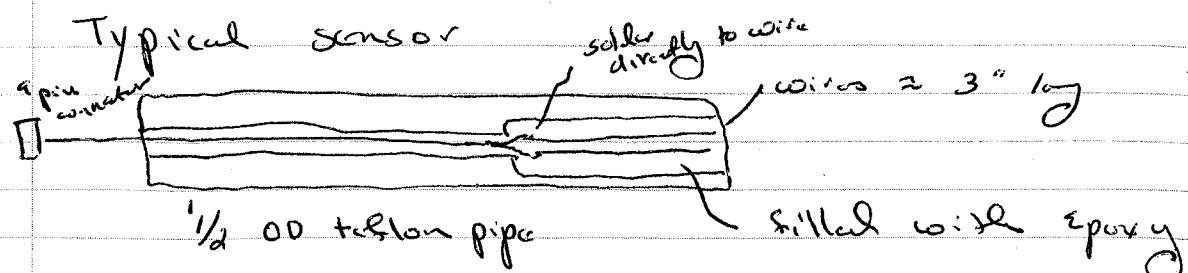
040704-6



Alloy -22, 1121°C

Rg T Pz  
04/07/04

A.7.04



Epoxy ARONCO products Inc  
2300-A 10:100 ratio  
2300-B

A.9.04

Polished C-22 crucice specimens to  
600 grit or 15 micron  
Hunt # 2277-8-3175

Polished 316L crucice specimens to  
600 grit or 15 micron  
Hunt P80746

Assembled 3 cells per instructions  
(except for the C22: 316 crucice  
specimens.)

A.10.04 Took 2 specimens from Arctic ERON  
316L P80746 Specimen 1: 2 (notebook  
378 p12). Repolished and used for  
this experiment

Made 2 specimens from 304L Ht # T0954  
Polished to 600 grit or 15 micron

Rogers  
09/13/04

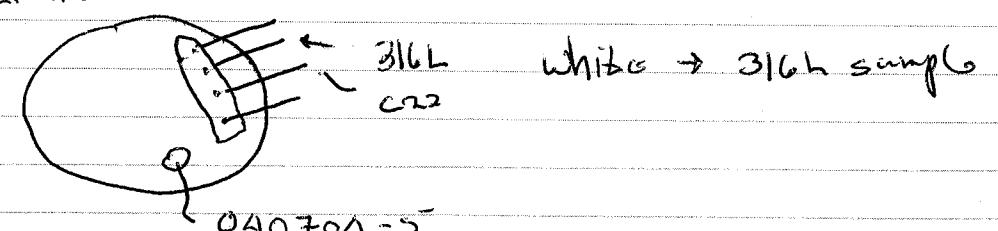
A.11.04 Made J-13 x 100 stock solution

KCl	0.634 g/L	Lot # 005573
NaCl	0.565 g/L	Lot # 035421
NaNO <sub>3</sub>	0.868 g/L	Lot # 020809
Na <sub>2</sub> SO <sub>4</sub>	2.116 g/L	Lot # 035451
NaHCO <sub>3</sub>	9.581 g/L	Lot # 028966
NaF	0.336 g/L	Lot # 006679

stock 502N  
10mL stock 502N Diluted to 1000mL = J-13

29.22 g of NaCl in 990mL H<sub>2</sub>O + 10mL x 100 J-13  
29.67 g of NaCl in 990mL H<sub>2</sub>O : 10mL x 100 J-13  
29.02 g of NaCl in 990mL H<sub>2</sub>O : 10mL x 100 J-13  
28.87 g of NaCl in 990mL H<sub>2</sub>O : 10mL x 100 J-13

C22 specimen Stuart loaded e is on  
green yellow wire in Consarcia cell (316L side)  
RPT 4.15.04



Rogers  
4.15.04

From page 256-

The coupons of control in all cells were given to Dr. Stuart Birnbaum to properly conserve the coupon surface

and to take <sup>SEM</sup> picture at UTSA. 157 days  
on Aug. 22/03 (page 46)

A coupon was placed in the dummy cell

that were used since at least

7/10/03 (See page 202, Book 287).

(See also page 46, book 287)

The dummy cell has been maintained with nutrients and N<sub>2</sub> bubbling all the way to 1/16/2004.

4.21.04 Filled J13 water + 0.5M NaCl  
(600 Pseudomonas cell mixture of strains)

Pictures are in electronic format and stored in the folder of this note book.

Some pictures were used in a final report see pages 292 of this book.

J. Young  
2/17/06

Three-Cell Tests (see page 278) 265  
<sup>dry</sup>

4.22.04 1450	Pseudomonas cell SS316L SS316L	- 316L white red	orange	white green	10/6/04
4.23.04 0815	red bold creviced -0.090A/d -0.0357/d -0.0640/d -0.0225/d	creviced -0.0044/d -0.0847/d	creviced -0.1093/d -0.0847/d	bold creviced -0.1128/d -0.1221/d -0.1104/d -0.1158/d	0.0300/d 0.0402/d

Filled J13 to 0.5M NaCl consortia Vibrio  
Pseudomonas, SRB, Thiobacilli (Marine  
Strain)

4.26.04 1215	Pseudomonas cell SS316L	SS316L - SS316L red bold yellow creviced	white creviced	orange creviced white bold creviced	1223 ~ C22 C22 Pltn
4.27.04 1330	-0.1809 -0.1903 -0.1902 -0.2081 -0.2594 0.2461 0.1931	-0.205 -0.208 -0.2113	-0.208 -0.2081	-0.2594 0.2461 0.1931	-0.2601 0.2489 0.1945

Pseudomonas cell is contaminated. Reassembled cell and removed the infected from cell. Rebuilt one of the glass rods with the C22 specimens.

