RECORD

308 --- Q200006080001
Scientific Notebook #138

Scientific Notebook detailing maintenance and checks carried out on the microbial Culture collection being maintained for MMS/MIC project # 20-5704-044

Activities performed by Dr. Peter Angell.

Initial procurement (Source) of bacterial strain will be entered, followed by dates of routine Sub culturing, with details of medium used (Batch # + manufacturer) as well as periodic checks on purity to include colony morphology, Gram stain shape, motility + such other biochemical tests as deemed necessary

Dr. Angell has a Batchelor of Science (Honours) from the University of Surrey in Microbiology + a Ph.D from the Same University also in microbiology

Cultures of bacteria are known to become contaminated with time, (particularly the anaerobic Sulphate-reducing bacteria) therefore a separate note book detailing transfer + purity checks is to be maintained in addition to the experimental note book to provide assurance that quality of culture is maintained

3-8-95 Peter J Angell

3-8-95

Marine Agar plates made using Difco Marine Agar. Lot # 568895B (exp at 97) with 27.55g dissolved in 500ml RO water. Autoclaved for 25min @ 121°C 21 psi. 30 plates poured. Designate MA1, 3 discarded + 2 placed in 30°C incubator for Sterility check.

5 cultures received (shipped UPS blue) from Center for Environmental Biotechnology) univ of Tennessee

1) Vibrio natriegens from ATCC # 14048
2) Oceanospirillum from B.L. Hte (NRL, Stennis)
3) G1Y isolated by P. Angell from a failed copper pipe in a hospital in germany identified as Pseudomonas putrefaciens by API 20NE test but with 99% certainty (Int. Biodet 27:135-145)
4) G1B isolated by P. Angell from a failed copper water pipe in a hospital in germany identified as an atypical Ps. putrefaciens by the National Collection of Industrial & marine Bacteria (NCIMB) (Aberdeen, Scotland) (Int. Biodet 27:135-143, Angell Ph.D thesis)
5) G1W isolated by P. Angell from a failed copper water pipe in a hospital in germany identified as an atypical Strain close to Ps. solanacearum by NCIMB (Int. Biodet 27:135-143, Angell Ph.D thesis)

Each culture streaked onto MA1 & incubated at 30°C (11:45) for subsequent purity checks

3-8-95 peter Angell

3-9-95

Cultures plated yesterday 3-8-95 & placed at 30°C where checked. @ 13:20

1) V. natriegen. good growth isolated colonies white/buff in colour/entire
2) Oceanospirillum isolated white colonies
3,4,5 no growth yet CNO.B these strains normally grown on Nutrient Agar as fresh water strains ∴ testing to see if grow at 1% NaCl of MA.

Note of Bacterial characterisation:
Nomeneleture is subject to change in bacteriology as new strains are found & new tests become available the strains tested here are either ATCC strains in which case the original citation paper given reason for its naming is given in ATCC catalogue. otherwise the papers describing the bacteria are listed. Bergey's Manual of Systematic Bacteriology is to be used as the authoritive source for bacterial classification. This will be used along with Cowan & Steel's Manual for the identification of Medical Bacteria. This manual lists the materials & procedures for the standard laboratory biochemical/physiological tests used for the characterisation of bacteria Tests in this note book follow these procedures which have citation for the publication of the test.

3-9-95 peter Angell

3-10-95

Made Nutrient Agar (1.5%) plates using Difco (Bacto Nutrient agar) lot # 136222JG (exp Sep 97) dissolved 15.5g in 500ml RO water boiled to dissolved & autoclaved at 121°C / 21psi for 25 min. 29 plates poured designated NA1, 1 placed at 30°C as sterility check.

3-10-95

— " —

Made Iverson's medium plates (Iverson (1966) Applied Microbiology, 14, 529.

500ml H_2O (RO)

TSA (Difco Lot # 580566JB)	20g.
Bacto-agar (Difco cert.)	2.5g
Sodium lactate	2ml
$MgSO_4 \cdot 7H_2O$ (Fisher Lot# 947401)	1g.
$FeSO_4 \cdot 7H_2O$ (Fisher Lot# 946178)	0.25g

boiled to dissolve & autoclaved at 121°C/21psi for 25 min, 30 plates poured designated I1

— " —

G1W, G1Y & G1B as per page 2 were restreaked for isolated colonies from original slants received from CEB Univ of Tennessee onto NA1 plates and incubated at 30°C 13-15.

Plates from 3-8-95 checked as before growth of Oceanospirillum & v. natriegens still no growth of G1W, G1B, & G1Y on NA1.

3-10-95

3-14-95

Sterility check on NA1 @ 30°C good no growth

G1B - 3-10-95 good isolated growth of brown/orange colonies, punctiform were isolated globular were heavy growth

G1W - 3-10-95 good isolated growth of white/translucent colonies, globular.

G1Y - 3-10-95 good growth of isolated globular yellow colonies.

Oceanospirillum 3-8-95. isolated buff/tan colonies globular

V. natriegens ~~3-8-95~~ isolated white/cream globular colonies

Gram Stain prepared using difco gram stain kit ~~lot #~~ CV 70064, GI 71056, GD 72077 GS 73063 - Kit designated gram stain set 1 Fisherbrand Gram ✓ QC Slide used with Staph. aureus & E. coli as controls Lot # 179 Exp 9-96

1) V. natriegens
2) Oceanospirillin
3) G1W
4) G1Y
5) G1B

i) Slide air dried & heat fixed
ii) 2 min Gram Crystal violet (CV) rinse tap H_2O
iii) 1 min Gram Iodine (GI) rinse tap H_2O
iv) rinse Gram Decolouriser (20-30 sec) rinse H_2O
v) Safranin 30 sec, rinse & dry (air)

Gram -ve control - red cells clumped ∴ no shape discernable

Gram +ve control blue/violet cocci 3-15-95

1) V. natriegens red (gram -ve) short rods
2) Oceanospirillum rods slight twist? possibly Gram +ve not sure will need to recheck this.
3) G1W - red (gram -ve) short rods
4) G1Y - red (gram -ve) short rods forming long chains
5) G1B - red (gram -ve) very long chain forming filamentous mat

Replated (Streak for isolated) G1Y, G1B & G1W onto MA1 & incubated at 30°C 09:45

Replated (Streaked for isolation) V. natriegens & Oceanospirillum onto MA1 & incubated at 30°C 09:50 3-16-95

Plated Oceanospirillum onto MA1 from original CEB short designated OC2 incubated @ 30°C 09:55

Marine agar plate + Cu made using Difco Marine Agar Lot # 56889JB (exp out 97) with 27.55g dissolved in RO H_2O with 0.75g $CuCl_2$ added. auto claved at 121°C 14psi for 30 min & poured 30 plates designated MCu1, one plated in incubator at 30°c for sterility check.

Oceanospirillum (3/8/95) plated onto MCu1 OC2 also plated onto MCu1 at 14:15 & incubated @ 30°C. Oceanospirillum is Cu^{2+} tolerant ∴ used as a semi selective medium.

3-14-95

check on plates 3-14-95 G1B no growth
G1Y - limited growth punctiform yellow colour
G1W - limited growth punctiform white/translucent colour
OC2 on MA1, off white colonies 1mm ø
V. nat (MA1) white colonies isolated 1.5mm ø
Oceanos MA1 off white colonies isolated 1mm ø
OC2 (MCu1) grey colonies 2-3mm ø globular
Oceano MCu1 grey colonies 2-3mm ø globular
(N.B Cu known to increase EPS production).

3-15-95

Sulphate-Reducing Bacteria (SRB) Sulphate reduction under anaerobic conditions is indicated by the formation of black iron sulphide in either lactate/acetate medium or Iverson's medium.

Tubes with Black iron sulphide in Lactate/acetate medium were brought from CEB/Univ Tennessee. 0.1ml spread plates were made of cultures as follows onto I1 plates:
2 @ Desulfovibrio vulgaris (MB) isolate from failed pipe at TVA power plant.
2 @ Desulfovibrio vulgaris ATCC # 29579
2 @ Desulfovibrio desulfuricans ATCC # 29577
2 @ Desulfovibrio desulfuricans ATCC # 27774
from here on D. will be short for Desulfovibrio following tradition of both the American Society of Microbiology (ASM) & General Society of Microbiology cultures placed in GasPak 100 anaerobic system with a GasPak plus catalyst System (Lot # 1006408500 exp Sep 96) & incubated at 30°C

3-16-95

3-17-95 Gram Stain checked on Oceanospirillum 3-23-95
on MA1 & MCu1 (3-14-95) & OC2 on MA1 & MCu1
(3-14-95) protocol cs page #6 (3-14-95) Same
Stains no Gram ✓ slide used instead used
Microscope Slide

Oceanospirillum on MA1 Gram -ve (Red) rods slight curve
" on MCu1 Gram -ve (Red) rods slight curve.
OC2 on MA1 (Gram -ve (Red) rods slight curve
OC2 on MCu1 Gram -ve (Red) rods Slight curve
∴ Clarified problems seen on 3-14-95 with
this organism's Gram Stain shown to be
Gram -ve which is correct.

—//—

Anaerobic plates of SRB (3-16-95) no Sign of growth
yet, few black Specks visible on Some plates
probably not growth, System not opened & left to incubate
at 30°C 3-17-95

—//—

3-20-95 All plates in anaerobic jar from 3-16-95 were
black from Sulphide production from outside
& jar numerous punctiform colonies were visible
on the plates. No colony growth with
D. Sulphuricans 29577 ∴ this one not streaked but
returned for further incubation. All other streaked
for isolation on I1 medium & returned to anaerobic
gar Gas pak Lot # 100644085001 (exp Sep 96). incubated
at 30°C 3-20-95

streaked check on MCu1 ok no growth
cultures from 3-8-95 & 3-10-95 detached. Received
3 new cultures from Ron Nealson's lab
designated MR-1, MR-4 & SP200. They are all
Strain of Shewanella putrefaciens (Gram -ve) rods.
MR-1 is freshwater Strain isolated from Oneida
Lake, NY used utilization of Nitrate, Nitrite,
Thiosulfate, Fe(III), Sulfite, Glycine, Fumarate,
DMSO, Mn(IV) & others, does not grow on glucose
MR-4 Similar MR-1 but will use glucose &
isolated from black Sea.
SP200 is Similar but a marine Strain initial
Studies of this Strain by P. Angell at CGB. TN
showed it to have good corrosive abilities.
SP200 was plated onto MA1 for characterization
& incubated at 30°C
MR-1 & MR-4 were plated onto MA1 for characterization
& incubated at 30°C
all plates from 3-14-94 were Subed onto
respective medium (Oceanospirillum only put onto
MCu1 not MA1) 2 plates per culture
Oceanospirillum, MCu1, V. natriegens MA1
(G1W, G1Y & G1B NA1, 1 plate per culture)
(1 plate of MA1 discarded due to mold)

SRB's in anaerobic Jar plate black with Fe Sulphide
will leave Hill Monday 27th March 1995 to
Sub culture.

3-23-95

3-28-95 Anaerobic jar opened & all plates (3-20-95) & 2 from (3-16-95) D. desulfuricans 29577 which previously had no growth. all showed isolated colonies & black colouration excess of H_2S produced. this might be a problem as whole plate black.

4-5-95 colonies isolated colonies were picked from each plate & restreaked for isolation plates sealed with parafilm as a test. placed in anaerobic jar a catalyst as per 3-16-95 & 3-20-95 vacuum lid jar sealed & incubated at 30°C. old plate left exposed to air to clear black from agar leaving colonies discernable cutting black sq clear, containment. After clearing the agar it was obvious that all isolated colonies were black indicating the presence of SRB's with no contaminants.

5-5-95

3-28-95

3-29-95 1 plate made of each of the aerobic cultures onto respective medium.

oxidase test on plates (3-23-95) using BB2 oxidase Reagent dropper (lot # 412024 exp 30 Dec 96) all aerobic cultures tested positive.

Tried catalase test using 4% peroxide from 30% H_2O_2 (lot # 902183A) (no date) all showed negative ∴ suspect hydrogen peroxide was not fresh!! will reorder & try again.

3-29-95

LB medium made according to Nealsons as follows

Lactate	Lot#	2ml. (60% Syrup)
Tryptone	(6B2785B dec 99)	10g
Yeast extract	(5969553B nov 99)	5g
NaCl	(947723)	10g
18 mΩ water to		1L.

Split into 500ml portion one dispensed (9ml). into tubes, other added agar 7.5g boiled & all medium autoclaved for 30 min 14psi 121°C, agar poured to plates labelled LBA2

4-5-95

Nutrient agar made 15.5g (Difco Lot# 136225G expd) dissolved in 500ml distilled water, boiled to dissolve agar & autoclaved in 500ml Duran for 30 min at 14 psi 121°C, cooled to 40°C & poured on plates labelled NA2.

LB media 1 liter made as above 500ml with added agar 7.5g boiled & autoclaved in 500ml Duran (30 min 14psi, 121°C) cooled to 40°C poured into petri dishes & labelled LBA2. other 500ml of broth dispensed as 5 x 100ml in 100ml duran & autoclaved (30 min. 14psi, 121°C) labelled LB2

4-6-95

4-7-95 Routine culture transfer on streak plates as follows all from culture (3-29-95)

G1W & G1Y	2 plates each on NA2
oceanospirillum	2 plates on NCu1
V. natrigen	2 plates on NA1
SP200	1 plate each on NA1 & LBA1
MR1 & MR4	1 plate each on NA2 & LBA1

anaerobic jar (3-28-95) opened all plates black but clear individual black colonies were visible darker black than plates. purity of strains was confirmed. all replated onto 11 plates till broth medium is available. noted that parafilm was hard to get off plate very sticky hard sealed no purpose -. not used. 1 plate from each sp was reincubated in Anaerobi jar catalyst Lot # 1006L085001 exp Sep 96 used as before. incubated at 30°C

4-7-95

4-14-95 15.5 g nutrient Agar (Lot #13622JG exp Sep 97) was dissolved in 500 ml of 18.1 MΩ water + autoclaved at 121°C 14psi for 30 min. cooled + 30 plates poured designated NA3

4-14-95

4-20-95 Made 1L of LBA medium

Lactate	2 ml.	
Tryptone	10 g	622785B
Yeast extract	5 g	596955B
NaCl	10 g	947725
Agar	15 g	

18.1 MΩ water 1 l.
Split into two 500 ml medium bottle + autoclaved at 121°C 14 psi for 20 min poured as plates designated LBA3

4-20-95

4-21-95 Routine transfer of stock culture (4-7-95) as follows

MR1, MR4 SP00	2 plates each on LBA3
G1W, G1Y	2 plate each on NA3
oceanospirillum	2 plates on NCu1
V. natrigens	2 plates on NA1

500 ml of 1.5% Nutrient Agar Difco lot # 13622JG exp Sep 97 was prepared. 15.5 g dissolved in 500 ml of 18.1 MΩ water autoclaved for 30 min at 121°C 14 psi + poured into petri dishes designated NA4

500 ml of Marine Agar Difco Lot # 56889-JB exp Oct 97 prepared 27.55 g in 500 ml of 18.1 MΩ water autoclaved for 30 min at 121°C 15 psi + poured into petri dish designated MA2.

4-21-95

4-26-95 plate of GIB & MR1 heard not grown from 4-21-95 :- new plate & made from old cultures, other plate which had grown heard catalase test done using 4.5% (3ml H_2O_2 & 17ml H_2O) H_2O_2 from batch # 932032 (30% H_2O_2) received 4/11/95

Result as follows

vibrio natriegens -ve

MR4 weak +ve

SP200 -ve

GIW -ve

oceanospirillum +ve.

4-26-95

4-26-95 Nutrient agar 1.5% Difco lot # ~~1326835~~ 1326225G (15.5g) dissolved in 500ml of 18.1 MΩ H_2O & atc dosed at 121°C 15psi for 30 min cooled to 45°C & poured as plate NA5

4-26-95

4-28-95 made Lactate/Acetate broth for SRB

Sodium Acetate	2.8g	950091
Ascorbic acid	0.1g	947449
$MgSO_4.7H_2O$	0.5g	946409
Na_2SO_4	0.5g	901219
KH_2PO_4	0.5g	950082
$FeSO_4.7H_2O$	0.1g	946178
NaCl	7.0g	946723
Yeast extract	1.0g	596955

NH_4Cl	0.5g	895252
18.2MΩ H_2O to	1 Litre	
Na Lactate	2.91ml	

put on hot plate stirrer & brought to boiling with 5% H_2 95% N_2 bubbled through boiled for 15min 10ml dispensed into tubes gassed with 5% H_2 95% N_2 & stoppered & capped autoclaved 121°C 15psi 20min

1 litre of LB broth made

Lactate	2ml	
Tryptone	10g	622785B
yeast extract	5g	596955B
NaCl	10g	946723

1 litre 18.1 MΩ water

10ml dispensed into tubes 80, rest in 2 x 100ml media bottle & all autoclaved 20min 121°C 15psi. designated LB3

Routine culturing of bacteria streaked for isolation as follows

Ocean & v.natriegens	2plates each on MA2
MR1, MR4 & SP200	2 plates each on LBA2
GIW GIB GIY	2plate each on NA5

GIY from plate possibly with growth, doubtfull

4-28-95

5/4/95 made up nutrient agar 1.5% Difco 13262256 (15.5g) in 500ml, auto claved for 35 min at 121°C 15 psi poured onto plates designated NA6

5-11-95

made up marine Agar Difco 568895JB 27.65g in 500 ml of 18.1 MΩ H_2O autoclaved 55 min at 121°C 15 psi, poured onto plate designated MA3

made 500 ml of LBAgar as follows
Hostate 1ml
Tryptone 5g 622788JB
Yeast extract 2.5g 596955JB
NaCl 5g 947723
Agar 7.5g
autoclaved 35 min 121°C 15 psi poured as plate designated LBA4

5-6-95 [signature]

5-6-95 transferred loops of colonies from plate anaerobically incubated on ~~3-28-95~~ 4-28-95 into Sterile anaerobic bacteriode/arcobacter Medium 4-28-95 1ml of this Suspension inoculated into 9ml of Sterile anaerobic bacteriode/arcobacter medium (tubes) and incubated at 30°C

5-23-95

Routine Subculture of aerobic cultures as follows
MRI, MRH + SP200 2 plates each on LBA4
oceanospirillum 2 plates on MACu7
V. natriegens 2 plates on ~~PA~~ MA3

GIW & GIB 2 plates each on NA6

5-6-95 [signature]

all SRB Sent - cultivated from broth inoculated 5-6-95 onto fresh Sterile anaerobic L/A medium

MRI noted not to have grown on plate MRH also problematic .. reisolated from previous plates

5-11-95 [signature]

5-12-95 Routine Subculturing of aerobic plates
GIW & GIY 2 each on NA6
oceanospirillum 2 on MACu1
V. natriegens 2 on MA3
SP200, MRI, MRH 2 each on LBA4

5-12-95 [signature]

Routine Subculture of aerobic plates
GIW & GIY 2 each on NA.5
oceanospirillum 2 on MACu2
V. natriegens 2 on MA3
SP200, MRI & MRH 2 each on LBA2

5-23-95 [signature]

5-24-95 Made 2 x 500ml of LB agar
Tryptone 5g
Yeast Extract 2.5g
NaCl 5g
Agar 7.5g
Lactate 1ml
18.1 MΩ H_2O 500ml.
autoclaved 121°C 15psi for 35 min, cooled and poured as plates LBA5

5-24-95

6-02-95 Routine Subculturing of aerobic plates from 6-23-95
G1W + G17 2 plates each on NA6
MR1 + MR4 2 plates each on LBA5
SP200 + V. natriegens 2 plate each on NA3
oceanospirillum 2 plates on MAcuZ

Routine Subculturing of anaerobic tubes from 5-11-95
1ml of each of following cultures into 9ml of fresh lactate/acetate medium (anaerobic)
D. desulfuricans 29577 + 27774
D. vulgaris MB + 295779.

6-2-95

6-12-95 Routine Subculturing of aerobic plate from 6-02-95
G1W + G17 2 plate each on NA6
oceanospirillum 2 plates on MAcuZ
V. natriegens 2 plates on NA3
SP200 1 plate on each of NA3 + LBA5

MR1 2 plate on LBA5
MR4 1 plate each on LBA5 + NA6.

6-12-95

6-19-95 Made 2 x 500ml of LB agar.
Tryptone 5g
Yeast Extract 2.5g
NaCl 5g
Agar 7.5g
forgot the lactate!!
18.1 MΩ H_2O 500ml.
autoclaved 121°C 15psi for 30 min, cooled to 45°C & poured as plates LBA6

made 500 ml of Nutrient agar 1.5% autoclaved as above & poured as plate NA7.

6-19-95

6-20-95 plate of MR1 & MR4 made 6-12-95 did not grow ∴ restreaked both plate from 6-02-95 onto LBA6 & incubated to revive the cultures.

6-20-95

6-23-95 3 tubes of anaerobic medium inoculated with 0.5ml of D vulgaris 295779 (6-02-95) & incubated at 30°C

one of the MR1 plate from 6-20-95 had grown but none of MR4
MR1 plated streaked onto 4 plate of LBA6

G1B streaked onto 2 plates of NA7
V. natriegens streaked onto 2 plates of NA7
MR1 streaked onto 3 plates of LBA6 to try & re isolate

[signature] 6-23-95

6-27-95 Routine gram stain carried out on two cultures isolated from chemostat sterile (pp 33 S.N.B #136) & purified (pp 43. S.N.B #138) position 1 yellow/brown, 2. white, 3. V. natriegens (6-23-95) 4. oceanospirillum (6-12-95) 5 MR1 (6-23-95) 6. G1B (6-23-95) on gram ✓ slide lot # 179 (Exp. 9-96) using technique & stain described on pp 5 this S.N.B (Scientific Note Book)

C+ stains blue/violet coccoid ✓ correct
C- " red coccoid/short rods ✓ correct
1 red coccoid/short rods: forming chains, 2, 3, 4 cells
2 red coccoid cells clumping
3 red short rods
4 red short rods
5 Red rods
6 Red rods forming "pseudo" hyphae.

oxidase test using BBL oxidase reagent dropper (lot # 412024 Exp. Nov 96)
1) +ve, 2) +ve 3) +ve 4) +ve
5) +- 6 ±

Catalase tested using 4.5% H_2O_2 made up 6-26-95 and stored in dark at 4°C

1) v. positive 2) v. positive
3) w. positive 4 w. positive
5) negative. 6) positive

[signature] 6-27-95

6-28-95 Hugh & Leifsons medium was made following Cowan & Steel's manual for the identification of medical bacteria
peptone 1g
NaCl 2.5g
K_2HPO_4 0.15g
Agar 1.5g
water 500ml
Bromthymol blue 0.2% aq sol 15 ml (should have been 7.5)

Solids dissolved by heating, cooled pH adjusted to 7.1 & Bromthymol blue added autoclaved along with tubes & ~~15ml of~~ 5g of Dextrose in 15ml of water @ 121°C 15 PSI for 30 min
cooled to 45°C + ~~glu~~ dextrose added. dispensed aseptically into tubes.

[signature]

6-29-95 ~~two~~ duplicate tubes of Hugh & Leifson's medium were stab inoculated with each of the cultures of aerobic bacteria currently held in collection: MR1, G1W, G1B, SP200, oceanospirillum, V. natriegens & light colonies for chem

are tube of each duplicate pair was topped of (≈3cm) of sterile petrolatum and all tubes incubated at 30°C.

Routine Subculturing of aerobic bacteria

GIW + GIB 2 plate each on NA7
MRI + SP200 2 plates each on LBA6
V. natrigen 2 plate on MA3
oceanospirillum 2 plates on MCU2
chemo light + dark plate each on LBA6.

6-24-95

7-10-95 DIF test read from 6-29-95

Strain	Sealed	open	result
GIW	Yellow	Yellow	fermentative
GIB	Yellow	Yellow	fermentative
MRI	Blue	blue	
SP200	Blue	blue	—
oceano	yellow	yellow	fermentative
V. nat	blue	yellow	oxidative
light chemo	blue	yellow	oxidative
dark chemo	yellow	yellow	fermentative

very questionable ??

7-10-95

7-11-95 Routine Subculturing of aerobic bacteria

GIW + GIB 2 plates each on NA7
MRI + SP200 2 plates each on LBA6
V. natrigens 2 plates on MA3
Oceanospirillum 2 plates on MCU2
chemo light + dark 2 plate each on LBA6.

7-11-95

7-17-95 PB ROUTINE SUBCULTURING OF AEROBIC BACTERIA

GIW + GIB 2 PLATES EACH ON NA7
SP200 2 PLATES ON LBA6
V. NATRIGENS 2 PLATES ON MA3
OCEANOSPIRILLUM 2 PLATES ON MCU2

MRI, CHEMO LIGHT, AND CHEMO DARK WERE NOT CULTURED BECAUSE NO GROWTH WAS OBSERVED ON PLATES SPREAD 7-11-95. PLATES FROM 7-11-95 WERE RETURNED TO INCUBATOR. TEMP SETPOINT CHECKED AT 30 °C.

V. NAT. Plates showed cloudiness within agar. Check of plates from 7-11-95 showed similar cloudiness (ppt?).

Paul Bertelli 7-17-95.

21 Jul 95 PB

The MRI, CHEMOLIGHT, and CHEMODARK plates spr PB 21 Jun as streaked on 7/11/95 still do not exhibit any observable growth. There is clear evidence of biomass transfer to the medium. I will streak 1 plate each of MRI, chemolight and chemodark from cultures of ~~7 liters~~ to see if growth can be initiated. 6/29/95 PB 7/21/95

8/25/95

CHEMO LIGHT 1 plate on LBA6
CHEMO DARK 1 plate on LBA6
MRI 1 plate on LBA6
(from 7 liter 6/29 PB 95 7/21/95 cultures.)

plates transferred to incubator @ 30 °C. 8/30/95

Pearl Berg Gillespie 7/21/95

8/1/95 made transfer of all aerobic plates
G+B & GLW 2 each on NA8
SP200 & ~~oceano~~ v. natriegens 2 each on MA2
oceanospirillum 2 on MA1

8/1/95

9/13/95

8/1/95 Made 500-ml of R2A agar as defined
lot # 5259350. 9.1 g in 500ml 18.1 MΩ H_2O autoclaved poured 30 plate labelled R2A1

8/17/95

Routine aerobic subculture as follows:

G+B & GLW 2 plate each on NA8
~~oceano~~ SP200 2 plates on LBA7
oceano & v. natrigen 2 (one) plate each NA8

8/16/95

Made 2 x 500ml of LB agar (as earlier) & poured 60 plate LBA7.
Made 500 ml of Marine agar Difco lot 5638891 37.55g in 500ml of H_2O. poured 30 plate MA3

8-25-95

Routine subculturing as follows

G+B & GLW 2 plates each on NA8
SP200 2 plates on LBA7
oceanospirillum & v. natrigens 2 plate each on MA3

8/30/95

Routine subculturing of aerobic plate as follows
G+B & GLW 2 plate each on ~~NA8~~ LBA7
SP200 2 plates on LBA7
oceanospirillum & v. natrigens 2 plates each of MA3

anaerobic cultures subcultured in fresh bottle of lactate/acetate medium 0.5ml x 2 per culture
29577, 27774, D. desulfuricans
MS, 295779 D. vulgaris

9/6/95

9/15/95 New cultures of Shewanella putrefaciens MR1 & MR4 arrived from Dr. Nealson's lab (plated 9/7/95) repleted onto LB plate provided by Dr. Nealson's lab & incubated at 30°C

9/12/95

9/15/95 made 2 x 500 ml of LB agar
Tryptone 5g NaCl 5g
yeast extract 2.5g Agar 7.5g
500 ml of 18.1 MΩ H_2O & 1 ml lactate
autoclaved at 121°C 14 psi for 35 min cooled to 45°C & poured as plates labelled LBAB

9/22/95 Made 2 x 500 ml of 4% Nutrient Agar (Difco Lot # 1362256 exp Sep 97) 15.5g in 500 ml 18.1 MΩ H_2O, autoclaved for 35 min @ 121°C 14 psi. cooled to 45°C & poured as plates NA9.

9/29/95

routine Subculturing of aerobic cultures
SP200, MR1, MR4 2 each on LBAB
oceanospirillum & V. nitrogen 2 each on MH3
G1B & G1W 2 each on NAB

9/22/95

10/09/95

9/25/95 made 1L PBS. 400 ml Phosphate buffered Saline 2 tablets (Sigma lot 45H8917) dissolved in 400 ml of water & dispensed into tubes 10 & 20 ml per tube & autoclaved 9/25/95

Made up 50 ml of gelatin suspending medium modified from Evans as follows (2.5g NB left out & replaced by LB medium)

Gelatin (difco lot 594805 JH)	5g
Inositol (Difco lot 756125A)	2.5g
NaCl (fisher lot 947723)	0.5g
Tryptone (Difco lot 622785B)	0.5g
Yeast Extract (Difco lot 5968 JB)	0.5g (2x LB)
Na Lactate (fisher lot 963605)	.5ml (10x LB)
18.1 MΩ H_2O	50ml

Brought to boil to dissolve gelatin, cooled and dispensed 3ml per tube and autoclaved at 121°C 14 psi for 35 min.

9/28/95

Routine Subculturing of aerobic plates carried out as follows:
SP200, MR1, MR4 2 plates each on LBAB
oceanospirillum & V. nitrogen 2 plate each on MH3
G1W & G1B 2 plates each on NA9.

9/29/95

Routine Subculturing of aerobic plate carried out as follows:
SP200, MR1 2 plates each on LBAB
oceanospirillum & V. nitrogen 2 plate each on MH3
G1W & G1B 2 plate each on NA9

10/09/95

10/18/95 Routine Sub-culture of aerobic plate of follow

MRI & SP200 2 plate each on LBA9
V. natrigen & oceanospirillum 2 plate each of MA3
GIW & GIB 2 plate each on NA9

10/18/95

10/24/95 Routine Subculture of aerobic plate as follow

MRI & SP200 2 plate each on LBA9
V. natrigen & oceanospirillum 2 plate each on MA3
GIW & GIB 2 plate each on NA9

also
GIW, GIB, V. nat, oceano 1 plate each on NA9
MRI 2 plate on LBA9
SP200 1 plate on LBA9
incubated at 30°C in anaerobic jar

10/24/95

10/27/95 Made 500ml of R_2A agar Difco Lot # 525928
9g in 500ml of 18.1 mΩ water autoclaved
poured as plate labeled R_2A2

Made 500 ml of Marine tyge 2216 Difco
lot # 568895B 27.55g in 500 ml of 18 mΩ
water boiled to dissolve autoclaved & poured
as plate MA4

Made 500 ml of LBA agar
5g NaCl (H977723) 2.5g Yeast Extract (H5969558)
3g Tryptone (H6227858) 1ml Na Lactate (H943605)
500 ml of 18.1 mΩ H_2O autoclaved poured as
plate LBA1

Made 400 ml of PBS 2 Sigma 45H8917 Tablets
in 400 ml of 18.1 mΩ H_2O dissolved and
dispensed into tubes 10 ml & 20 ml.

10/30/95

10/30/95 MRI, V. natrigen, SP200 all spread anaerobically
GIB, GIW & oceanospirillum did not.

10/30/95

11/2/95 routine Subculture of aerobic plates
MRI & SP200 2 plates each on LBA1
V. natrigen & oceanospirillum 2 plate each on MA4
GIW & GIB 2 plate each on NA9

10/2/95

11/2/95 made 400 ml of PBS & distributed into 20 ml
tubes 2 Sigma tablet lot# 45H8917 in 400 ml
of 18.1 mΩ H_2O

11/2/95

11/4/95 Stock plate learn made from MRI 11/2/95

11/4/95

11/6/95 Stock plate from 11/6/95 of MRI showed good growth so restreaked on LBA1 plate & incubated at 30°C ready to inoculate MRITHIO4 on 11/6/95

11/6/95

11/6/95 made 500ml of LB agar as follow
5g NaCl (#947723) 2.5g Yeast Extract (#5969538)
5g Tryptone (#6227853) 1ml lactate (#943605)
7.5g Agar 500ml 18.1 MΩ H_2O

autoclaved at 121°C 14psi for 35 min cooled to ≈45°C & poured as plate LBA2

11/6/95

11/10/95 Routine Subculture of aerobic plate borders GIW & GIB 2 plate each on NA9
Oceanospirillum & V. natriegens 2 plate each on MA4
MRI & SP2A 2 plate each on LBA2

11/10/95

11/6/95 Made 500ml of LB agar as follow
5g NaCl (#947723) 2.5g Yeast Extract (#5969538)
5g Tryptone (#6227853) 1ml lactate (#943605)
7.5g Agar 500ml H_2O

autoclaved at 121°C 14psi for 40min cooled to ≈45°C & poured as plate LBA3

500ml of 1:6W diluent tryp Made as follow

Difco Lot # 1362256 15.0g in 500ml of H_2O autoclaved at 121°C 14psi for 40min cooled to ≈45°C & poured as plate LBA1.

Preparation of MNB (Myers Nealsen Broth) for anaerobic culture of MRI in presence of thiosulphate

900ml of 18.1 MΩ H_2O
1ml of trace minerals (SNB 134 p74)
0.1g $FeSO_4 \cdot 7H_2O$
2mM 0.316g of Sodium thiosulphate
0.1g Casamino acids
100ml of Basal medium (SNB 134 p47)

brought to boil & degassed with 95%N_2 5%H_2 gas mix for 20 min.
dispensed in 10ml aliquots in vial & using a four place gas station gassed with 95%N_2 5%H_2 (≈10sec with cap in place) septum sealed & crimped with Aluminium cap & autoclaved at 121°C 14psi for 20 min

11/6/95

11/6/95 MRI scraped from plate 11/10/95 & dispersed in tube of sterile MNB & mixed then 1ml transfer to fresh anaerobic MNB & both incubated at 30°C

11/6/95

11/7/95 Routine Sub culture of aerobic plate

GIW & GIB 2 plate each on NA4
SP200 & MR1 2 plates each on LBA3
creeno spirth & v. malaya 2 plate each on MA3

growth had occurred visibly in MR1 Broth culture MNB in the original vial, open to O_2 ∴ in further tube of anaerobic MNB Medium where each would be with 0.5ml of the 11/6/95 culture

11/7/95

11/20/95 A vane Solution of SP200 was made & 0.1ml injected into 2 anaerobic vial of MNB & incubated at 30°C

11/20/95

11/22/95 500ml of R_2A agar made with 9.1g Difco R_2A (Lot # 525935D) in 500ml of H_2O autoclaved at 121°C 14psi for 35 min & poured as plate R_2A3.

made 400ml of PBS 2 sigma tablets. Lot # 45H6917 dissolved in 400ml of $18.1 M\Omega$ water dispensed into 10 x 20ml aliquot & autoclaved

11/22/95

12/5/95 Routine Sub culture of aerobic plates
MR1 & SP200 2 plate each on LBA4
GIW & GIY 2 plates each on NA7
creeno spirth & v. malaya 2 plate each on MA4,

12/5/95

12/12/95 Subculture of NOP1 1, NOP1 2 & NOP1 3 from 11/1/95 2 plates of each on ~~LBA~~ NA7 & R_2A3 for characterisation

12/12/95

12/13/95 carried out oxidase test on NA7 plate of NOP1 1,2,3 made 12/12/95 using BBL oxidase reagent dropper lot # 612026 (exp 30 NOV 96).
NOP1 1 weak +ve
NOP1 2 strong +ve
NOP1 3 weak +ve

made Gram stain on above cultures (spot 1,2,3) & also MR1 (4), SP200 (5) & Oceano (1) on Fisherbrand Gram slide lot # 179 (exp 9-96) using reagents & method as described p5 this note book. examined using Nikon microscope 100x objective
G- red rod shaped cells
G+ blue short rods, (too much red dear however)
control not reliable :- restained using new slide & new bacteria
2nd set better results are G-B Some + ve control still weak

C- red rods ∴ gram -ve ✓
C+ blue rods (weak) ∴ gram +ve ✓
1 NOPI-1 red short rods ∴ gram -ve
2 NOPI 2 red thin rods ∴ gram -ve
3 NOPI 3 red thin rods ∴ gram -ve
4) MRI red short rods gram -ve
5) SP200 red short rods gram -ve
6) ocean red long, tapered rod gram -ve

12/13/95

12/15/95 Routine subculture of aerobic plates as follow
MRI + SP200 2 plate each on LBA4
V. natrigen + oceanospirillum 2 plate each on MA4
GIW + GIB 2 plates each on NA1

transferred 3 anaerobic cultures of MRI from 12/17/95 which showed some possible denken to fresh. MNB anaerobic medium

12/18/95

12/27/95 Routine cultures of aerobic plate from 12/15/95 as follows
MRI + SP200 2 plate each on LBA5
V. natrigen + oceanospirillum 2 plate each on MA4
GIW + GIB 2 plate each on NA2

transferred NOPI 1, NOPI 2, + NOPI 3 from R2A plates (12/12/95) to fresh R2A4 plate 2 plates of each 3 gram

12/27/95

12/29/95 made 500 ml of LB agar
7.5g Agar, 5g NaCl
5g Tryptone 2.5g Yeast Extract
500 ml of H_2O 1 ml Lactate
autoclaved for 45 min at 121°C 14 psi + cooled to 45°C + poured as plate LBA6

used 4.5% H_2O_2
NOPI 1 catalase +ve
NOPI 2 Catalase -ve
NOPI 3 Catalase +ve

12/29/95

1/3/96 made 400 ml of PBS (2 x Sigma PBS tablet lot #42548891 + dispensed as 20 ml aliquot in tube + autoclaved.

1/3/96

1/5/96 made 500 ml of LB agar as follows
Lactate #943605 1 ml Agar 7.5g.
NaCl. #947723 5g Yeast Extract #5969453 2.5g
Tryptone #6227853 5g H_2O 500 ml
autoclaved 35 min @ 121°C 14 psi cooled to 45°C + poured as plate LBA7.

routine aerobic subculturing as follow
MRI + SP200 2 plates each on LBA7
V. harveyi + oceanospirillum 2 plates each on MA4
GIW + GIB 2 plate each on NA2
NOPI 1, NOPI 2, + NOPI 3 2 plates each on R2A4
also 1 plate each of NOPI, NOPI 2, + NOPI 3

on R_2A plate for anaerobic culture in anaerobic jar at 30°C

1/5/96

1/11/96 Made 500ml of modified R2A broth (R_2B) as follows

Yeast extract	0.25g	Peptone	0.25g
Casamino acids	0.25g	Dextrose	0.25g
Potassium phosphate dibasic	0.15g	Magnesium Sulfate	0.025g

500ml of H_2O autoclaved for 35 min at 121°C & 14psi

1/11/96

1/18/96 Made 200ml of PBS - 1 Sigma tablet (45H8917) in 200ml of 18.1 MΩ H_2O & dispensed in 10ml aliquots into tubes & autoclaved 20 min @ 121°C 14psi

Made 500ml of Marine Agar as 27.55g of Difco lot# 5688953 in 500ml of water brought to boil & then autoclaved for 30min at 121°C 14psi & cooled to 45°C & poured as plate MA5

1/18/96

1/24/96 Made plate of WOPI 1 - V on R2A agar WOPI IV isolated from WOPI-433-24c 4J (10°C 48hr) & WOPI V from WOPI-433-24c II as above broth made 1/22/96

1/24/96

2/2/96 Made routine subcultures of aerobic plates as follows

GIB & GIW	2 plate each on WA2
oceanospirillum & V. natriegens	2 plate each on MA5
SP200	2 plate on LBA8
WOPI 1 - V	2 plate each on R_2A5

Made 500ml of R_2A labelled R_2A6

2/2/96

2/14/96 Made 500ml of R2A using 9.1g Difco R2A (lot # 5259350) autoclaved 35 min poured as plate R_2A7

Made 500ml of LB Agar as follows

7.5g	Agar	5g	NaCl
2.5g	Yeast Extract	5g	Tryptone

1ml Luriola (60% Glycerol) 500ml RO H_2O

autoclave 35 min poured as plate LBA9.

Made 400ml of PBS 2 Sigma tablets (45H8917) in 400ml 18.1MΩ H_2O. dispensed 10x10ml & 15 x 20ml Autoclaved

2/19/96

2/20/96 Routine Subcultures of aerobic plates (2/19/96) as follows

WOPI (I, II, III, IV, V)	2 plates each R_2A7
oceanospirilli & V. natriegens	2 plates each MA5
SP200 & MK1	2 plate each on LBA9
GIW & GIB	2 plate each on WA2

2/20/96

As a result of budget changes decided by the Nuclear Regulatory Commission for the remaining of FY 96, the Engineered Barrier System Experimental Research (EBSER) Project has been closed. Starting from January 20, 1996, the experimental research activities have been reoriented according to the prioritization of technical needs described in the Key Technical Issue of Container Life and Source Term.

The task that replaces EBSER, titled Container Life and Source Applied Technical Investigations, includes the continuation of long-term experimental studies on corrosion and stress corrosion cracking of candidate waste package materials and the effect of microbial activity on the long-term performance of these materials. The scientific notebooks for activities under EBSER will continue to be used for similar activities that are contained in the new task.

2/23/96

3/8/96 Routine Subculture of aerobic poured plates

G1W & G17 2 plate each on NA2
SP200 2 plate on LBA9
Oceanospirillum & Vibrio natriegens 2 plate each on MA6

3/8/96

3/15/96 made 500ml of Nutrient agar Difco lot # 136223 G 15.5g in 500ml H_2O autoclaved at 121°C 14psi for 1hr (used 4 x 100ml) cooled & poured as plate NA3

made 500ml of Marine Agar Difco lot # 568895 D 27.55g in 500ml H_2O autoclaved as part of above run, cooled & poured as plate MA6

made 500ml of LB agar (Lactate) as follow

Agar	7.5g	Tryptone	5g
NaCl	5g	yeast extract	2.5g

Lactate 60% Syrup 1ml 500ml H_2O autoclaved in above run, cooled & poured as plate LBA1

Also made 500ml of TSB using 7.5g in 500ml of H_2O autoclaved with above run & used as blanket check for pH probe.