Re assembled Pseudomonas cell, autoclaved every component including infected. Replaced soil to cover luger probe.

Re assembled J13 + 0.5M KCl + stem former  
+ SRB. autoclaved every component including infected.

Notes by Stuart Birnbaum see Pages 27-56  
S. Notebook # 675.

J. Young  
10/6/04

Project: Datasheet  
Dated: Dunn 5/6/02  
SwRI-GNWRA 5/6/02  
Phone: (210) 522-6090  
Fax: (210) 522-5184  
e-mail: ddunn@swri.org

CNWRA Drawing 20-01402-571-006 rev. 1  
Dimensional tolerances +/-0.005"  
unless otherwise specified  
16 rms surface finish  
Crevice Repassivation Specimen

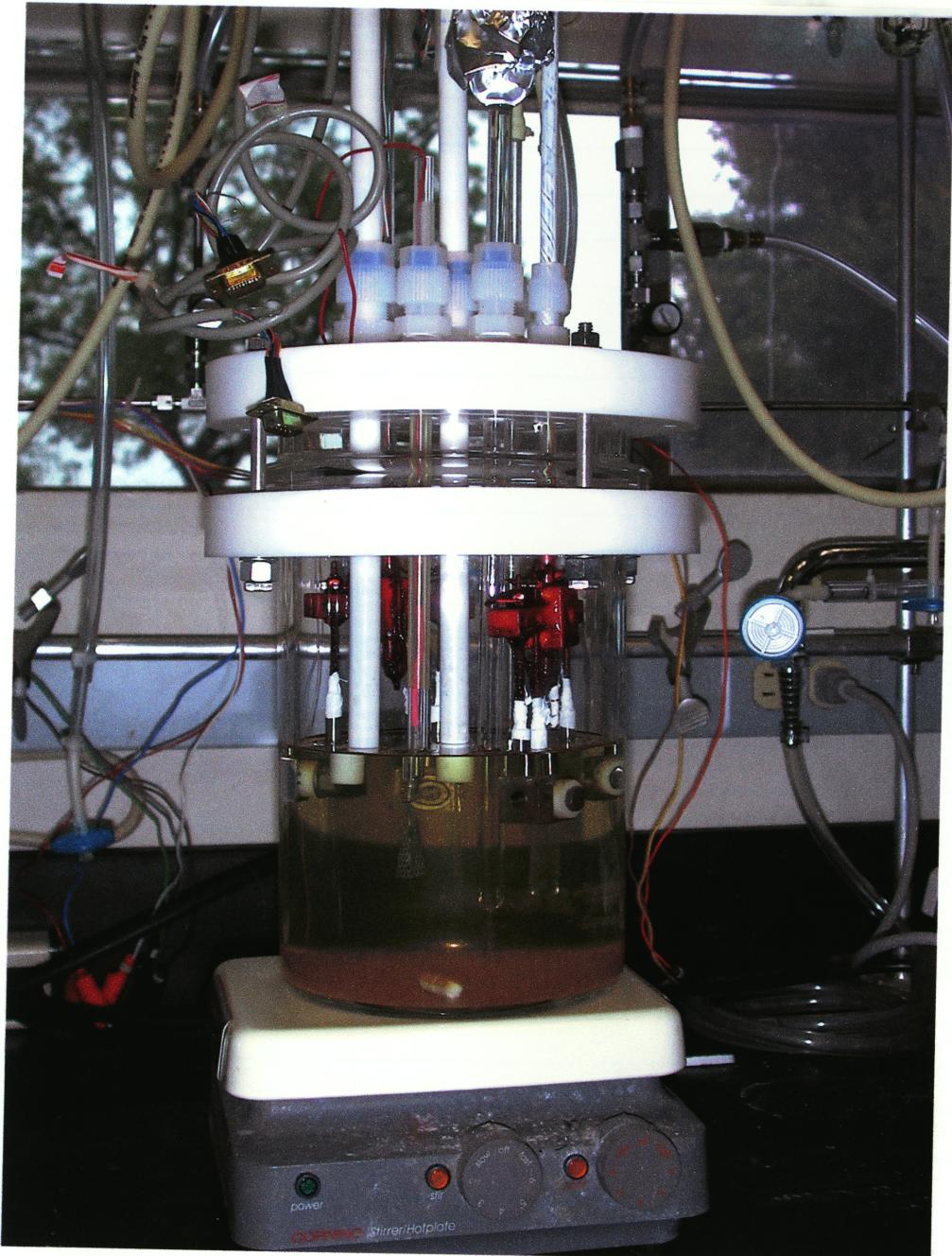
To be completed at time of order:  
Material: 316L  
Heat: P507A6  
Specimen Orientation:  
Other:

Procedure 10S-W1-82A Specification CC31MS  
Project # J.C. # 79480 EQUIPMENT  
TOTAL PCS. INSPECTED 24 Cal 30 LC-2  
TOTAL PCS. ACCEPTED 24 Tad Gage  
TOTAL PCS. REJECTED 0  
"NR #" IF REJECTS NA  
INSPECTOR APR DATE 13 2004

Daniel Dunn 5/6/2002  
Initiated by: D. Dunn Date  
Reviewed by V. Jain Date  
B. Mabrito 5/6/2002  
QA Approval B. Mabrito Date

Made these specimens for suturing use.

Roger T. Myard  
4/26/04



Picture of Pselomonas cell taken on 4/21/04 after initial fill.

Roger T. Myard  
4/26/04