3/15/96

3/16/96 Lactate/Acetate medium anaerobic for SRB

N-acetate	2.8g	Sodium lactate	2.91ml (60% Syrup)
Yeast Extract	1g	Ascorbic Acid	0.1g
$MgSO_4 \cdot 7H_2O$	0.5g	Na_2SO_4	0.5g
K_2HPO_4	0.5g	NH_4Cl	0.5g
$FeSO_4 \cdot 7H_2O$	0.1g	NaCl	7g

1000ml H_2O
boiled & degassed for 15 min with 5% H_2 95% N_2 dispensed into tubes (9ml) & degassed & stoppered autoclaved for 20 min at 14 psi 121°C.

3/12/96

5/10/96 Made routine transfer of Aerobic plate as follow

G1B & G1W	2 plates	each on	NA3
oceanospirillum	2 plate	on	MA6
V. natriegens	2 plate	on	MA6
SP200	2 plate	on	LBA9

5/10/96

5/5/96 Made LB Agar (500ml) as follows:

NaCl	5g	Tryptone	5g
Yeast Extract	2.5g	Agar	7.5g
Na Iodate (60%)	1ml	H_2O	500ml

autoclaved for 30 min at 14psi 121°C cooled to 45° poured as plate LBA1

5/1/96

5/20/96 Made 500ml of Nutrient agar: 15.5g of Difco 165% Nutrient Agar. Lot# 1362256 in 500 ml RO water. autoclaved 30 min @ 14psi 121°C. cooled & poured as plate NA4

Made 500ml of Marine agar: 27.55g of Difco Marine Agar 2216 Lot# 5688956 in 500 ml of RO water boiled & autoclaved at 14psi 121°C for 30 min cooled to 45°C & poured as plate MA7

5/20/96

5/21/96 Routine Sub culture of aerobic plates as follows

GIB & GIW	2 plates each on NA4
oceanospirillum	2 plates on MA7
V. diazogenes	2 plate on MA7

Entries will now be made in the Scientific notebook by Alice Stone trained in Biology/microbiology at UTSA & Supervised

in the lab by Dr. Peter Ingall

5/21/96

5/21/96 Made 1 Liter of 9K media as follows:

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

which was then dissolved in 700ml of distilled water. 1ml of 10N H_2SO_4 was added to adjust the pH to 2.0-2.5 and then autoclaved at 14psi 121°C for 45min.

$FeSO_4 \cdot 7H_2O$ 946178 44.22g was added to 300ml of distilled water and heated. The mixture was then filtered with an ACRO 50A 0.2um Lot # 1705 for sterilization

5/21/96 Made 400m/s of Pbs as follows: 2 phosphate buffered saline tablets added to 400m/s of RO water. Dispensed in 20ml aliquots and autoclaved for 30 minutes at 14psi 121°C. PBS tablet Lot # 45118917

Alice Stone

5/21/96 Made 50ml of gelatin-suspending media as follows:

gelatin powder	lot# 59480JH	5.0g
NaCl	947723	0.5g
yeast extract	59695JB	0.5g
peptone	63928JB	0.8g
inositol	75642JA	2.5g

H_2O 50ml

solids were dissolved by gentle heating and mixture was then distributed to 6ml. bottles in 3ml volumes which were then autoclaved for 20min at 14psi and 121°C.

Alice Stone

5/21/96 Made 1000ml of Lactate/acetate medium as follows:

sodium acetate	lot# 950091	2.8g
sodium lactate	943605	2.91ml
yeast extract	59695JB	1.0g
ascorbic acid	947449	0.1g
$MgSO_4 \cdot 7H_2O$	947409	0.5g
Na_2SO_4	901213	0.5g
K_2HPO_4	950082	0.5g
NH_4Cl	895752	0.5g
$FeSO_4 \cdot 7H_2O$	946178	0.1g
NaCl	947723	7.0g
H_2O	1000ml	

boiled and degased with 95% N_2 & 5% H_2, dispensed into 9ml aliquots and autoclaved for 20min at 121°C 14psi Alice Stone 5/21/96

5/23/96 Made 2x 500ml of modified R2A broth (R2B) as follows:

yeast extract	lot# 59695JB	0.25g
protease peptone	63928JB	0.25g
casamino acids	70866JB	0.25g
dextrose	64682JA	0.25g
soluble starch	953432	0.25g
sodium pyruvate	955829	0.15g
potassium phosphate	950082	0.15g
magnesium sulfate	947409	0.025g

per 500ml of H_2O. Autoclaved for 45 min at 14psi and 121°C.

Alice Stone

Routine anaerobic subcultures as follows:

2 tubes of 29594	D. vulgaris
2 tubes of MB	D. vulgaris
2 tubes of 27774	D. desulfuricans
2 tubes of 29577	D. desulfuricans

in fresh bottles lactate/acetate media.

Alice Stone

Made 200ml of PBS + 1% sodium pyrophosphate media as follows:

1 PBS tablet lot# 45H8917
sodium pyrophosphate 64H1002 0.2g
20ml 18 1mΩ H_2O; dispensed in 10ml aliquots. stirred and autoclaved for 20min at 14psi and 121°C

5/23/96 Alice Stone

5/27/96 Made 500ml of R2A agar as follows:
9.1g R2A Agar lot # 52593JD
500ml 18.1mΩ H_2O
autoclaved for 30min at 14psi and 121°C. Poured as plates labeled R2A8
Alice Stone 5/27/96

5/28/96 Routine subculture of aerobic plates as follows:
GIB & GIW 2 plates each on NA4
oceanospirillum 2plates on MA7
V. natrigens 2plates on MA7

Made a lawn of each organism as follows:

GIB & GIW	each on	NA4
oceanospirillum	on	MA7
V. natrigens	on	MA7

Made 100ml of marine broth as follows:
marine broth lot # 51401JA 3.74g
100ml 18mΩ H_2O was stirred and dispensed in 10ml aliquots to small tubes. Autoclaved for 25min at 14psi and 121°C.

Made 100ml of LB broth as follows:

tryptone	lot # 62278JB	1.0g
NaCl	947723	1.0g
yeast extract	59695JB	0.5g

100ml 18mΩ H_2O stirred and dispensed in 10ml aliquots to small tubes. Autoclaved for 25min at 14psi and 121°C.
5/28/96 Alice Stone

6/3/96 Made 400ml of PBS as follows:
Added 2 phosphate buffered saline tablets (lot # 45H8917) to 400ml of 18.1mΩ H_2O. Stirred to dissolve tablets and dispensed in 10ml aliquots. Autoclaved for 30min at 14psi and 121°C.

Routine subculture of aerobic plates as follows:
GIB 2 plates on NA4
GIW 2 plates on NA4
oceanospirillum 2 plates on MA7
V. natrigens 2 plates on MA7

Made a lawn of each organism as follows:

GIB & GIW	each on NA4
oceanospirillum	on NA4
V. natrigens	on NA4

Made 1000ml of R2B as follows:

yeast extract	lot # 59695JB	0.50g
protease peptone	63928JB	0.50g
casamino acids	70866JB	0.50g
dextrose	64682JA	0.50g
soluble starch	953432	0.50g

sodium pyruvate	955829	0.30 g
potassium phosphate	950082	0.30 g
magnesium sulfate	947409	0.05 g

Autoclaved for 45 min at 14psi and 121°C.

Alice Stone 6/3/96

6/4/96 Made 500ml of R2A agar as follows:

R2A agar	lot# 52593JD	9.1 g
18.1 mΩ H_2O		500 ml

Autoclaved for 30min at 14 psi and 121°C. Poured as plates labeled R2A9.

Alice Stone

Made 500ml of minimal media as follows:

sodium lactate	lot# 943605	0.28g
Casamino acid	70866JB	0.025g

500 ml 18.1 mΩ H_2O. Boiled and dispensed in 50ml aliquots. Degased with 95% N_2 & 5% H_2O. Autoclaved for 20min at 121°C and 14psi.

Alice Stone

6/4/96 suspended oceanospirillum & V. natriegens in 1.5ml of MB pH4 grown made 6/3/96 and mixed in 3ml of gelatin suspending medium pH2 melted @ held at 37°C mixture plated as 20 µl drops in 3 small petri dishes frozen @ -20°C & freeze dried overnight

6/5/96

Made 1 liter of 9K media as follows:

$(NH_4)SO_4$	lot# 953346	3.0g
KCl	946305A	0.1g
K_2HPO_4	950082	0.5g
$MgSO_4 \cdot 7H_2O$	947409	0.5g
$Ca(NO_3)_2$	961948	0.01g

was dissolved in 700ml of 18.1mΩ H_2O. 1ml of 10N H_2SO_4 was added to adjust the pH. Autoclaved for 60min at 121°C and 14psi.

Made 300ml of $FeSO_4$ supplement by adding 44.22g of $FeSO_4 \cdot 7H_2O$ Lot# 946178 in 300ml of 18.1 mΩ H_2O filtered thru a 0.2 µm filter into 9K above

Suspended GIW & GIB in 1.5 ml of MB pH4 from plates seeded 6/3/96 then mixed 0.5 ml of 3 ml of gelatin suspending medium pH2 mixture used to form 20 µl drops on a small petri dish & frozen then freeze dried

Checked viability of freeze dry specimens by placing one chip of each in a broth media.

1 chip oceanospirillum in marine broth.
1 chip V. natrigens in marine broth.
1 chip GIB in LB
1 chip GIW in LB

6/5/96 Alice Stone

6/10/96

Made 500ml of R2A agar as follows: 9.1g Difco R2A agar lot # 52593JD in 500ml 18.1mΩ H_2O. Autoclaved for 45min at 121°C and 14psi. Cooled and poured as plates labeled R2A1.

Made 500ml of nutrient agar as follows: 15.5g of Difco 1.5% Nutrient agar lot # 13622JG in 500ml 18.1mΩ H_2O. Autoclaved 45min at 121°C and 14psi. Cooled and poured as plates labeled NA5.

Made 500ml of marine agar as follows: 27.55g of Difco Marine agar 2216 lot # 56889JB in 500ml 18.1mΩ H_2O. Autoclaved 30min at 121°C and 14psi. Cooled and poured as plates labeled MA8.

Routine subcultures of aerobic plates:

G1B	2 plates on NA4
G1W	2 plates on NA5
oceanospirillum	2 plates on MA7
V. natrigens	2 plates on MA7

Plated out freeze dried specimens:

oceanospirillum	on MA7
V. natrigens	on MA7
G1B	on NA5
G1W	on NA5

6/11/96

Made 400ml of PBS as follows: Added 2 phosphate buffered saline tablets (lot # 45H8917) to 400ml of 18.1mΩ H_2O. stirred and dispensed in 10ml & 20ml aliquots. Autoclaved for 30min at 121°C and 14psi.

Alice Stone 6/10/96

Made 2 x 500ml of R2B as follows:

Yeast extract	lot # 59695JB	0.25g
protease peptone	63928JB	0.25g
casamino acids	70866JB	0.25g
dextrose	64682JA	0.25g
soluble starch	953432	0.25g
sodium pyruvate	955829	0.15g
potassium phosphate	950082	0.15g
magnesium sulfate	947409	0.025g

per 500ml 18.1mΩ H_2O. Autoclaved for 45min at 121°C and 14psi.

Made 100ml of nutrient broth as follows: Difco nutrient broth lot # 79113JF 0.8g in 100ml 18.1mΩ H_2O. Dispensed in 10ml aliquots and autoclaved for 30min.

Made 10% thiobacillus medium as follows:

$(NH_4)_2SO_4$	lot # 953346	0.08g

KH_2PO_4	lot# 946016A	0.04g
$MgSO_4 \cdot 7H_2O$	947409	0.016g

placed in 700ml of 18.1mΩ H_2O and Autoclaved for 45 min.

$FeSO_4 \cdot 7H_2O$	946178	2.0g

placed in 300ml 18.1mΩ H_2O along with 0.20ml $1N H_2SO_4$ and filter sterilized. 700ml and 300ml solutions were combined and placed in 2x 500ml media bottles.

Alice Stone 6/11/96

6/12/96 Plated out GIB & GIW freeze dried specimens in nutrient broth tubes.

Received plates of SP200, MR1 & MIR4 from Dr. Ken Nealson's Lab. plated onto LB plates supplied with culture & onto LBA1 plate. total 3 plates per culture incubated at 30°C

6/12/96

6/13/96 Made 500ml of LB Agar as follows:

NaCl	lot# 947723	5.0g
Yeast Extract	59695JB	2.5g
tryptone	62278JB	5.0g
Agar (Difco)		7.5g
lactate 60%	943605	1ml

500ml 18.1mΩ H_2O Autoclaved for 30 min at 121°C and 14psi. Poured as plates labeled LBA2.

Made MN Agar as follows:
50ml 10x modified MN medium
5ml of 10x NaCl
0.5ml trace minerals
7.5g Agar
7.2ml 0.2% Bromthyol blue sol.
into 450ml 18.1mΩ H_2O. pH checked and found to be 7.00. Autoclaved 30 min at 14psi and 121°C and poured as plates labeled MNA1

Alice Stone 6/13/96

6/14/96 Made 600ml of PBS as follows:
Added 3 PBS tablets (lot# 45H8917) to 600ml 18.1mΩ H_2O. Stirred and dispensed in 10ml & 20ml aliquots. Autoclaved for 30min at 121°C and 14psi.

Alice Stone 6/14/96

6/17/96 Routine subcultures of aerobic plates

GIB	2	plates on NA5
GIW	2	plates on NA5
oceanospirillum	2	plates on MA8
V. natrigens	2	plates on MA8
SP200	2	plates on LBA2
MR1	2	plates on LBA2
MR4	2	plates on LBA2

Made a lawn of each organism as follows:

oceanospirillum	on MA8
V. natrigens	on MA8
GIB	on NA5
GIW	on NA5
SP200	on LBA2
MR1	on LBA2
MR4	on LBA2

Alice Stone 6/17/96

6/18/96 Made 500 ml of R2A agar as follows: 9.1g Difco R2A agar lot # 52593JD in 500ml 18.1mΩ H_2O. Autoclaved for 45 min at 121°C and 14psi. Cooled and poured as plates labeled R2A2.

Made 2 x 500ml R2B as follows:

yeast extract	lot # 59695JB	0.25g
protease peptone	63928JB	0.25g
casamino acids	70866JB	0.25g
dextrose	64682JA	0.25g
soluble starch	953432	0.25g
sodium pyruvate	955829	0.15g
potassium phosphate	950082	0.15g
magnesium sulfate	947409	0.025g

per 500ml 18.1mΩ H_2O. Autoclaved for 45min at 121°C and 14psi

Made 500 ml of LB agar as follows:

NaCl	lot # 947723	5.0g
yeast extract	59695JB	2.5g
tryptone	62278JB	5.0g
Agar		7.5g
lactate	943605	1ml

500ml 18.1mΩ H_2O. Autoclaved for 30min at 121°C and 14psi. Poured as plates labeled LBA3

Suspended MR1, MR4, ½ SP200 in 1.5ml of LB (p.44/138) from plates seeded 6/17/96. Then took a 0.5ml sample from each and dispensed in 3ml of gelatin suspending medium (p. 42/138). Vortexed and placed 20ul drops on a small petri plate. 20ul Samples were then frozen and freeze dried overnight.

Alice Stone 6/18/96

6/20/96 Made 400 ml of PBS as follows: Added 2 phosphate buffered saline tablets (lot # 45H8917) to 400ml of 18.1mΩ H_2O. Stirred and dispensed in 20ml & 10ml aliquots. Autoclaved for 45 min at 121°C and 14psi.

Made 500ml of LB agar as follows:

NaCl	lot# 947723	5.0g
yeast extract	5969JB	2.5g
tryptone	62278JB	5.0g
Agar		7.5g
lactate 60%	943605	1ml

500ml 18.1 mΩ H_2O. Autoclaved for 30 min at 14psi & 121°C. Poured as plates labeled LBA4.

6/21/96

made 100ml of LB broth as follows

NaCl	lot# 947723	1.0g
Yeast extract	5969JB	0.5g
tryptone	62278JB	1.0g
lactate	943605	0.2ml

100 ml 18.1 mΩ H_2O.. Autoclaved for 30 min at 14psi & 121°C.

Made 1L of lactate/acetate (10%) medium as follows:

sodium acetate	lot# 950091	0.28g
sodium lactate	lot# 943605	0.29ml
yeast extract	59695JB	0.10g
ascorbic acid	947449	0.01g
$MgSO_4 \cdot 7H_2O$	947409	0.05g
Na_2SO_4	901213	0.05g
K_2HPO_4	(895752)	0.05g
NH_4Cl	950082	0.05g
$FeSO_4 \cdot 7H_2O$	946178	0.01g
NaCl	947723	0.7g

1000 ml 18.1 mΩ H_2O. Autoclaved for 45 min at 121°C & 14psi.

6/24/96

Made 500ml of R2A agar as follows:
9.1 g Difco R2A agar lot# 52593JD in 500ml 18.1 mΩ H_2O. Autoclaved for 45 min at 121°C and 14psi. Cooled and poured as plates labeled R2A3. Alice Stone 6/24/96

Placed 1 freeze dried chip of MR1, MR4, & SP200 in 10ml tubes of LB broth.
Alice Stone 6/20/96

Plated out MR1, MR4, SP200 from LB broth onto LBA3 to check for viability & contamination.
6/21/96 Alice Stone

Routine subcultures of aerobic plates

G113	2 plates on NA5
G1W	2 plates on NA5
oceanospirillum	2 plates on MA8
V. natrigens	2 plates on MA8
SP200	1 plate on LBA4
MR1	1 plate on LBA4
MR4	1 plate on LBA4

Alice Stone
6/24/96

Pages 56 and 57 Are Intentionally
Left Blank

6/25/96 Made 600 ml of PBS as follows: Added 3 PBS tablets lot # 45H8917 to 600 ml 18.1 mΩ H_2O. Stirred and dispensed in 10 ml & 20 ml aliquots. Autoclaved for 45 min at 121°C and 14 psi.

Alice Stone 6/25/96

6/26/96 Routine subcultures of Ym isolates (YmI 1 - YmI 8).

Alice Stone 6/26/96

6/27/96 Made 500 ml of LB agar as follows:

NaCl	lot #947723	5.0 g
yeast extract	5969JB	2.5 g
tryptone	62278JB	5.0 g
agar		7.5 g
lactate	943605	1 ml

500 ml 18.1 MΩ H_2O. Autoclaved for 45 min at 14 psi and 121°C. Poured as plates labeled LBA5.

Made 500 ml R2A agar as follows: 9.1 g Difco R2A agar (lot # 52593JD) in 500 ml 18.1 MΩ H_2O. Autoclaved for 45 min at 14 psi and 121°C. Poured as plates labeled R2A4.

Made 500 ml of nutrient agar as follows: 15.5 g Difco 1.5% NA lot # 13622JG in 500 ml 18.1 MΩ H_2O Autoclaved for 45 min at 14 psi and 121°C. Poured as plates labeled NA6.

Performed Gram Stain and Oxidase test on Ym isolates. Preliminary results:

YmI-1	short rods, G+, ox+
YmI-2	short rods, G+, ox-
YmI-3	short rods, G+, ox-
YmI-4	med-long rods, G+, ox+, poss. spore former
YmI-5-1	short rod, G-, ox-
YmI-5-2	short rod, G-, ox-
YmI-6	short rod, G+, ox-
YmI-7	long rod, G-, ox+ (?)
YmI-8	short & long rods, G-, ox-

6/27/96 Alice Stone

6/28/96 Made 600 ml of PBS as follows: added 3 PBS tablets (lot # 45H8917) to 600 ml 18.1 mΩ H_2O. Stirred and dispensed in 10 ml & 20 ml aliquots. Autoclaved for 45 min at 121°C and 14 psi.

Alice Stone

7/1/96 Routine subcultures of Ym isolates and aerobic plates

Gram stained YmI-9 & YmI-10

YmI-9 G+, short rods, spore former

YmI-10 G+, polymer producer, short rods

Alice Stone

6 7/2/96 Made 500 ml of LB agar as follows:

NaCl	lot# 947723	5.0 g
yeast extract	596916	2.5 g
tryptone	622781B	5.0 g
agar		7.5 g
lactate	943605	1 ml

500 ml 18.1 mΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi, (LBA6)

Made 500 ml of R2A as follows:
9.1 g Difco R2A agar (lot # 525931D) in 500 ml 18.1 mΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi. Poured as plates labeled R2A5.
7/2/96 Alice Stone

7/5/96 Made 2 x 500 ml R2A agar as follows: placed 9.1 g Difco R2A (lot #525931D) per 500 ml 18.1 mΩ H_2O. Autoclaved for 45 min at 14 psi and 121°C. Poured as plates labeled R2A6

Bacteroid isolates made by Prof. P. Long's staff in Div. of Biol Sciences under as part of DOE microbial characterization of YM were received @ 10 am on 4th July 1996 by Peter Engell @ home having been shipped by U.S. Mail Express by chief glacier work site off Y.M has Vega (packed 3rd July 1996) Samples were on slants or in FeOx media packed - cardboard tubes in side an insulated

box cooled with freezer packs. Samples were identified as follows

YM 10-M-02,
YM 10-A-02
YM 10-M-05
YM 10-Q-06
YM 10-H-12

two tubes labelled FeOx these were additionally subsequently labelled as YM FeOx -1 & YM FeOx -2 on 5th July samples were brought to lab & streaked onto fresh R2A plates & FeOx tubes 2:0,1 inoculated into 5 ml of 9x this medium all samples incubated at 30°C [signature] 7/5/96

Made 2 x 500 ml R2B as follows:

yeast extract	lot# 59695JB	0.25 g
protease peptone	63928JB	0.25 g
casamino acids	70866JB	0.25 g
dextrose	64682JA	0.25 g
soluble starch	953432	0.25 g
sodium pyruvate	955829	0.15 g
potassium phosphate	950082	0.15 g
magnesium sulfate	947409	0.025 g

per 500 ml 18.1 mΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi.
Alice Stone 7/5/96

Made 500 ml of marine agar as follows:
27.55 g of Difco Marine Agar 2216 lot #56889JB in 500 ml 18.1 mΩ H_2O. Autoclaved 45 min at 121°C and 14 psi. Cooled and poured as plates labeled MA9.

Made 600 ml of PBS as follows:
Added 3 PBS tablets ~~lot~~ #45H8917 to 600ml 18.1 mΩ H_2O. Stirred and dispensed in 10 ml & 20 ml aliquots. Autoclaved for 45 min at 121°C and 14 psi.

Made 500ml LB agar as follows:

NaCl	~~lot~~ #947723	5.0g
yeast extract	5969JB	2.5g
tryptone	6227BJB	5.0g
agar		7.5g
lactate	943605	1.0ml

500 ml 18.1 mΩ H_2O. Autoclaved 45 min at 121°C and 14 psi: (LBA7)

Routine subculture of aerobic plates as follows:

Ym I 1 - Ym I 10	on R2A5
MR1, MR4, SP200	on LBA6
oceanospirillum	on MA8
V. natrigens	on MA8
GIB & GIW	on NA6

Alice Stone 7/8/96

7/9/96 Made 500ml of Hughes & Lefson's media as follows

peptone	lot# 6378BJB	1.0g
NaCl	947723	2.5g
K_2HPO_4	950082	0.15g

Agar		1.5g

500ml H_2O. Solids dissolved by heating, cooled and pH adjusted to 7.1. 0.2% aqueous solution of Bromthymol blue was added and autoclaved along with tubes. 5g of dextrose lot # 64682JA was added to 15ml 18.1 mΩ H_2O. Dextrose sol was 0.2um filter sterilized and added to media. Dispensed in 10 ml aliquots. 7/9/96

7/10/96 Innoculated Hughes & Lefson's deeps with YM-Isolates (1 – 10) and (Ym-10-A-04, Ym-10-H-12, Ym-10-H-5, Ym-10-Q-06, & Ym-10-M02). Each bacteria was innoculated twice & one was covered with sterile petroleum to create anaerobic conditions.

Plated Ym isolates on LBA6.

Made 1L of 9K media as follows:

$(NH_4)SO_4$	lot# 953346	3.0g
KCl	946305A	0.1g
K_2HPO_4	950082	0.5g
$MgSO_4 \cdot 7H_2O$	947409	0.5g
$Ca(NO_3)_2$	961948	0.1g

was dissolved in 700ml 18.1 mΩ H_2O and Autoclaved for 60 min at 121°C and 14 psi. Made 300 ml of $FeSO_4$

supplement by adding 44.22 g of $FeSO_4 \cdot 7H_2O$ lot # 946178 in 300 ml 18.1 MΩ H_2O. Filter sterilized (0.2um) and added to 9K media.

Alice Stone 7/10/96

7/15/96 Made 500ml of LB agar as follows:

NaCl	lot# 947723	5.0g
yeast extract	5969JB	2.5g
tryptone	62278JB	5.0g
agar		7.5g
Na lactate	943605	1.0ml

500ml 18.1 MΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi. Cooled and poured as LBA8.

Alice Stone 7/15/96

7/16/96 Made 500ml of R2A as follows: 9.1g Difco R2A agar (lot#52593JD) in 500ml 18.1 MΩ H_2O. Autoclaved for 45 min at 14 psi and 121°C. Poured as plates labeled R2A7.

Made 1L of lactate/acetate media as follows:

sodium acetate	lot# 950091	2.8g
sodium lactate	943605	2.99ml
yeast extract	5969SJB	1.0g
ascorbic acid	947749	0.1g
$MgSO_4 \cdot 7H_2O$	947409	0.5g
Na_2SO_4	901213	0.5g

K_2HPO_4	950082	0.5g
NH_4Cl	895752	0.5g
$FeSO_4 \cdot 7H_2O$	946178	0.1g
NaCl	947723	7.0g
H_2O	1000ml 18.1 MΩ H_2O	

boiled and degased with 95% N_2 & 5% H_2. Dispensed into 9ml aliquots and autoclaved for 30 min at 121°C and 14psi

Alice Stone 7/16/96

7/17/96 Routine subculture of anaerobic bacteria as follows:

D. vulgaris 25779
D. desulfuricans 27774
D. vulgaris MB
D. desulfuricans 29577

0.5ml of each placed in fresh lactate/acetate media prepared on p. 64/138.

Alice Stone 7/17/96

7/20/96 Subcultured aerobic plates of SP200, Ym-10-A-04, Ym-10-H-12, Ym-10-0-06 Ym-10-H-05, & Ym-10-M-02.

Alice Stone 7/20/96

7/22/96 Routine subculture of aerobic plates as follows:

G1B	2 plates on NA6
G1W	2 plates on NA6

oceanospirillum	2 plates on MA9
V. natrigens	2 plates on MA9
Ym-10-A-04	1 plate on R2A7
Ym-10-H-12	"
Ym-10-H-05	"
Ym-10-M-02	"
Ym-10-Q-06	"
SP200	1 plate on LBA8
MR1	"
MR4	"
YmI-1 - YmI-10	1 plate ea on R2A7

Innoculated 6 variants of modified J13 media with 0.5 ml of D. vulgaris 295779.

Alice Stone 7/22/96

7/23/96 Made 500 ml of LB agar as follows:

NaCl	lot# 947723	5.0g
yeast extract	5969JB	2.5g
tryptone	62278JB	5.0g
agar		7.5g
Na lactate	943605	1.0 ml

500 ml 18.1 MΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi. Cooled and poured as LBA9.

Made 2 x 500 ml of R2A as follows: 9.1g Difco R2A agar (lot# 525933D) per 500 ml 18.1 MΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi. Cooled

and poured as plates labeled R2A8.

Made 2 x 500 ml R2B as follows:

yeast extract	lot# 59695JB	0.25g
protease peptone	63928JB	0.25g
casamino acids	70866JB	0.25g
dextrose	64682JA	0.25g
soluble starch	953432	0.25g
sodium pyruvate	955829	0.15g
potassium phosphate	950082	0.15g
magnesium sulfate	947409	0.025g

per 500 ml 18.1 MΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi.

Alice Stone 7/23/96

7/24/96 Checked growth present in 6 variants of mod. J13 media innoculated with 0.5 ml D. vulgaris 295779 (p. 66/138).

1	Basal	—
2	Basal + 10 ppm YE	+
3	Basal + 1 ppm AA	+
4	Basal + 10 ppm AA	+
5	Basal + 10 ppm YE & AA	+
6	Basal + 10 ppm YE + 1 ppm AA	+

Alice Stone 7/24/96

7/26/96 Routine subculture of Ym-FeOx-1, Ym-FeOx-2 & biotic into 5 ml of 9k thio media. Incubated @ 30°C. Alice Stone

7/29/96 Routine subculture of aerobic plates as follows:

G1B	2 plates on NA6
G1W	2 plates on NA6
oceanospirillum	2 plates on MA9
V. natrigens	2 plates on MA9
Ym-10-A-04	1 plate on R2A8
Ym-10-H-12	"
Ym-10-H-05	"
Ym-10-M-02	"
Ym-10-Q-06	"
SP200	1 plate on LBA8
MR1	"
MR4	"
YmI-1 - YmI-10	1 plate on R2A8

Alice Slone 7/29/96

7/30/96 Made 500ml of R2A as follows: 9.1 g Difco R2A agar (lot# 52593JD) in 500ml 18.1MΩ H2O. Autoclaved for 45 min at 121°C and 14psi. Poured as plates R2A9.

Made 600 ml of PBS as follows: Added 3 PBS tablets lot # 45H8917 to 600ml 18.1MΩ H2O. Stirred and dispensed in 10ml & 20ml aliquots. Autoclaved for 45 min at 121°C and 14psi.

Alice Slone 7/30/96

8/7/96 Routine subculture of aerobic plates as follows:

G1B & G1W	2 plates ea. on NA6
oceanospirillum	2 plates on MA9
V. natrigens	2 plates on MA9
Ym-10-A-04	1 plate on R2A8
Ym-10-H-12	"
Ym-10-H-05	"
Ym-10-M-02	"
Ym-10-Q-06	"
SP200	1 plate on LBA9
MR1 & MR4	"
YmI-1 - YmI-10	1 plate ea on R2A8

Alice Slone 8/7/96

8/8/96 Made 2 x 500 ml of R2A as follows: 9.1g Difco R2A agar (lot# 52593JD) per 500 ml 18.1MΩ H2O. Autoclaved for 45 min at 121°C and 14 psi. Poured as plates labeled R2A1

Made 500ml of LB agar as follows:

NaCl	lot# 947723	5.0 g
yeast extract	5969JB	2.5 g
tryptone	62278JB	5.0 g
agar		7.5 g
lactate	943605	1.0 ml

500 ml 18.1MΩ H2O. Autoclaved for 45 min at 121°C and 14 psi.

Cooled and poured as plates labeled LBA1

Made 500 ml of nutrient agar as follows: 15.5 g Difco 1.5% NA lot# 136222G in 500 ml 18.1 MΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi. Cooled and poured as plates labeled NA7.

Made 500 ml of marine agar as follows: 27.55 g of Difco marine agar 2216 lot# 5689JB in 500 ml 18.1 MΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi. Cooled and poured as plates labeled MA1.

Alice Stone 8/8/96

8/9/96 Made 2×500 ml R2B as follows:

yeast extract	lot# 59695JB	0.25 g
protease peptone	63928JB	0.25 g
casamino acids	70866JB	0.25 g
dextrose	64682JA	0.25 g
soluble starch	953432	0.25 g
sodium pyruvate	955829	0.15 g
potassium phosphate	950082	0.15 g
magnesium sulfate	947409	0.025 g

per 500 ml 18.1 MΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi.

Streaked Ym-10-A-04, Ym-10-H-12, Ym-10-H-05, Ym-10-M-02, & Ym-10-Q-06 on LBA to check for T12B.

Alice Stone 8/9/96

8/12/96 Routine subculture of aerobic plates as follows:

G1B & G1W	2 plates ea on NA6
oceanospirellum	2 plates on MA9
V. natriegens	2 plates on MA9
Ym-10-A-04	1 plate on R2A9
Ym-10-H-12	"
Ym-10-H-05	"
Ym-10-M-02	"
Ym-10-Q-06	"
SP200, MR1, MR4	1 plate ea on LBA9
YmI1-YmF10	1 plate ea on R2A9

Alice Stone 8/12/96

8/15/96 Made 400 ml of PBS as follows added 2 PBS tablets (lot# 45H8917) to 400 ml 18.1 MΩ H_2O. Stirred and dispensed in 10 ml & 20 ml aliquots. Autoclaved for 45 min at 121°C and 14 psi.

Prepared 500 ml of peptone water as follows:

peptone	lot# 63928JB	5.0 g
NaCl	lot# 951450	2.5 g

into 500 ml 18 MΩ H_2O. Dissolved solids by heating. Adjusted pH to 8.0 and boiled for 10 min. Adjusted pH to 7.2. Added 5 ml of Bromthymol Blue indicator and autoclaved for 20 min at 121°C and 14 psi. Dissolved 5 g of dextrose (lot # 646823A) in 50 ml of 18 MΩ H_2O and 0.2 um filter sterilized. Added to sterile peptone water along with indicator. Distributed into sterile test tubes

Alice Stone 8/15/96

8/16/96 Innoculated peptone water with Ym-10-H-12, Ym-10-H-05, Ym-10-A-04, Ym-10-Q-06, Ym-10-M-02, & YmI-1 – YmF-10.

Results from quick TRB check indicate that Ym-10-Q-06 or Ym-10-H-12 may be TRB. Alice Stone

8/19/96 Results from glucose acid production test indicate all organisms are acid producers

Made 500ml of R2A as follows: 9.1 g. Difco R2A agar (lot # 525931D) in 500ml 18.1 MΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi. Poured as plates labeled R2A2.

Made 500ml of LBA as follows

NaCl	5.0 g
yeast extract	2.5 g
tryptone	5.0 g
agar	7.5 g
lactate	1.0 ml

500ml 18.1 MΩ H_2O. Autoclaved for 45 min at 121°C and 14 psi. Plates labeled LBA2.

Routine subcultures of aerobic plates as follows:

G16 & G1W	2 plates ea on NA6
oceanosparillum	1 plate on MA9
V. natriegens	1 plate on MA9
Ym-10-A-04	1 plate on R2A9
Ym-10-H-12	"
Ym-10-H-05	"
Ym-10-M-02	"
Ym-10-Q-06	"
SP200, MR1 & MR4	1 plate ea on LBA9

Alice Stone 8/19/96

8/26/96 Performed routine subcultures of all aerobic plates same as before
Alice Stone 8/26/96

8/29/96 Made 500ml of R2A as follows: 9.1g Difco R2A agar (lot# 52593JD) in 500ml 18.1MΩ H2O. Autoclaved for 45 min at 121°C and 14psi. Poured as plates labeled R2A3.

Made 500 ml of Levan test media as follows: placed 15.5g of nutrient agar in ~~50~~ ml of 18.1MΩ H2O. Placed 25g of Dextrosen in 50ml of 18.1 mΩ H2O. Autoclaved both bottles for 20 min at 121°C and 14psi. Combined as they cooled and poured as plates labeled LPI.
Alice Stone 8/29/96

8/30/96 streaked LPI plates with YmI-1 – YmI-10 to check for possible EPS producers
Alice Stone 8/30/96

9/3/96 Performed routine subcultures of all aerobic plates same as before. Alice Stone 9/3/96

9/5/96 streaked LPI plates with YmI-1 → YmI-10 to check for possible polymer producers.

Made 500ml of R2A as follows: 9.1g Difco R2A agar (lot# 52593JD) in 500ml 18.1MΩ H2O. Autoclaved for 45 min at 121°C and 14psi. Poured as plates labeled R2A4.
Alice Stone 9/5/96

9/9/96 Performed routine subcultures of all aerobic plates; same as before.
Alice Stone 9/9/96

9/16/96 Performed routine subcultures of all aerobic plates, same as before

Made 2X500ml of R2A as follows: 9.1g Difco R2A agar (lot# 52593210) per 500ml 18.1MΩ H2O. Autoclaved for 45 min at 121°C and 14psi. Poured as plates labeled R2A5.
Alice Stone
9/16/96

Made 500ml of R2B as follows:

yeast extract	lot# 59695JB	0.25g
protease peptone	63928JB	0.25g
casamino acids	70866JB	0.25g
dextrose	64682JA	0.25g
soluble starch	953432	0.25g
sodium pyruvate	955829	0.15g
potassium phosphate	950082	0.15g
magnesium sulfate	947409	0.025g

per 500ml 18.1MΩ H_2O. Autoclaved for 45min at 121°C and 14psi. Placed 8ml aliquots in test tubes.

Made 200ml of gelatin-suspending media as follows:

gelatin	lot# 59480JH	20.0g
NaCl	947723	2.0g
yeast extract	59695JB	2.0g
peptone	63928JB	3.2g
inositol	75642JA	10.0g

in 200ml 18.1MΩ H_2O. Solids were dissolved by gentle heating and mixture was distributed in 3ml aliquots to small test tubes. Autoclaved for 45min at 121°C and 14psi.

Alici Stone 9/16/96

9/23/96

Made 500ml of R2B as follows:

yeast extract	lot# 59695JB	0.25g
protease peptone	63928JB	0.25g
casamino acids	70866JB	0.25g
dextrose	64682JA	0.25g
soluble starch	953432	0.25g
sodium pyruvate	955829	0.15g
potassium phosphate	950082	0.15g
magnesium sulfate	947409	0.025g

into 500ml 18.1MΩ H_2O. Autoclaved for 1hr at 121°C and 14psi.

Made 500ml R2A as follows:
9.1g Difco R2A agar lot# 52593JD into 500ml 18.1MΩ H_2O. Autoclaved for 45min at 121°C and 14psi. Poured as plates R2A6

Made 500ml of LBA as follows:

NaCl	5.0g
yeast extract	2.5g
tryptone	5.0g
agar	7.5g
lactate	1.0ml

into 500ml 18.1MΩ H_2O. Autoclaved for 1hr at 14psi & 121°C. Poured as plates LBA3

Made 500 ml of nutrient agar as follows: 15.5 g Difco 1.5% NA lot# 136221G in 500ml 18.1 mΩ H_2O. Autoclaved for 1hr at 14psi and 121°C. Poured as plates NA8.

Made 500 ml marine agar as follows: 27.55g of Difco agar 2216 lot# 56889JB in 500 ml 18.1 MΩ H_2O. Autoclaved for 1hr at 121°C and 14psi. Poured as MA2

Routine subcultures of aerobic plates as follows:

G1B	2 plates on NA7
G1W	2 plates on NA7
Oceanospirillum	2 plates on MA1
V. natrigens	2 plates on MA1
SP200, MR1, & MR4	2 plates ea on LBA2
Ym-10-M-02,	1 plate ea on R2A5
Ym-10-A-04	"
Ym-10-H-05	"
Ym-10-H-12	"
Ym-10-Q-06	"
YmI-1 → YmI-10	"

9/25/96

Made 1000 ml of lactate/acetate medium as follows:

sodium acetate	lot# 950091	2.8g
Sodium lactate	943605	2.91ml
yeast extract	54695JB	1.0g
ascorbic acid	947449	0.1g
$MgSO_4 \cdot 7H_2O$	947409	0.5g
Na_2SO_4	901213	0.5g
NH_4Cl	895752	0.5g
$FeSO_4 \cdot 7H_2O$	946178	0.1g
NaCl	947723	710g

1000 ml 18.1 MΩ H_2O. Boiled & degased with 95% N_2 & 5% H_2. Dispensed in 9ml aliquots and autoclaved for 28 min at 121°C and 14psi.

Alice Stone 9/23/96

9/25/96

Routine subcultures anaerobic bacteria as follows:

D. vulgaris 25779
D. desulfuricans 27774
D. vulgaris MB
D. desulfuricans 29577

0.5 ml of each placed in fresh lactate/acetate media (p. 79/138.

Alice Stone 9/25/96

9/30/96 Routine subculture of aerobic plates performed as before on p. 78/138.

Made 2x500 ml of R2A as follows: 9.1 g Difco R2A agar lot # 5259310 per 500 ml 18.1 MΩ. H_2O. Autoclaved for 45 min at 121°C and 14 psi poured as plates labeled R2A7.

Made 500 ml R2B as follows:

yeast extract	0.25 g
protease peptone	0.25 g
casamino acids	0.25 g
dextrose	0.25 g
soluble starch	0.25 g
sodium pyruvate	0.15 g
potassium phosphate	0.15 g
magnesium sulfate	0.025 g

into 500 ml 18.1 MΩ H_2O. Autoclaved for 1 hr at 121°C and 14 psi.

Alice Stone 9/30/96

10/1/96 Made 1 L of lactate/acetate media as follows:

sodium acetate	2.80 g
sodium lactate	2.91 ml
yeast extract	1.00 g
ascorbic acid	0.10 g
$MgSO_4 \cdot 7H_2O$	0.50 g
Na_2SO_4	0.50 g
K_2HPO_4	0.50 g
NH_4Cl	0.50 g
$FeSO_4 \cdot 7H_2O$	0.10 g
NaCl	7.00 g

into 1 L 18.1 MΩ H_2O.

Alice Stone 10/1/96

10/7/96 Routine subcultures of aerobic plates performed as before on p. 78/138.

Made 500 ml of R2A as follows: 9.1 g Difco R2A agar lot # 5259310 per 500 ml 18.1 MΩ H_2O. Autoclaved for 1 hr at 121°C and 14 psi. Poured as plates labeled R2A8.

Made 500 ml of LBA as follows:

NaCl	5.0 g
yeast extract	2.5 g
tryptone	5.0 g
agar	7.5 g
lactate	1.0 ml

500 ml 18.1 MΩ H_2O Autoclaved for 45 min at 121°C and 14 psi. Poured as plates labeled LBA3.

Alice Stone 10/7/96

10/16/96 Routine subculture of aerobic plates performed as before on p. 78/138.
Alice Stone 10/16/96

10/21/96 Routine subculture of aerobic plates performed as before on p. 78/138.

5/11/96 Made 500ml of R2B & poured as plates labelled as R2A9
made 400ml of PBS & dispensed into tube (20ml) autoclaved.
5/11/96

14/11/96 Routine Subculture of aerobic plate as per p. 78, from plates 10/21/96.
14/11/96

21/11/96 Made 500ml of R2A agar poured as R2A1
21-11-96

11/12/96 Made 500ml of R2A agar poured as plate R2A2

made 9L basal medium

$(NH_4)_2SO_4$	953346	3.0g
KCl	946305A	0.1g
K_2HPO_4	950082	0.5g
$MgSO_4 \cdot 7H_2O$	951425	0.5g
$Ca(NO_3)_2$	961948	0.1g

dissolved in 700ml of 18.2 MΩ H_2O

2.11.1996

2-1-97

8.1.97

21/1/97

28/1/97

autoclaved for 60 min @ 121°C @ 14psi
made 300ml of $FeSO_4$ (44.22g @ 946175)
& filter sterilized into stock.,
11-12-96

made 500ml of R2A agar poured as plates R2A3
21/12/96

Make 2x 500ml of R2A agar plate poured as R2A4.
2-1-97

Made 400ml of PBS & dispensed into tubes (20ml) autoclaved
8-1-97

made 2x 500ml of R2A agar plates poured as ~~R2A4~~ R2A5 (autoclaved for 45min) M. Hill 21/1/97

prepared 400mL of PBS (400mL of nanopure with x2 tablets of phosphate buffered saline tablets lot # 45H8917 placed on magnetic stirer. Placed 20mL per tube & autoclaved for 30 min).
M. Hill
28/1/97

31/1/97 prepared 400 mL of PBS (400 mL of nannopure with x2 tablets of phosphate buffered saline tablets lot # 45H8917 placed on magnetic stirrer. ~~Placed in autoclave for 30 min~~. Prior to placing into autoclave measured 2.40g of $Na_4P_2O_7$ & added to 240 mL of above PBS. Dispensed into 40 mL ~~aut~~ aliquots. Remainder of PBS dispensed in x5 40 mL aliquots. Placed in autoclave for 30 min. M. Hill 31/1/97

M. Hill 31/1/97

M. Hill 31/1/97

4/2/97 made 2 x 500 ml of R2A agar plates poured as R2A6 (autoclaved for 45 min) M. Hill 4/2/97

13/2/97 prepared PBS (100 ml of nannopure with 1 tablet of phosphate buffered saline ~~lot~~ # 45H8917) 1.6 g of $Na_4P_2O_7 \cdot 10H_2O$ lot # 64H1002 was added after removing 40 ml of pure PBS. Afterwards, soln was poured into aliquots (40 ml/aliq.) Placed in autoclave for 30 min.
M. Hill 13/2/97

17/2/97 prepared 400 ml of PBS (400 ml of nannopure with x2 tablets of phosphate

buffered saline tablets lot # 45H8917 placed on magnetic stirrer. Added 2.40g of $Na_4P_2O_7 \cdot 10H_2O$ lot # 64H1002 to 240 ml of PBS. Dispensed into aliquots (40ml) while remainder of PBS was dispensed ~~in~~. Placed in autoclave for 30 min.
M. Hill 17/2/97

20/2/97 prepared 400 ml of PBS as follows (400 ml of nannopure with x2 tablets of phosphate buffered saline tablets lot # 45H8917 placed on magnetic stirrer and placed in autoclave for 30 min).
M. Hill 20/2/97

21/2/97 prepared x2 500 ml of R2A agar plates poured as R2A7 - autoclaved for 45 min.
M. Hill 21/2/97

29/4/97 prepared x2 500 ml of R2A agar as follows: 9.1g of R2A agar added to ~500 ml of deionized H_2O. Autoclaved for 45 min. Poured as R2A8.
M. Hill 29/4/97

5/5/97 prepared 400 ml of PBS as follows: 2 tablets of phosphate buffered saline tablets lot # 45H8917 were added to 400 ml of 18.1 MΩ H_2O. Autoclaved for 30 min @ 121°C & 14 psi. Prior to autoclaving PBS was dispensed (20 ml) into glass tubes. M. Hill 5/5/97

28/5/97 prepared 400 ml of PBS as follows: 2 tablets of phosphate buffered saline tablets lot # 45H8917 were added to 400 ml of 18.1 MΩ H_2O. Dispensed 40 ml of PBS into 100 ml media bottles. Autoclaved for 30 min @ 121°C 14 lb/in^2. M. Hill 28/5/97

4/8/97 prepared x2 500 ml of R2A Agar as follows: 9.1 g of R2A agar, lot # 52593JO was added to ~500 ml of deionized H_2O. Autoclaved for 45 min @ 121°C, 14 psi. Poured as R2A9 plates. M. Hill 4/8/97

13/8/97 prepared 400 ml of PBS as follows: 2 tablets of phosphate buffered saline tablets lot # 45H8917 into 400 ml of 18.1 MΩ H_2O. Added 2.40 g of

sodium pyrophosphate lot # 64H1002 to 240 ml of PBS. Dispensed 40 ml of 1% sodium pyrophosphate soln into 100 ml aliquots. Autoclaved for 30 min @ 121C, 14 psi. M. Hill 13/8/97

9/15/97 prepared 400 ml of PBS as follows: 2 tablets of phosphate buffered saline tablets lot # 45H8917 into 400 ml of 18.1 MΩ H_2O. Dispensed PBS into 20 ml tubes & autoclaved for 30 min @ 121°C, 14 psi. M. Hill 9/15/97

10/20/97 prepared x2 500 ml R2A agar as follows: 9.1 g of Difco R2A agar lot # 52593JO into 500 ml of deionized H_2O. Autoclaved for 45 min @ 121°C 14.1 psi. Poured as R2A10. M. Hill 10/20/97

11/21/97 dispensed remainder of PBS prepared on 9/15/97 (p 87 of this notebook) into 20 ml tubes and autoclaved for 30 min @ 121°C, 14 psi. M. Hill 11/21/97

12/19/97 prepared 200 ml of PBS as follows: 1 tablet of phosphate buffered saline lot # 45H8917 added to 200 ml of 18.1 MΩ H_2O. Autoclaved for 45 min @ 121 °C 14 psi.

prepared x2 500 ml R2A agar as follows: 9.1 g of Difco R2A agar lot # 52593 JD into 500 ml of deionized H_2O. Autoclaved for 45 min @ 121 °C, 14 psi. M. Hill 12/19/97

R2A agar poured as plates R2A11. M. Hill 12/19/97

1/3/98 prepared 200 ml of PBS as follows: 1 tablet of phosphate buffered saline lot # 45H8917 added to 200 ml of 18.1 MΩ H_2O. Added to PBS prepared on 12/19/97 p 88 of this notebook. Added 2.40 g of sodium pyrophosphate lot # 64H1002 to 240 ml of PBS. Dispensed 40 ml of 1% soln sodium pyrophosphate & PBS, respectively into 100 ml aliquots. Autoclaved for 30 min @ 121°C, 14 psi. M. Hill 1/3/98

4/2/98 prepared 400 ml PBS as follows: 2 tablets of phosphate buffered saline lot # 45H 8917 were added to 400 ml 18.1 MΩ H_2O. Dispensed into 40 ml tubes & autoclaved for 30 min @ 121°C, 14 psi. M. Hill 4/2/98

4/9/98 prepared 500 ml of R2A agar as follows: 9.1 g of Difco R2A agar lot # 52593 JD into 500 ml of deionized H_2O. Autoclaved for 45 min @ 121°C 14 psi. R2A agar poured as plates R2A12. M. Hill 4/9/98

8/28/98 prepared 500 ml of lactate/acetate as follows:

		Lot #
sodium acetate	14.00 g	950091
sodium lactate	14.55 g	943609
yeast extract	5.00 g	596950
ascorbic acid	0.50 g	947449
$MgSO_4 \cdot 7H_2O$	2.50 g	947409
Na_2SO_4	2.50 g	901213
K_2HPO_4	2.50 g	950092
NH_4Cl	2.50 g	895752
$FeSO_4 \cdot 7H_2O$	0.50 g	946178
NaCl	35.00 g	972174

into 500 ml 18.1 MΩ H_2O.
M. Hill 8/28/98

3/12/99 Lactate/Acetate Medium anaerobic for SRB:

			3/12/99 Lot #
Na Acetate	1.4 g	mH	950091
Yeast Extract	~~5.0~~ g	0.5 g	596955B
$MgSO_4 \cdot 7H_2O$	0.25 g	mH 3/12/99	947409
K_2HPO_4	0.25 g		950082
$FeSO_4 \cdot 7H_2O$	~~0.5~~ g	0.05 g	946678
Na Lactate	1.5 g	ml	943605
Ascorbic Acid	0.05 g		947449
Na_2SO_4	0.25 g		901213
NH_4Cl	0.25 g		895752
NaCl	3.5 g		972274

mH 3/12/99

into 500 ml deionized H_2O

boiled & degassed for 15 min with 5% H_2 & 95% N_2. Dispensed 9 ml's into tubes degassed & stoppered. Autoclaved for 10 min @ 121°C, 14.7psi gage.

Prepared 500 ml of Difco Nutrient Broth with 1.5% NaCl as follows:

Difco nutrient broth lot # 79113JF 4 g with 7.5 g of NaCl lot # 972274 into 500 ml deionized H_2O.

Dispensed 9 ml's into screw cap glass tubes. Placed in autoclave for 20 min @ 121°C, 14.7psi gage.

Prepared VPI Salt Sol'n for PYG broth as follows:

		Lot #
$CaCl_2 \cdot 2H_2O$	0.2 g	947250
$MgSO_4 \cdot 7H_2O$	0.2 g	947409
K_2HPO_4	1.0 g	950082
KH_2PO_4	1.0 g	946016A

$CaCl_2$ & $MgSO_4$ were added to 300 ml of deionized H_2O. Sol'n was mixed & brought up to 800 ml by adding deionized H_2O. K_2HPO_4 & KH_2PO_4 were then added to the sol'n. Sol'n was mixed & brought up to a volume of 1 L by adding deionized H_2O.

PYG Broth prepared as follows:

		Lot #
Peptone	20 g	63928JB
Glucose	10 g	99915JA
Yeast extract	10 g	596955JB
L-cysteine HCl	0.5 g	121H0359

into 1000 ml deionized H2O. 40 mls of the VPI salt sol'n was added to the PYG Broth. Autoclaved for 15 min @ 121°C, 14.7 psi gage. Allowed to cool under Nitrogen atmosphere.

M. Hill 3/12/99

SRB - Desulfovibrio vulgaris ATCC# 29579 received as freeze dried culture from ATCC 03/24/95 was revived in 9ml of lactate medium (p90) & incubated @ 30°C

EPS - Vibrio natriegens ATCC # 14048 received as freeze dried culture from ATCC 03/03/99 was revived in 9ml of 1.5% NB (p90) & incubated @ 30°C

APB - clostridium acetobutylicum ATCC # 43084 as freeze dried culture from ATCC 03/03/99 was revived in 9ml of YPG medium (p91 & incubated @ 30°C

3/12/99

Made 500 ml of Iversen's medium for plates (ph).

H_2O (RO)	500 ml
TSA (Difco Lot # 5805656)	20 g
Bacto Agar	2.5 g
Sodium lactate	2 ml
$MgSO_4 \cdot 7H_2O$ (Lot # 947409)	1 g
$FeSO_4 \cdot 7H_2O$ (lot # 946178	0.25 g

boiled to dissolve autoclaved at 121°C 14psi for 20 min poured as plates (I 1)

Made 500 ml of 1.5% NA for plates

H_2O (RO)	500 ml
Nutrient Broth (lot # 791125F)	4 g
NaCl (lot # 972274)	7.5 g
Bacto agar	7.5 g

change to 10 g 3/14/99

autoclaved at 121°C 14psi for 20 min poured as plates (NA 1)

3/12/99

prepared 20 ml of gelatin-suspending media as follows:

gelatin	lot # 59480JH	2 g
NaCl	972274	0.2 g
yeast extract	59695JB	0.2 g
peptone	63928JB	0.32 g
Inositol	75642JA	1 g

into 20 ml o deionized H_2O. Dissolved solids by heating.

Dispensed 4mls into screw-cap glass tubes & autoclaved @ 121°C, 14.7 psi guage for 10 min.
M. Hill 3/13/99

3/14/99 prepared 500mls of 1.5% nutrient broth agar as follows:

nutrient broth	791135F	4 g
NaCl	972274	7.5 g
Bacto Agar		10 g

into 500 ml deionized H_2O autoclaved for 20 min @ 121°C 14.7 psi guage. Poured on plates NA2.
M Hill 3/14/99

3/16/99 Prepared 400 mls of PBS as follows: 2 phosphate tablets lot # 45H8917 were added to 400 ml of 18.1 MΩ H_2O. Stirred to dissolve tablets autoclaved @ 121°C, 14.7 psig for 30 min.
M. Hill 3/16/99

3/23/99 On 3/14/99 streak plates of the V. natriegens & C. acetobutylicum cultures revived on 3/13/99 p 92 of this scientific notebook were performed. Both streak plates (mechanical dilution) were incubated for 48 hrs @ 31°C. On 3/16/99 the plates viewed under the stereoscope:

X10

C. acetobutylicum
(after incubation under aerobic conditions)
Note: it was observed that the sample was contaminated

X 10

C. acetobutylicum
(after incubation under anaerobic conditions)
Note: it was observed that the sample was contaminated.

X 10

V. natriegens
after incubation under aerobic conditions
Note: axenic culture for which colony characteristics were obtained

X 10

V. natriegens
after incubation under anaerobic conditions
Note: axenic culture

Because C. acetobutylicum was contaminated a 2nd streak plate was performed to obtain an axenic culture on 3/16/99. Sample incubated for 48 hrs. @ 31°C. After incubation the sample was viewed under the stereoscope.

X 10

C. acetobutylicum ?
incubation under aerobic conditions

Note: An axenic culture was obtained. Tests will be run to characterize the microorganism to verify that it is indeed C. acetobutylicum.

X10

V. natriegens

after incubation under anaerobic conditions
Note: axenic culture obtained for which colony characteristics will be noted.

On 3/19/99 a routine subculture was performed.

Because growth was not observed in lactate/acetate broth nor on Tween plates for the D. vulgaris resuscitated on 3/13/99 p 92 of this notebook, a 2nd vial of D. vulgaris ATCC # 29579 90-01 MED 1249 received freeze dried from ATCC 3/24/95

was revived in 9ml of lactate/acetate media p 90 of this notebook & incubated @ 31°C. M Hill 3/23/99

3/25/99 Routine subculture of V. natriegens in 1.5% Na Nutrient broth & on NA2 plate. C. acetobutylicum was subcultured in PYG broth & on NA2 plate. Incubated @ 31°C. M. Hill 3/25/99

4/1/99 Routine subculture of V. natriegens in 1.5% Na Nutrient broth & on NA2 plate. C. acetobutylicum was subcultured in PYG broth & on NA2 plate. Incubated @ 31°C. M. Hill 4/1/99

4/5/99 Prepared Modified Baar's Medium for SRB as follows:

Componet I — Lot # MB 915A 903

$MgSO_4$	2.0 g	947250
Sodium Citrate	5.0 g	905357A
$CaSO_4$	1.0 g	
NH_4Cl	1.0 g	895752

into 400 ml deionized H_2O

Componet II

K_2HPO_4	0.5 g	950082

into 200 ml deionized H_2O

Component III
Sodium lactate 3.5g 143605
Yeast extract 1.0g 5969558
into 400 ml deionized H_2O

measured pH = 7.446 after combing all 3 componets.

MH 4/5/99 ~~Three~~ Five mls were placed in glass tubes & purged with 95% N_2 & 5% Hydrogen Capped with septum seal & crimped. Autoclaved for 25 min @ 121°C 14.7 psig.

Component IV
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ 5g into 100 ml deionized H_2O.

placed component III in fridge for preservation.
M Hill 4/5/99

4/6/99 Boiled & purged remainder of components I, II & III prepared on 4/5/99. Three components had been placed in refridgerator overnight for preservation. Purged with 95% nitrogen & 5% hydrogen. Collected & dispensed 5 ml/s of components I, II & III (previously combined on

4/5/99 p 99-100 of this notebook into glass tubes. Tubes sealed with septum & crimped following a 2nd purge cycle of 95% nitrogen & 5% hydrogen. Autoclaved for 20 min @ 121°C, 14.7 psig.

Filter sterilized (0.2 µm) component IV & purged with 95% N_2 & 5% hydrogen. Using a sterile needle & syringe 0.1 ml of component IV was inoculated into each glass tube.

Remainder of media placed in fridge for preservation.

Resuscitated a vial of Desulfovibrio vulgaris received from ATCC on 4/2/99. ATCC # R 96-06 29579. D. vulgaris was resuscitated in lactate/acetate & modified Baar's medium, respectfully. Incubated under anaerobic conditions @ 31°C.
M Hill 4/6/99

4/9/99 Performed routine subculture of V. retrigens & C. acetobutylicum

as described on p 99 of this notebook. M. Hill 4/9/99

4/14/99 Performed routine subculture of D. vulgaris in lactate/acetate & Baars Medium, respectively. Performed streak plate on Iverson's agar. Incubated plate under aerobic conditions @ 30°C. This was done because the media has not yet turned black. If the microorganism is indeed an SRB, then growth should not occur under aerobic conditions.

M Hill 4/14/99

4/16/99 Performed routine subculture of V. natriegens & C. Acetobutylicum as described on p 99 of this notebook.

M. Hill 4/16/99

4/19/99 Iverson plate streaked on 4/14/99 p 102 of this notebook shows no signs of growth after incubation under aerobic conditions

(which is expected of D. vulgaris). Additionally, the subculture performed on 4/14/99 p 102 of this notebook (Baar's Modified Medium) has turned black, indicating the presence of a viable SRB. The lactate/acetate cultures have not, to this date, turned black. Perhaps Modified Baar's Medium will make a better substrate than the lactate/acetate. M. Hill 4/19/99

4/21/99 Performed routine subculture of D. vulgaris in Baars Medium (modified) & lactate/acetate, respectively. Incubated @ 31°C.

M Hill 4/21/99

Additionally, the odor of rotten eggs was detected during the subculturing process which further substantiates that an SRB is present. A photograph of the media (61k) was taken & will be inserted into the notebook @ a later date. M. Hill 4/21/99

4/22/99 Performed routine subculture of *V. natriegens* & *C. acetobutylicum* as described on p 99 of this notebook.
M Vill 4/22/99

4/28/99 Performed routine subculture of *D. vulgaris* in Baar's (Modified) Medium. Incubated @ 31 °C.

Prepared 500 ml of Difco Nutrient Broth as follows:

4.00 g nutrient broth lot # 79113JF into 500 ml deionized H_2O

to 100 ml of above sol'n 5 g of NaCl lot # 972274 were added

to 100 ml of orginal nutrient broth p 104 of this notebook 10 g of NaCl lot # 972274 were added

the procedure was repeated with 15 g & 20 g of NaCl (same lot #). Dispensed 9 ml in screw-tubes. Autoclaved 20 min 121°C, 14.7 psig. M Vill 4/28/99

M H 4/28/99

On 4/27/99 sterilized whatman filter paper (#1) & wooden sticks by autoclaving for 30 min @ 121 °C, 14.7 psig. M Vill 4/28/99

4/29/99 Chloride Toleration Test

Inoculated nutrient broth amended with 5, 10, 15 & 20% NaCl concentrations prepared on 4/28/99 p 104-105 of this notebook with *V. natriegens* (4/22/99 subculture used as inoculum). Additionally, 3 positive controls (1.5 NaCl nutrient broth) were inoculated. One of (+) controls will be used as routine subculture of *V. natriegens*. Incubated @ 31°C.
M. Vill 4/29/99

4/30/99 prepared 1000 ml of PYG broth as described on p 91-92 of this notebook.

Chloride Toleration Test - set up test as described on 4/28/99 & 4/29/99 p 104-105 of this notebook except that PYG broth was used in place of nutrient broth & *C. acetobutylicum* was the inoculum.

Temperature toleration test

Test was performed in duplicate 2 tubes per genus. Routine subculture of *V. natriegens*, *C. acetobutylicum* & *D. vulgaris* was performed. Samples were incubated @ room temperature 19 °C. Temperate & Rh was monitered with a white Box temp./Rh logger. M. Hill 4/30/99

5/3/99 ## Results of Temperature Tests

Chart recorder indicated that temperature had varied from 19 °C to 13.9 °C from 4/30/99 to 5/3/99. Growth visible for *D. vulgaris* inoculated over the aforementioned temp. range. Growth visible (cloudy) for *V. natriegens* @ above temp. range. Note: Baar's Modified Medium (*D. vulgaris* was light green on 4/30/99 & blk on 5/3/99). P4G broth was not cloudy suggesting *C. acetobutylicum* did not grow @ the above temp. ranges. Note: *D. vulgaris*

& *V. natriegens* exhibited 5/3/99 growth in both test tubes. *C. acetobutylicum* did not exhibit growth (clear) in both test tubes. The same tubes (P4G) inoculated on 5/3/99 will be incubated @ 31 °C to see if growth occurs.

Salt Toleration Tests

Checked *V. natriegens* which were inoculated on 4/29/99 p 105 of this notebook. Growth now visible (cloudy) for all 3 test tubes with 10% NaCl concentrations. The 10% NaCl con'c. did not exhibit growth (clear) on 4/30/99, however the 3 + controls & 3 5% NaCl concentration test tubes did exhibit growth on 4/30/99, which suggests that *V. natriegens* grows faster in the lower (1.5% & 5%) NaCl con'c., respectively. Will continue to incubate test tubes so that a false negative is not reported for the 15% & 20% NaCl con'c. tubes which to date do not exhibit

signs of growth (clear) for all 3 15% & 20% NaCl conic. tubes.

Checked C. acetobutylicum which were inoculated on 4/30/99. Growth visible in all 3 5% NaCl conic & all 3 + controls (cloudy). Growth not visible (clear) in all 3 10%, 15% & 20% NaCl conic. tubes, respectively. Will continue to incubate @ 31°C to avoid reporting a false negative for the 10%, 15% & 20% NaCl conic., respectively.

Prepared Modified Baars Medium as described on p 99-col 1 of this notebook except that 5%, 10%, 15% & 20% NaCl concentrations were prepared, respectively, in triplicate for salt tolerance tests.

Prepared TSB for Ron Green's project as follows: 15 g of TSB lot # 588555JE was added to 500 ml deionized H_2O.

Dispensed 20 ml in screw cap tubes & autoclaved for 20 min @ 121°C, 14.7 psig. Additionally swabs were autoclaved along with the TSB. MJW 5/3/99

5/4/99 MH 5/5/99 Prepared Halophilic Clostridium Broth as follows:

Solution I

Composition per liter MH 5/16/99

			Lot #
✓✓	NaCl	105.0 g	~~947323~~ 972274
✓✓	KCl	7.5 g	946305A
✓✓	L-Glutamic Acid	4.0 g	946193
✓✓	Casamino Acids	2.0 g	708166JB
✓✓	Nutrient Broth	2.0 g	791130F
✓✓	Yeast Extract	2.0 g	596955JB
✓✓	$FeSO_4 \cdot 7H_2O$	2.0 mg	946178
✓	Resazurin	1.0 mg	7H1310
✓✓	NaOH 2.5 N	12.5 mL	8289-02
✓	Nitrilo/Mg/Na/Mn Sol'n	10 mL	

Added 1.5 g of nitriloacetic acid to 500 ml deionized H_2O.

MH 5/5/99 ~~Adjusted pH to 6.5 with KOH~~

Added the following to 500 mL DI H_2O:

✓	$MgSO_4 \cdot 7H_2O$	3.0 g	947409
✓	NaCl	1.0 g	972274

✓	$MnSO_4 \cdot H_2O$	0.5 g	951425
✓	$FeSO_4 \cdot 7H_2O$	0.1 g	946178
✓	$CoCl_2 \cdot 6H_2O$	0.1 g	990214
✓	$CaCl_2$	0.1 g	947250
✓	$ZnSO_4 \cdot 7H_2O$	0.1 g	951602
mH stated ✓	$CuSO_4 \cdot 5H_2O$	0.01 g	9514540
✓	$AlK(SO_4)_2 \cdot 12H_2O$	0.01 g	126H1008
✓	H_3BO_3	0.01 g	949358A
✓	$Na_2MoO_4 \cdot 2H_2O$	0.01 g	990041

prepared sol'n 2 as follows:

		Lot #
$MgCl_2 \cdot 6H_2O$	20.3 g	936846
$CaCl_2 \cdot 2H_2O$	7.35 g	947250

per 100 ml DI H_2O.

mH 5/5/99

Combined nitriloacetic sol'n with Mg/NaCl/Mn sol'n. Added 10 ml of the aforementioned sol'n to solution 1 p 109 of this notebook.

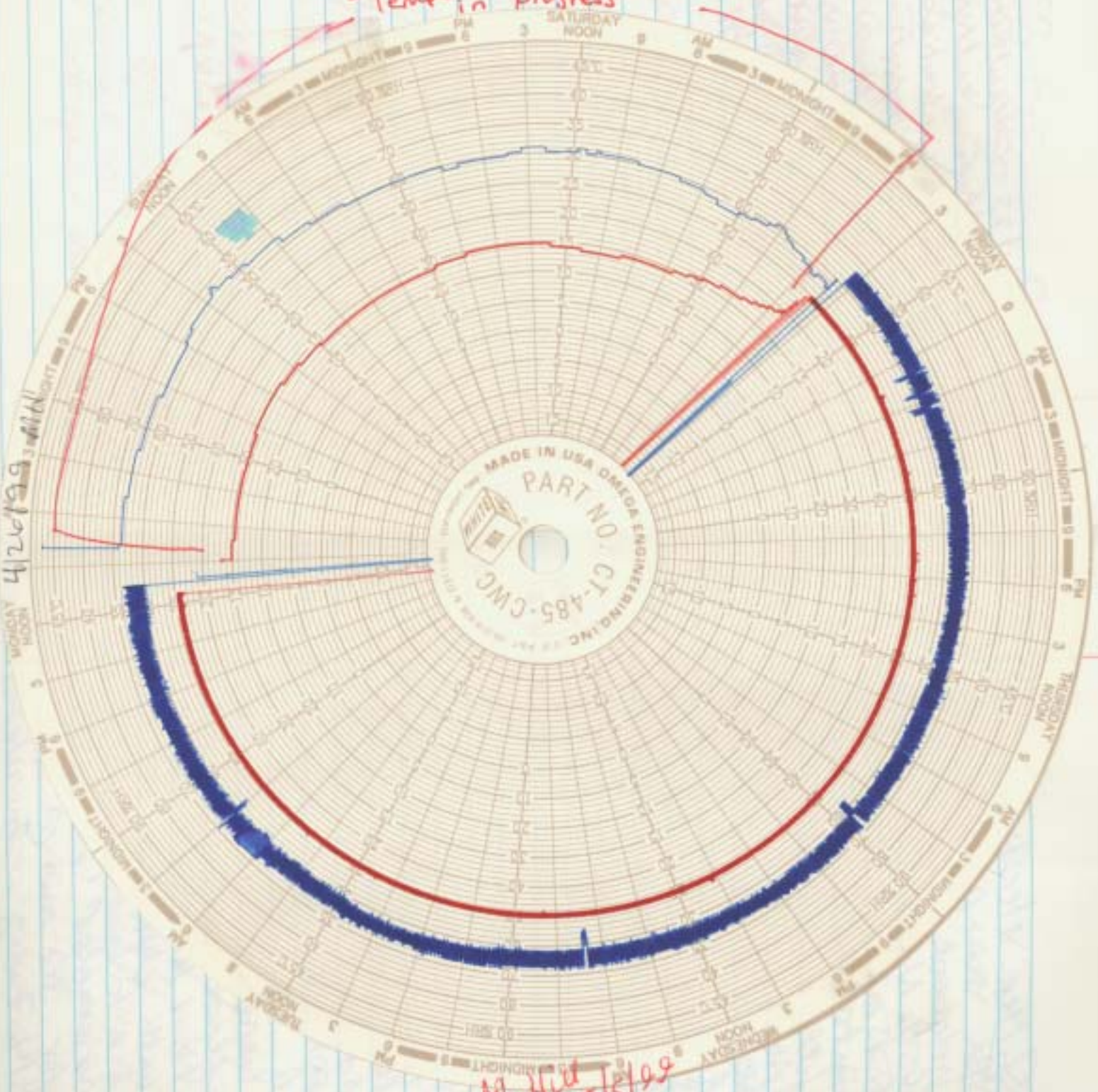
Placed sol'n 1, 2 & combined Mg/NaCl/Mn sol'n in fridge.

On 4/30/99 oxidase test performed on V. natriegens. V. natriegens oxidase +.

On 5/4/99 subculture performed using cultures cultivated under chloride concentrations of 5 & 10%, respectively. This was done to ensure that microorganisms were viable. Subcultured on Nutrient agar (1.5% NaCl) plates.

M Hill
5/5/99

chart recording temp. variation during temp. tolerance tests p 106-107 of this notebook.



4/26/99

M. Hill 5/5/99

5/6/99 Filter sterilized Componet IV see p 100 of this notebook & purged with 95% N_2 5% H_2. Inoculated 0.1 ml of componet IV into septum sealed vials filled with Modified Baars Medium p 108 of this notebook. Inoculated 0.1 ml of *D. vulgaris* in each Septum sealed glass vial for Salt toleration test. Incubated @ 31°C.

Prepared nutrient broth as follows:
Nutrient broth 2 g
into 250 ml DI H_2O.

5/7/99 to 100 ml of nutrient broth sol'n 12.5 g of NaCl were added. Prepared 5% sol'n as follows: 5 mls of 12.5 weight % NaCl were added to 250 ml flask with 95 ml of 18.1 MΩ H_2O added. Topped with cheesecloth/cotton twine & autoclave paper. 3 flask assembled & autoclaved for 30 min @ 121°C, 14.7 psig. Inoculated each flask with 1 ml of Vibrio/10% NaCl sol'n. # 1.

Prepared x2 500 mls MH 5/14/99 of 1.5% Nutrient ~~broth agar~~ (NaCl) as follows: 5/6/99

Nutrient broth	7911 3JF	24 g
NaCl	972274	7.5 g
Bacto Agar		10 g

into 500ml DI H_2O autoclaved for 20 min @ 121°C, 14.7 psig. Poured as NA3 plates.
MHill 5/6/99

5/7/99 Prepared 5 & 10% NaCl concentrations of P4G & nutrient broth respectively as described on p 104 & 105 of this notebook.

Prepared 5% sol'n of P4G & nutrient broth (both amended with 5% NaCl) as follows: 25 mls of 5% NaCl P4G broth was added to 475 ml of 18.1 MΩ H_2O. Nutrient broth was prepared the

Same as above. Additionally 475 ml of 18.1 MOhms H_2O was added to 3rd aliquot. All 3 aliquots were Sterilized @ 121°C, 14.7 psig.

MHill 5/7/99

5/10/99 On 5/9/99 sterilized x2 500 ml batch reactors which will be used for growth curves, Autoclaved @ 121°C, 14.7 psig for 30 min. Added 5% Nutrient broth & PYG both ~~amyx~~ MH 5/10/99 amended with 5% NaCl, respectively.

MH 5/11/99 Performed routine subculture of in 1.5% NaCl, 5% & 10% Nutrient broth (Vibrio natriegens) & PYG, 5% NaCl PYG & 10% NaCl PYG (Clostridium acetobutylicum).

Salt Tolerance Test (D. vulgaris)

On 5/7/99 the 3 (+) controls were black, however to date no growth is visible in the 5, 10, 15 & 20% NaCl conic

in Modified Baar's Medium.

On 5/7/99 3 additional flasks were prepared as described on p 114 of this notebook, except that PYG broth & C. acetobutylicum were introduced. Placed in Shaking H_2O bath set A @ 31°C 100 Shakes/min.

MH 5/10/99

On 5/10/99 dispensed 5 ml of Baar's Modified Medium p 99 of this notebook into glass tubes. Purged with 95% N_2 5% H_2 sealed with septum & crimped. Autoclaved for 25 min @ 121°C, 14.7 psig. Additionally Whatman #1 filter paper & wooden sticks were sterilized @ 121°C, 14.7 psig for 25 min.

Prepared 1000 ml of component IV (Modified Baar's Medium) as follows: 50g of $Fe(NH_4)_2(SO_4)$ lot # 951022 into 1000 ml 18.1 MΩ H_2O. Filter sterilized (0.2 μm filter) & purged with 95% N_2 5% H_2. Inoculated 0.1 ml of filter sterilized

$Fe(NH_4)_2(SO_4)_2$ into septum Sealed Vials filled with 5ml of Modified Baar's Medium (p117 of this notebook).

Inoculated one septum Sealed vial 3% NaCl (Mod. Baar's Med) with D. vulgaris. Incubated @ 31°C. M Hill 5/10/99

5/11/99 Adjusted room temperature 45min prior to setting up batch reactors & set up White Boy temp/RH recorder which will monitor temp & RH during the operation of the batch reactors. NA3 plates placed in oven to dry for 1 hour. Autoclaved Pipettes for 30 min @ 121°C, 14.7 psig. Additionally, centrifuge tubes were Sterilized as described above. Batch reactors purged with 95% N_2 5% H_2 2 hrs prior to operating batch reactors. Media in batch reactors is clear.

Prepared x2 500ml of

1.5% nutrient broth agar as follows:

		lot #
Bacto Agar	10.0 g	
Nutrient broth	4.0 g	791130F
NaCl	7.5 g	972274

into 500 ml DI H_2O. Autoclaved for 25 min @ 121°C, 14.7 psig. Poured as plates NA4.

Prepared 400 ml of PBS as follows 2 PBS tablets lot # 45H8917 were added to 400ml 18.1 MΩ H_2O & autoclaved for 25 min @ 121°C 14.7 psig.

Collected 500 µL from batch reactors every hour on the hour after inoculating with 50µL of V. parviperis & C. acetobutylicum, respectively.

Performed plate counts following the procedures of Miles & Misra on inoculum & samples collected from batch reactors. M Hill 5/11/99

Prepared 4% NaCl Baar's Medium soln. Inoculated D. vulgaris cultivated in 3% NaCl p 118 of this notebook. Incubated @ 31°C.

Continued collecting samples from batch reactors every hour on the hour. Performed Miles & Misra plate counts. Prepared 500 mls of 1.5% nutrient agar following the procedures discussed on p ~~120~~ mtl 5/12/99 119 of this notebook. mtl 5/12/99

5/13/99 Batch reactor for Vibrio natriegens is clear which suggests that inoculation was unsuccessful on 5/11/99. Additionally, no growth is visible on plates, however growth is visible on plates performed on inoculum. It is suspected that inoculum got dispersed on threads on cover. Will re-run with D. vulgaris batch reactor.

Growth visible for D. vulgaris in 4.5% NaCl/Baar's mtl 5/13/99

medium inoculated on 5/12/99 p 119-120 of this notebook. Prepared 5% NaCl/Baar's Medium as follows: 5 g of NaCl lot # 97 2274 were added to 100ml Baar's Medium p 108 of this notebook. Purged with 95% N_2 5% Hydrogen. Sealed with septum & crimped. Autoclaved for 25 min @ 121°C, 14.7 psig. Inoculated each tube (filled with 5mls of 5% NaCl/Baar's) with 0.1um filter sterilized $Fe(NH_4)_2(SO_4)_2$ on 5/10/99 p117 of this notebook. Inoculated 4 tubes with D. vulgaris cultivated in 4% NaCl/Baar's.

Plate Counts for C. acetobutyl.

date	time	CFU/ml	
5/11/99	inoc t_0	1.13×10^7	triplicate plates
	13:00	1.28×10^7	
		1.04×10^7	
5/11/99	14:00 t_1	*392 ~~325~~ mtl 5/11/99	singularly 1 plate
5/11/99	15:00 t_2	325	
5/11/99	16:00 t_3	333	
5/11/99	17:00 t_4	333	
5/11/99	18:00 t_5	333	
5/11/99	19:00 t_6	317	
5/11/99	20:00 t_7	400	

5/11/99	21:00 t_8	*	325
5/11/99	22:00 t_9		400
5/11/99	23:00 t_{10}		300

*indicates droplets merged during dilution (unreliable count/questionable).

Note: plate counts performed after 48 hrs of incubation @ 31°C.

	√ natriegens		Plate Counts
	date	time	CFU/mL
triplicate	5/11/99	13:00 t_0	7.92×10^7
	5/11/99	inoc	6.25×10^7
	5/11/99		9.00×10^7
singular plates	5/11/99	14:00 t_1	0
	5/11/99	15:00 t_2	0
	5/11/99	16:00 t_3	0
		17:00 t_4	0
		18:00 t_5	0
	"	19:00 t_6	0
		20:00 t_7	0
		21:00 t_8	0
		22:00 t_9	0
	5/11/99	23:00 t_{10}	0

M Hill 5/13/99

5/14/99

Plate Counts for C. acetobutyl.

date	time		CFU/ml
5/12/99	8:00	19	1.25×10^3
5/12/99	9:00	20	1.35×10^3
5/12/99	10:00	21	1.30×10^3
5/12/99	11:00	22	1.72×10^3
5/12/99	12:00	* 23 MH 5/14/99	~~5.50×10^4~~ 5.67×10^5
5/12/99	13:00	* 24	2.08×10^4
5/12/99	14:00	* 25	
5/12/99	15:00	26	5.50×10^4
5/12/99	16:00	*	

* indicates droplets merged during dilution (unreliable count/questionable)

Problem was that agar did not dry after 1 hr in incubator. However, it was observed that the agar dried after 4 hrs in the incubator.

Prepared 6 flasks as follows: 95 mls 18.1 MΩ H_2O 3 flasks were filled with 5mls of 15% NaCl NB (nutrient broth) & 3 flasks were filled with 5mls of 15% NaCl PYG broth. This was done because i) flasks in shaker were cloudy (PYG) & NB flasks had slime. Six flasks autoclaved for 25 min

at 121°C, 14.7 psig. Growth also seems visible (slime) in 15% NaCl broth subcultures. Thus, 3 15% NaCl broth (PYG) cultures were sterilized along with the 6 flasks (p 123 of this notebook). 5/15/99 mH 5/15/99

Plate Counts for V nat.
All plates performed on 5/12/99 from 8:00-16:00 showed no growth.

Note medium prepared for 6 flasks (p 123 of this notebook) were prepared as follows:
3 @ Lot #
15 g NaCl 972274
into 100 ml PYG broth p 105 of this notebook. Stirred & dispensed 9 ml's into glass tubes prior to autoclaving.
3 @ the same as above except that nutrient broth replaced PYG broth.
Nutrient broth was prepared on 5/7/99 as follows: 4 g Difco nutrient broth Lot # 791 3JF into 500 ml DI H_2O. MtHill 5/14/99
Additionally, 4 tubes in-

oculated with D. vulgaris on 5/13/99 p 121 of this notebook exhibited growth. MtHill 5/14/99

Plate Counts C. acetobutylicum

date	time	CFU/ml
5/13/99	9:00	*
5/13/99	10:00	* 1.17×10^6
5/13/99	11:00	* 2.08×10^6
5/13/99	12:00	1.50×10^6
5/13/99	13:00	2.83×10^6
"	14:00	2.58×10^6
	15:00	4.42×10^6
5/13/99	16:00	2.67×10^6

* indicates droplets merged during dilution

Collected last sample from batch reactor (C. acetobutylicum) @ 16:00 on 5/15/99. Terminated gas & stirrer.

6 flasks assembled on 5/6 & 5/7/99 p 114 & 117 all were + for growth @ 12.5% NaCl (cloudy). Thus, 6 flasks prepared on 5/14/99 p 123 & 124 were ~~tube~~ mH 5/15/99 inoculated with 1 ml, respectively, of bacteria/nutrient broth (x3) & bacteria/PYG broth previously amended with 12.5% NaCl x3
indicates tests were run in triplicate
MtHill 5/15/99

5/16/99 Plate Counts C. acetobutylicum

date	time	CFU/ml
5/14/99	9:00	*
5/14/99	11:00	4.75×10^6
5/14/99	13:00	5.67×10^6
5/14/99	15:00	2.07×10^6

* indicates droplets merged during dilution plating

Performed subculture of V. natriegens in 1.5% NaCl/nutrient broth, 5% NaCl/nutrient broth & 10% NaCl nutrient broth. Performed a similar subculture for C. acetobutylicum except that PYG broth was used. D. Vulgaris subcultured in 5% NaCl Baar's medium. M Hill 5/16/99

5/17/99 Plate Counts C. acetobutylicum

date	time	CFU/ml
5/15/99	16:00	1.55×10^6

Prepared 5% NaCl/nutrient broth soln as follows: 5 g of NaCl lot # 972274 into 100 ml of nutrient broth p124 of this notebook. 25 mls of the 5% NaCl/nutrient broth soln was added to 475 ml of DI H_2O. Autoclaved soln

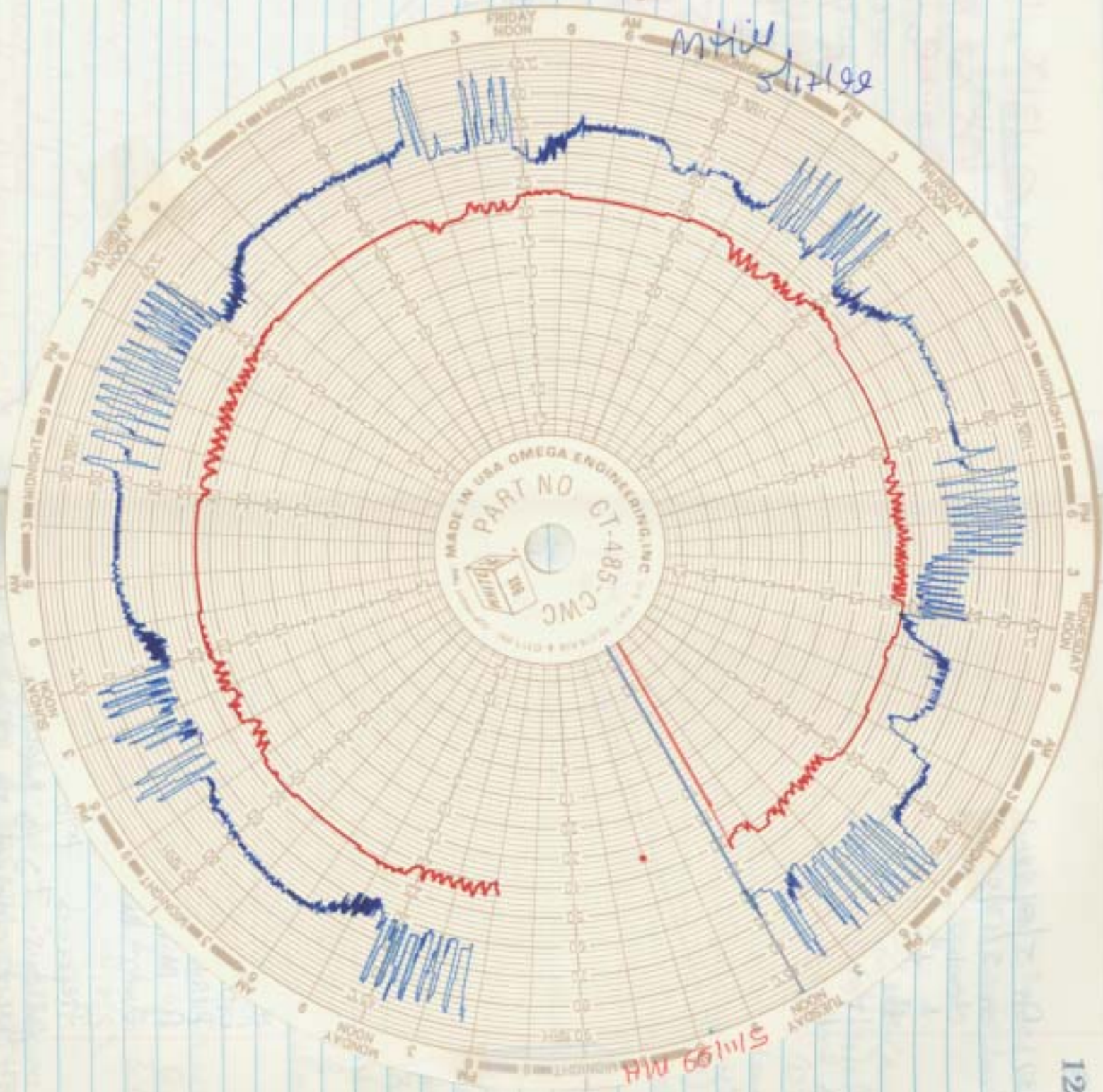
for 30 min @ 121°C, 14.7 psig.

Autoclaved 475 ml of 18.1 MΩ H_2O for 30 min @ 121°C, 14.7 psig.

Autoclaved 2 batch reactors @ 121°C, 14.7 psig. Added 5% NaCl nutrient broth soln to 1 & added 475 ml of DI H_2O to other. Additionally, 5 tubes of 5% NaCl/Baar's p121 of this notebook were added to the 2nd batch reactor. M Hill 5/17/99

Subcultured C. acetobutylicum in 1.5% NaCl/PYG broth (screw tube) M. Hill 5/17/99

Temp chart during operation of Batch Reactor
Inoculated with C. acetobutylicum.



5/19/99

On 5/18/99 batch reactor prepared on 5/17/99 for D. vulgaris appeared cloudy. Reactor was disassembled & flakes were found floating on the surface. Upon further inspection it was observed that the flakes had a gritty feel. It is interpreted that although the reactor & sol'n were allowed to cool prior to the introduction of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, the reactor had not cooled enough to prevent ppt. System autoclaved for 30 min @ 121°C, 14.7 psi & assembled. 475 ml of 18.1 MΩ H_2O were dispensed into an aliquot & 25 ml of 5% NaCl pt MH 5/18/99 Baar's prepared as follows 5 g of NaCl lot # 972274 were added to 100 ml Baar's p 108 of this notebook.

On 5/18/99 Vibrio natriegens (50 µL) was inoculated into the batch reactor filled with 500 ml of nutrient broth amended with 5% NaCl. Plate count following the procedures of Miles & Misra were collected (500 µL) every hour.

Prepared 7% NaCl/Baar's as follows: 7 g of NaCl lot #972274 were added to 100 ml of Baar's p 108 of this notebook. Purged with 95% N_2, 5% H_2 & autoclaved for 121°C, 14.7 psig for 20 min. MH 5/19/99

Prepared component IV of Baar's as follows: 25 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ were added to 500 ml of 18.1 MΩ H_2O. Filter sterilized (0.2 µm Sartorius filter) into pre-autoclaved vessel. Inoculated sterile Baar's medium (amended with 7% NaCl) after purging $Fe(NH_4)_2(SO_4)_2$ sol'n with 95% N_2, 5% H_2. 0.1 ml of $Fe(NH_4)_2(SO_4)_2$ sol'n was introduced to sterile Baar's medium.

Prepared 5% NaCl/Baar's as follows: 5 g of NaCl lot # 972274 were added to 100 ml of Baar's p 108 of this notebook 25 ml of the 5% NaCl/Baar's sol'n was added to 475 ml of 18.1 MΩ H_2O as described on p 130 of this notebook was autoclaved for 20 min @ 121°C, 14.7 psig. MH 5/19/99

500 µL of $Fe(NH_4)_2(SO_4)_2$ sol'n was introduced to batch reactor. D. vulgaris (50 µL) inoculated in batch reactor @ 24:00 on 5/18/99.

PBS prepared as follows: 2 tablets of PBS lot # 45H8917

were added to 400 ml of 18.1 MΩ H_2O. Dispensed 2700 µL of PBS into tubes purged with 95% nitrogen 5% hydrogen sealed with septum & crimped autoclaved for 20 min @ 121°C, 14.7 psig. Performed serial dilution as follows 10^{-1} through 10^{-6} x5. Inoculated each tube with roughly 300 µL SB batch/PBS ~~reactor~~ sol'n. MHill 5/19/99

After speaking with P. Angell, he suggested performing a single dilution series using 5% NaCl Baar's Med. So on 5/19/99 a 5% NaCl Baar's sol'n was prepared as described previously p 131 of this notebook. Inoculated SRB plates (Invasors) in gas chamber adding 1 packet of Gas Pak Plus lot # 10068125008. Incubated @ 31°C.

On 5/18/99 inoculated 7% NaCl/Baar's with *D. vulgaris* previously sub-cultured in 5% NaCl/Baar's.

Transferred 5% NaCl Nutrient broth to Wheaton tube & purged with 95% Nitrogen 5% hydrogen placed septum & crimped autoclaved for 20 min 121° 14.7 psig. 5% NaCl Nutrient broth previously prepared on 5/7/99 p 115 of this notebook. MHill 5/19/99

5/21/99

On 5/20/99 no growth was visible in batch reactors so stopped gas flow. After speaking with P. Angell it was decided that the growth curves need not be performed for *V. natriegens* & *C. acetobutylicum* due to budget constraints. Gas flow resumed to batch reactors additionally a 3rd batch reactor was prepared as follows: 5 g of NaCl lot # 972274 with 100 ml of PYG broth p105 of this notebook. 25 mls of the above sol'n was added to 475 ml of 18.1 MΩ H_2O & autoclaved @ 121°C 14.7 psig. The batch reactor vessel was autoclaved along with the PYG/5% NaCl broth for 25 min @ 121°C, 14.7 psig as mentioned previously. MHill 5/21/99

Growth positive for *D. vulgaris* subcultured in 7% NaCl/Baar's p 132 of this notebook.

Subcultured *V. natriegens* & *C. acetobutylicum* in 1.5% Nutrient broth, 5% NaCl nutrient broth, 10% NaCl nutrient broth and PYG, 5% NaCl PYG & 10% NaCl PYG, respectively. Incubated @ 31°C. on Hill 5/21/99

5/24/99 Plate Counts SRB

date	time	CFU/ml
3/18/99	24:00 to	5.25×10^5
3/19/99	9:00	0
3/19/99	10:00	TFTC

inoculated C. acetobutylicum (2 ml) into 3rd batch reactor. M Hill 5/24/99

5/25/99 Prepared PYG broth as follows:

	lot #
peptone 20 g	63928JB
dextrose 10 g	99915JA
yeast extract 10g	59695JB
L-cysteine 0.5g	19H1152

added 40 ml of VPI salt soln p91 of this notebook & filled to 1000 ml with DI H_2O. Stored in fridge for preservation.

Prepared 5% NaCl/Baar's as follows: 5g of NaCl lot # 972274 was placed in graduated cylinder & Baar's p 108 of this notebook was added up to 100 ml. Purged with 95% nitrogen, 5% hydrogen sealed & placed in autoclave for 20 min. @ 121°C, 14.7 psig

M Hill 5/25/99

/nutrient broth

Prepared 17.5% NaCl by adding 17.5 g NaCl lot # 972274 nutrient broth added up to 100 ml. Added 5% of 17.5% NaCl/nutrient broth to 95 ml of 18.1 MΩ H_2O.

Prepared 17.5% NaCl/PYG the same as described above.

Both soln's placed in autoclave @ 121°C, 14.7 psig for 20 min.

M Hill 5/25/99

A 3rd soln of 100% 17.5% NaCl/PYG was placed in a 3rd flask sterilized as described above.

M Hill 5/25/99

5/27/99 On 5/26/99 inoculated 3 flasks prepared on 5/25/99 p 134 & 135 of this notebook with bacteria. (V. natriegens in 17.5% NaCl nutrient broth & C. acetobutylicum in 17.5% NaCl PYG broth). Placed in shaking water bath set @ 31°C 100 strokes/min

Performed routine subculture of D. vulgaris in 5% NaCl/Baar's after adding 0.1 ml of $Fe(NH_4)_2(SO_4)_2$ purged with 95% nitrogen 5% hydrogen mixture. $Fe(NH_4)_2(SO_4)_2$

filter sterilized using a 0.2 µm filter.
M Hill 5/27/99

5/28/99 performed routine subculture of *V. natriegens* in 1.5% NaCl/nutrient broth, 5% & 10% NaCl nutrient broth. Performed routine subculture of *C. acetobutylicum* in PYG broth & 5% & 10% NaCl PYG broth. Incubated @ 31°C.
M Hill 5/28/99

6/1/99 Prepared nutrient broth as follows:

lot #

4g of nutrient broth 79113JF into 500 ml DI H_2O.

Added 20g mtkd 11/99 of NaCl lot # 972274 to 100 ml of nutrient broth. Added 5% mtkd 6/1/99 5 mls of 20% NaCl/nutrient broth mixture to 95 mls of 18.1 MΩ H_2O. (5% sol'n of 20% NaCl/nutrient broth was dispensed in 1 flask. A second flask was prepared using 100% of the 20% NaCl/nutrient broth sol'n.

20g of NaCl lot # 972274

were added to 100 mls of PYG broth p134 of this notebook. A 5% sol'n of 20% NaCl/PYG & a 100% sol'n of 20% NaCl/PYG were prepared as described for 20% NaCl nutrient broth sol'ns on p136 of this notebook.

40 mls of PBS prepared on 5/11/99 p119 of this notebook were transferred to a 100 ml aliquot & autoclaved @ 121°C, 14.7 psig.

4 flasks containing 5% & 100% sol'n of 20% NaCl/nutrient broth & 20% NaCl/PYG broth respectively were autoclaved for 20 min @ 121°C 14.7 psig.

Inoculated flasks with *V. natriegens* & *C. acetobutylicum* respectively.

To 40 mls of PBS 2 mls of *V. natriegens*, *C. acetobut.* & *D. vulgaris*, respectively were added. Consortium of bacteria used in pressure test.
M Hill 6/1/99

6/3/99 Inoc #'s for pressure test

	date	time	cfu/ml
V. nat	6/1/99	n/a	6.58×10^6
C. aceto	6/1/99	n/a	2.08×10^7

Growth + for C. acetobutylicum cultivated in batch reactor.

No growth visible in batch reactor for V. natriegens so will increase to 10% soln of 5% NaCl nutrient broth & will reinoculate reactor.

Prepared 5% NaCl nutrient broth as follows:

5 g of NaCl lot # 972774 were added to 100mls of nutrient broth p 136 of this notebook. Dispensed 25mls in 100ml aliquot & remainder 9mls per screw-top tube. Autoclaved @ 121°C, 14.7psig for 20 min.

Performed oxidase test V natriegens + & C. aceto. negative.

M Hill 6/3/99

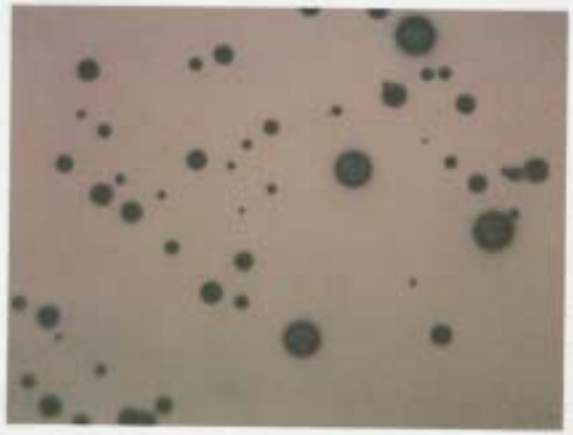
6/3/99 Note: Inoculated D. vulgaris into 7% NaCl Baar's 50 l'n. on 6/3/99 (See p 139 where initial entry was made in this notebook).

6/4/99 Inoc # for Pressure Test

	date	time	cfu/ml
D. vulg.	6/1/99	n/a	5.75×10^4

On 6/3/99 subcultured D. vulgaris in Baar's Med amended with 5 & 7% NaCl, respectively.

On 6/4/99 performed routine subculture of V. natriegens & C. acetobutylicum as described on p 136 of this notebook.



x10

D. vulgaris cultivated on Tryptone agar, @ 31°C under anaerobic atmosphere.

MH 6/4/99

* New 10% soln of NaCl in nutrient broth

Introduced 25 mls of 5% NaCl nutrient broth into batch

reactor. Inoculated 2 mls of V. natriegens into batch reactor. M Hill 6/4/99

6/8/99 On 6/6/99 prepared components I, II, & III of Baar's Medium as followed:

Componet I		Lot #
$MgSO_4$	2.0 g	947250
Sodium Citrate	5.0g	905357A
$CaSO_4$	1.0g	
NH_4Cl	1.0g	895752

into 400 mls DI H_2O.

Componet II

K_2HPO_4 0.5g 950082
into 200 mls DI H_2O

Component III

Sodium lactate	3.5g	943605
Yeast Extract	1.0g	59695JB

into 400 mls DI H_2O.

placed all 3 sol'ns (components I, II & III) in fridge for preservation.

On 6/7/99 disassembled pressure test. Performed plate count (in triplicate) on effluent to determine if microorganisms were able to tolerate rapid pressurization. M Hill 6/8/99

6/10/99 Performed oxidase test to check that V. natriegens survived pressure test: Results oxidase +

MH 6/11/99

V. natriegens following pressure test @ 500psig. Cultivated on 1.5% NaCl nutrient agar. MH 6/10/99

6/11/99 Prepared media for chemostat (C. acetobutylicum) as follows.

175 mls of P4G broth p 134 of this notebook amended with 5% NaCl lot # 972274 were added to 3500 mls of 18.1 MΩ H_2O. Soln autoclaved for 2 hrs @ 121°C, 14.7psig. MHill 6/11/99

6/13/99 C. acetobutylicum was successfully subcultured on 1.5% NaCl nutrient agar (~10^3 CFU/ml) following incubation in a 5% sol'n of 20% NaCl PYG broth @ 31°C in a shaking H_2O bath (~100 strokes/min). C. acetobutylicum oxidase negative. No growth visible in a 100% sol'n of 20% NaCl PYG broth.

Performed routine subculture of V. natriegens, C. acetobutylicum & D. vulgaris as described on p 136 & 135 of this notebook. Reinoculated D. vulgaris into 7% NaCl Baar's sol'n previously inoculated on 6/3/99 p139 ~~138~~ of this notebook. MH 6/13/99 MH 6/13/99

Prepared 1000 mls of $Fe(SO_4)_2(NH_4)_2$ Component IV of Baar's Medium as follows: MH 6/13/99
25 g of $Fe(SO_4)_2(NH_4)_2$ lot # 951022 were added to 500 mls of 18.1 MΩ H_2O. A 2nd batch was prepared as described above for a total of 1000 mls. Placed in fridge for preservation.

Prepared Baar's Medium for chemostat as follows:

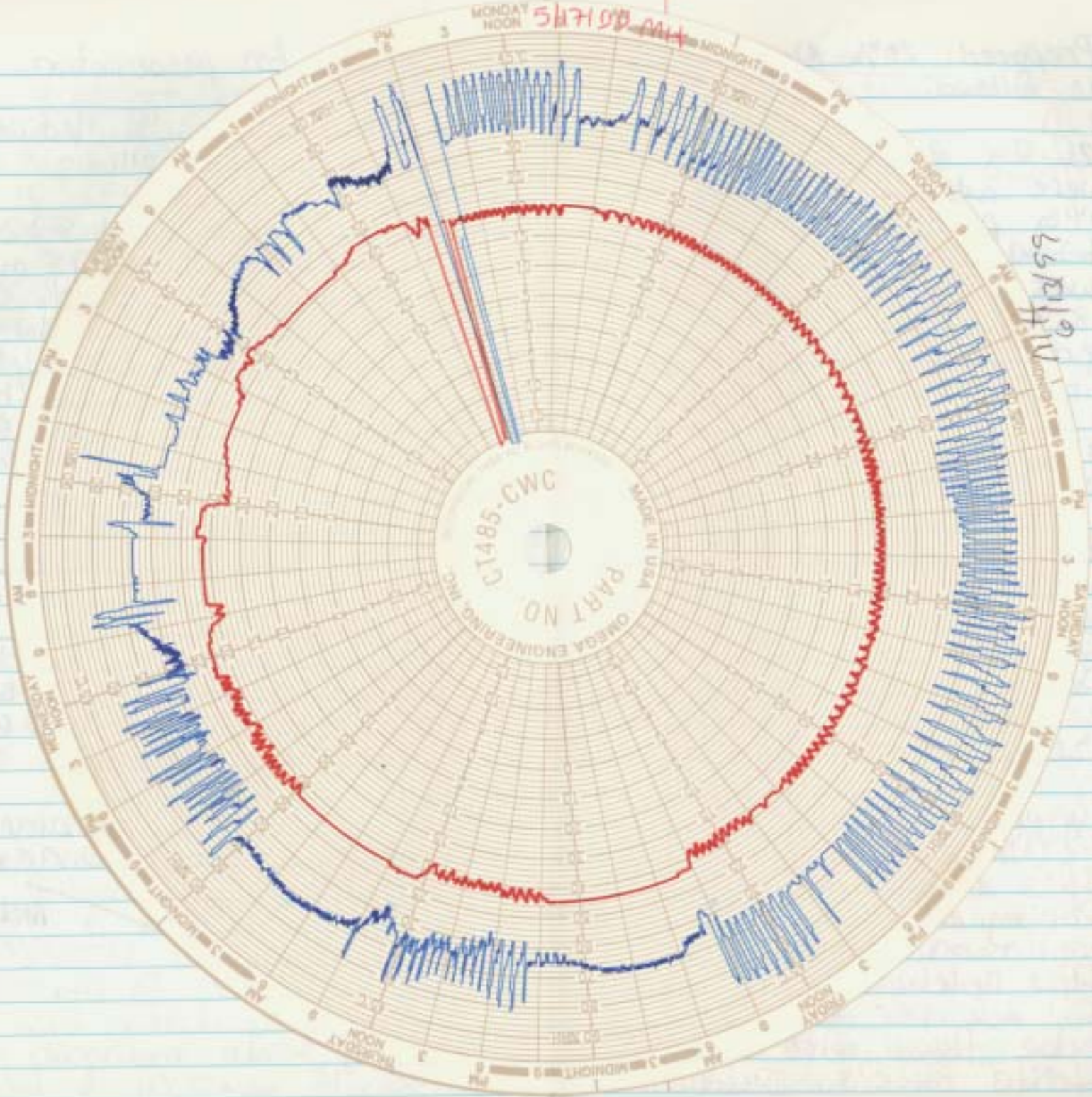
5 g of NaCl lot # 972274 were added to 175 mls of Baar's Medium p 140 of this notebook. Baar's amended with 5 g NaCl were added to 3,500 mls of 18.1 MΩ H_2O. Autoclaved for 2 hrs @ 121°C, 14.7 psig. MH 6/13/99

6/14/99 Filter sterilized 1000 mls of $Fe(NH_4)(SO_4)_2$ sol'n prepared on 6/13/99. Added 3.5 mls of filtered $Fe(NH_4)(SO_4)_2$ sol'n to Baar's Medium prepared on 6/13/99. M. Hill 6/14/99

Note entry out of order see chart attached to next page (144).

MH 6/14/99

Temp & RH data for V. natriegens & D. vulgaris
batch reactors p 130 & 131 of this notebook.
MH 6/13/99



6/15/99 Prepared 20% NaCl PYG broth as follows:

20.0 g of NaCl lot # 972274 were added to 100mls of PYG p 134 of this notebook. ~~4.5 mls~~ of the above sol'n was added to 8.55mls of 18.1 MΩ H_2O. Dispensed in screw top tubes & autoclaved for 25 min @ 121°C, 14.7psig.

MH 6/15/99 0.45 mls

9% NaCl / Baar's sol'n prepared as follows:

9 g of NaCl lot # 972274 were added to 100mls of Baar's. Dispensed in crimp-top tubes & purged with high purity nitrogen. Sealed tubes & autoclaved for 25min @ 121°C, 14.7psig.

Purged remainder of $Fe(NH_4)_2(SO_4)_2$ p 142 of this notebook with high purity nitrogen. Filter Sterilized sol'n on 6/14/99 p 143 of this notebook. Inoculated 0.1 ml per each crimp top tube filled with 5 mls of Sterile Baar's. Subcultured

6/16/99

6/18/99

D. vulgaris in 9% NaCl/ Baar's. Incubated @ 31°C.

M Hill 6/14/99

MH 6/15/99 6/15/99

Prepared 500mls of nutrient broth as follows:

4.0 g of nutrient broth lot # 791135F were added to 500mls of 18.1 MΩ H_2O.

prepared nutrient broth for Chemostat as follows:

17.5 g of NaCl lot # 972274 were added to 350 mls of nutrient broth p 147 of this notebook. The above sol'n was then added to 3,500 mls of 18.1 MΩ H_2O & autoclaved for 2 hrs @ 121°C, 14.7psig.

M Hill 6/16/99

On 6/17/99 prepared 2 flasks as follows: one with 5% PYG broth amended with 20% NaCl & the second with 10% PYG broth amended with 20% NaCl. Autoclaved @ 121°C, 14.7psig. Inoculated with 1 ml of bacteria

& placed in shaking H_2O bath.

Prepared a 2nd set of flasks as described above except that V. natriegens was introduced into the flasks & nutrient broth was substituted for PYG.

MH 6/18/99 On 6/17/99 prepared 10% nutrient broth amended with 5% NaCl as follows:

To 350 mls of nutrient broth p 147 of this notebook 17.5g of NaCl lot # 972274 were added. The above soln was added to 3,500 mls of 18.1 MΩ H_2O & autoclaved for 2 hrs @ 121°C, 14.7 psig.

MH 6/18/99 5%
On 6/18/99 prepared PYG broth amended with 5% NaCl as follows:

175 mls of PYG p 134 of this notebook were amended with 8.80g of NaCl lot # 972274 The above soln was added to 3,500 mls of 18.1 MΩ H_2O & autoclaved for 2 hrs @ 121°C, 14.7 psig.

performed routine subculture of V. natriegens, C. aceto. & D. vulgaris as described on p 135 + 136 of this notebook. MH 6/21/99 M Hill 6/18/99

6/24/99 Prepared 5% soln of Baar's amended with 5% NaCl as follows:

8.80g of NaCl lot # 972274 were added to 175 mls of Baar's p 140 of this notebook. The above soln was added to 3,500 mls of 18 MΩ H_2O & autoclaved for 2 hrs @ 121°C, 14.7 psig.

Prepared 1000 mls of $Fe(NH_4)_2(SO_4)_2$ as follows:

50g of $Fe(NH_4)_2(SO_4)_2$ lot # 951022 were added to 1000 mls of 18.1 MΩ H_2O.

Prepared a 5% soln of Baar's amended with ~~5%~~ MH 6/20/99 11% NaCl as follows:

11.02 g of NaCl lot # 972274 were added to 100 mls of Baar's p 140 of this notebook 0.25 mls of the above soln

was added to 5 mls of 18.1 MΩ H_2O Autoclaved for 20 min @ 121°C, 14.7 psig. M Vill 6/21/99

6/22/99 Filter sterilized 1000 mls of $Fe(NH_4)_2(SO_4)_2$ p 149 of this notebook. Added 3.5 mls of filter-sterilized $Fe(NH_4)_2(SO_4)_2$ to Baar's prepared on 6/21/99 p 149 of this notebook. Sol'n will be used for chemostat in 1st corrosion test.

Added 0.1 ml of filter-sterilized $Fe(NH_4)_2(SO_4)_2$ to 5% sol'n of Baar's amended with 11% NaCl p 149 of this notebook. Inoculated with D. vulgaris & incubated @ 31°C. in shaking H_2O bath 100 strokes/min.

Prepared 10% nutrient broth amended with 5% NaCl as follows:

17.5 g of NaCl lot # 972274 were added to 350 mls of nutrient broth p 147 of this notebook. The above sol'n was then added to 3,500 mls of 18.1 MΩ H_2O & autoclaved for 2 hrs @ 121°C 14.7 psig.

collected samples from chemostat housing V. natriegens to perform sterility check. M Vill 6/22/99

6/23/99 Prepared PYG broth as follows:

		Lot #
peptone	20 g	63928JB
glucose	10 g	99915JA
yeast extract	10 g	59695JB
L-cysteine HCl	0.5 g	19H1152

added 40 mls of VPI salt sol'n ~~to~~ MH 6/23/99 p 91 of this notebook & filled aliquot to 1000 ml level with 18.1 MΩ H_2O.

~~autoclaved for 2 hrs @ 121°C, 14.7 psig.~~ MH 6/23/99

Prepared Iverson agar as follows:

		Lot #
TSA	20 g	58056JB
Bacto Agar	2.5 g	
$MgSO_4 \cdot 7H_2O$	Pg	947409
$FeSO_4 \cdot 7H_2O$	0.25 g	946178

boiled to dissolve ingredients & autoclaved for 20 min @

121°C, 14.7psig. Poured as plates I2.

Prepared media for chemostat (C. aceto.) as follows:

8.8 g of NaCl lot # 972274 were added to 175 mls of PYG broth p 151 of this notebook. The above soln was added to 3,500 mls of 18.1 MΩ H_2O & autoclaved for 2 hrs @ 121°C, 14.7psig.

Prepared nutrient broth as follows:

4 g of nutrient broth lot # 79133F were added to 500 mls of 18.1 MΩ H_2O.

Contamination detected in one of chemostats (V. natriegens) so disassembled & washed with alconox. Sterilized vessel & lines by autoclaving for 25 min @ 121°C 14.7 psig.

prepared ~~5~~ mH 6/23/99 10% soln of nutrient broth amended with 5% NaCl

as follows:

5.0 g of NaCl lot # 972274 were added to 100 mls of nutrient broth p 152 of this notebook. ~~Tea~~ 50 mls of the above soln were added to 450 mls of 18.1 MΩ H_2O & autoclaved for 25 min @ 121°C 14.7psig.

mH 6/23/99

M Hill
6/23/99

6/24/99 Contamination confirmed for batch reactor (V. natriegens) disassembled on 6/23/99. Not only did the culture not look like V. natriegens but it was oxidase neg.

Inoculated sterilized vessel with axenic culture of V. natriegens. M Hill 6/24/99

6/25/99 Inoculated 3 mls of V. natriegens into chemostat reactor. M Hill 6/25/99

6/28/99 On 6/26/99 performed routine subculture of V. natriegens, C. Acetobuty. & D. vulgaris as described

m p 135 & 136 on this notebook, except that C. acetobutylicum was not subcultured in 5% & 10% NaCl/PYG both.

On 6/27/99 prepared Baar's for chemostat as follows:

8.80 g of NaCl lot # 972274 were added to 175 mls of Baar's p 140 of this notebook. The above sol'n was added to ~~175~~ mls 6/28/99 3,500 mls of 18.1 MΩ H_2O & autoclaved for 2 hrs @ 121°C, 14.7 psig.

On 6/28/99 prepared PYG as follows.

5.00 g of NaCl lot # 972274 were added to 100 mls of PYG broth p 151 of this notebook, dispensed 9 mls of the above sol'n into screw-top tubes & autoclaved for 20 min @ 121°C, 14.7 psig.

10.00 g of NaCl lot # 972274 were added to 100 mls of PYG broth p 151 of this notebook & autoclaved for

mh 6/28/99

20 min @ 121°C, 14.7 psig.

prepared 500 mls of $Fe(NH_4)_2(SO_4)_2$ as follows:

25.0 g of $Fe(NH_4)_2(SO_4)_2$ ~~were~~ lot # 951022 were added to 500 mls of 18.1 MΩ H_2O.

On 6/27/99 (Note entry out of order) prepared ~~5 mls of~~ 5% sol'n of Baar's amended with 14.5 g of NaCl lot # 972274 as follows:

14.5 g of NaCl lot # 972274 were added to a 100 ml graduated cylinder. Brought volume up to 100 mls with Baar's p 140 of this notebook. To 5 mls of 18.1 MΩ H_2O 0.25 mls of the Baars/14.5 g NaCl sol'n was added. Autoclaved for 15 min @ 121°C 14.7 psig. Added 0.1 ml of filter sterilized $Fe(NH_4)_2(SO_4)_2$ to Baar's (5% sol'n of Baar's/14.5 g NaCl). Inoculated with D. vulgaris previously subcultured in 11% NaCl/Baar's. Incubated in shaking H_2O bath set @ 31°C,

100 strokes/min.

MH 6/28/99

6/29/99 Prepared PYG media for chemostat as follows:

MH 6/29/99

8.80 g of NaCl lot # 972274 were added to 175 mls of PYG broth P 151 of this notebook. The above sol'n was then added to 3,500 mls of 18.1 MΩ H_2O & autoclaved for 2 hrs @ 121°C 14.7 psig.

MH 6/1/99

6/1/99

Prepared Baar's Media as follows:

Comp I

		Lot #
$MgSO_4 \cdot 7H_2O$	2.0 g	947250
Sodium Citrate	5.0 g	905357A
$CaSO_4$	1.0 g	
NH_4Cl	1.0 g	895752

into 400 mls 18.1 MΩ H_2O

Comp II

K_2HPO_4	0.5 g	950082

into 200 mls 18.1 MΩ H_2O

Comp III

Sodium lactate	3.5 g	943605
Yeast Extract	1.0 g	59695JB

into 400 mls 18.2 MΩ H_2O.

Combined components I, II & III & stored in fridge for preservation.

M Hill 6/29/99

MH 7/1/99

29 MH 7/1/99

On 6/30/99 prepared the following:

Mineral sol'n 1

		Lot #
K_2HPO_4	6.0 g	950082

into 1 L of 18.1 MΩ H_2O.

Mineral sol'n 2 (per L)

		Lot #
NaCl	12.0 g	972274
KH_2PO_4	6.0 g	946016A
$(NH_4)_2SO_4$	6.0 g	953346
$MgSO_4 \cdot 7H_2O$	2.4 g	947409
$CaCl_2 \cdot 2H_2O$	1.6 g	947250

On 6/30/99 prepared the following:

Sodium Carbonate Sol'n

		Lot #
Na_2CO_3	8.0 g	976472A

into 100 mL 18.1 MΩ H_2O

Wolfe's Mineral Sol'n

		Lot #
$MgSO_4 \cdot 7H_2O$	3.0 g	947409
Nitriloacetic Acid	1.5 g	85H0935
NaCl	1.0 g	972274
$MnSO_4 \cdot H_2O$	0.5 g	951425
$FeSO_4 \cdot 7H_2O$	0.1 g	946178
$CoCl_2 \cdot 6H_2O$	0.1 g	990214
$CaCl_2$	0.1 g	947250
$ZnSO_4 \cdot 7H_2O$	0.1 g	951602
$CuSO_4 \cdot 5H_2O$	0.01 g	951540
$AlK(SO_4)_2 \cdot 12H_2O$	0.01 g	126H1008
H_3BO_3	0.01 g	947358A
$Na_2MoO_4 \cdot 2H_2O$	0.01 g	990041

MH 7/1/99

Added nitriloacetic acid to 500 mls of 18.1 MΩ H_2O pH ~10. Added remaining components & brought volume to 1 L.

On 7/1/99 prepared L-cysteine-sulfide reducing agent as follows:

L-Cysteine HCl·H_2O 0.3 g lot # 19H1152 into 10mls 18.1 MΩ H_2O.

$Na_2S \cdot 9H_2O$ 0.3 g lot # 990385 into 10mls 18.1 MΩ H_2O.

Purged both sol'ns with high purity ~~nitrogen~~ MH 7/1/99 nitrogen & autoclaved for 15 min @ 121°C, 14.7 psig. Allowed to cool to 50°C in ~~shaking~~ H_2O bath set @ 50°C.

MH 7/1/99

On 6/30/99 (note entry out of order) combined components as follows:

Mineral sol'n 2	50 ml
Sodium Carbonate sol'n	50 ml
Mineral sol'n 1	25 ml
Wolfe's Mineral sol'n	10 ml
Resazurin (0.025% sol'n)	4 ml

brought volume to 980 mls.

Prepared 0.025% Resazurin soln as follows: 0.025 g of Resazurin lot # 7783703 into 100 mls 18.1 MΩ H_2O.

Dispensed 4.9 mls of Methanobacteria Medium into Balch tubes. Sealed & purged with 80% H_2 & 20% CO_2. Autoclaved for 15 min @ 121°C 14.7 psig. Note: Resazurin turned pink following purging with gas (80-190 count no purge).
MH 7/1/99

Prepared 1 additional soln MH 7/1/99 of L-cysteine sulfide reducing MH 7/1/99 agent as described on p 59 of this notebook except that the tubes were purged with high purity nitrogen for a longer period of time. MH 7/1/99

7/4/99 MH 7/4/99

On 7/2/99 revived M. ~~foric~~ formicicum ATCC # 33274 lot # 966751 in anaerobic atmosphere. Incubated @ 37°C.

On 7/4/99 prepared Baar's medium for chemostat as follows:

5 g of NaCl lot # 972274 were placed in a graduated cylinder. Brought volume up to 100 mls with Baar's p 156 of this notebook. Filled volume to 3,500 mls with 18.1 MΩ H_2O. Autoclaved for 2 hrs @ 121°C, 14.7 psig.

Prepared 2 separate Baar's soln's as follows:

17.5 g of NaCl lot # 972274 were placed in grad cylinder. Brought volume up to 100 mls with Baar's p 156 of this notebook. Added 0.25 mls of the above soln to 5 mls of 18.1 MΩ H_2O. Repeated the procedure above except that 20.0 g of NaCl lot # 972274 were used. Purged with high purity nitrogen, sealed, & autoclaved for 15 min (both soln's) @ 121°C, 14.7 psig. MH 7/4/99

Performed routine subculture of V. natriegens, C. acetobutylicum & D. vulgaris as described on p 135 & 136 of

this notebook. MH 7/4/99

7/6/99 Prepared 1000 mls of $Fe(NH_4)_2(SO_4)_2$ as follows:

50 g of $Fe(NH_4)_2(SO_4)_2$ lot # 957022 were added to empty aliquot. Brought volume to 1000 mls with 18.1 MΩ H_2O.

prepared P46 media for chemostat as follows:

0.80 g of NaCl lot # 972274 were placed in grad. cylinder. Added 175 mls of P46 broth P 151 of this notebook. Brought volume up to 3500 mls with 18.1 MΩ H_2O. Autoclaved for 2 hrs @ 121°C, 14.7 psig.

Filter sterilized $Fe(NH_4)_2(SO_4)_2$ & added 3.5 mls to Baars prepared on 7/4/99 P 160-161 of this notebook (Baar's media for chemostat). Added 0.1 ml of $Fe(SO_4)_2(NH_4)_2$ to Baar's modified with 17.5 g ± 20 g of NaCl (5% soln of Baar's p 161 of this notebook. Inoculated with D. vulgaris. Contamination by molds observed in chemostat reactor (V. natriegens.) Disassembled & reautoclaved. Added 25 g of NaCl lot # 972274 to 500 mls of nutrient broth. autoclaved for 20 min @ 121°C, 14.7 psig. Allowed to cool & inoculated with 3 mls of V. natriegens.

To date have successfully cultured C. acetobutylicum & V. natriegens in 5% soln of 2% NaCl nutrient. Sub-cultured major. Incubated @ 31°C. MH 7/6/99

7/9/99 prepared Baar's amended with 10% NaCl as follows:

10 g of NaCl lot # 972274 were added to 100 mls of Baar's p 56 of this notebook. Purged with nitrogen & autoclaved for 15 min @ 121°C, 14.7 psig.

Performed routine subculture of D. vulgaris, V. natriegens

MH 7/9/99

& C. acetobutylicum as described on p 135 & 136 of this notebook. M Hill 7/19/99

7/19/99 On 7/16/99 performed routine subculture of D. V. natriegens, C. acetobutylicum & D. vulgaris as described on p 135 & 136 of this notebook, except that D. vulgaris was not subcultured in media amended with 5% NaCl. D. vulgaris was subcultured, however in media amended with 9% NaCl.

On 7/18/99 purged glove box with high purity nitrogen & inoculated 0.1 ml of $Na_2S \cdot 9H_2O$/L-cysteine p/59 of this notebook (into Methanobacteria medium p 160 of this notebook). After Resazurin turned colorless subcultured M. arbicicum. M. Hill 7/19/99

7/23/99 Prepared component I of Baar's as follows:

		Lot #
$MgSO_4 \cdot 7H_2O$	2.0g	947250
Sodium Citrate	5.0g	905357A

		Lot #
$CaSO_4$	1.0g	
NH_4Cl	1.0g	895752

into 400 mls 18.1 MΩ H_2O

Comp II

K_2HPO_4	0.5g	950082

into 200 mls 18.1 MΩ H_2O

Comp III

Sodium lactate	3.5g	943605
Yeast Extract	1.0g	5969515

into 400 mls 18.1 MΩ H_2O.

Combined components I, II & III & stored in fridge for preservation.

Added 5.0g of NaCl lot # 972274 to 100 mls of Baar's p 164-165 of this Notebook. Dispensed 5 mls in tubes, purged with nitrogen & capped. Autoclaved for 25 min @ 121°C, 14.7 psig.

Prepared 500mls of nutrient agar as follows:

	Lot #	
Nutrient broth	79113JF	4.0 g
NaCl	972274	7.5 g
Bacto Agar		10.0 g

into 500 mls 18.1 MΩ H_2O. Autoclaved for 25 min @ 121°C, 14.7 psig. Poured as plates NA6.

performed routine subculture of V. natriegens, C. acetobutylicum & D. vulgaris as described in p 135 & 136 of this notebook, except that D. vulgaris was subcultured in media amended with 9% NaCl. M Vill 7/23/99

7/26/99 Prepared 1000 mls of $Fe(NH_4)_2(SO_4)_2$ as follows: (lot # 951022)
Added 1000 mls of 18.1 MΩ H_2O to 50.0 g of $Fe(NH_4)_2(SO_4)_2$. lot # 951022
Purged with high purity nitrogen following filter sterilization. Added 0.1 mL

of sterile $Fe(NH_4)_2(SO_4)_2$ sol'n to Baar's amended with 5% NaCl p 165 of this notebook.

Subcultured D. vulgaris in Baar's amended with 5% NaCl.
M. Vill 7/26/99

8/2/99 Prepared nutrient broth as follows:

8.02 g of nutrient broth lot # 79113JF were placed in 1000 ml aliquot. Filled volume up to 1000 mls with 18.1 MΩ H_2O. Stored in fridge for preservation.

prepared Methanobacteria Medium as follows:

Added 50 mls of Mineral sol'n 2 (p 157 of this notebook) 50 mls of sodium carbonate sol'n p 158 of this notebook 25 mls of mineral sol'n 1 p 157 of this notebook, 10 mls of Wolfe's mineral sol'n (p 158 of this note-

book 4 4 mls of Resazurin
p 160 of this notebook.
Dispensed in 1000 ml
aliquots. Brought volume
up to 980 mls with 18.1
MΩH2O. stored in fridge
for preservation.
M Hill 8/2/99

performed routine subculture
on M. formicicum & D.
vulgaris.
M Hill 8/2/99

8/5/99 On 8/4/99 prepared
nutrient broth for
abiotic corrosion test
as follows:

Added 35.0g of NaCl
lot # 947723 to 4L
Carboy. Added ~~77~~ 350 mls of nutrient
broth p 164 of this
notebook & brought
volume up to 3500 mls
with 18.1 MΩH2O.
Prepared PYG broth
as follows:

Added 35.0g of NaCl
lot # 947723 to 4L
Carboy. 175 mls of

PYG broth were added
p 151 of this notebook
& the volume was
brought up to 3500 mls
with 18.1 MΩH2O.

On 8/5/99 prepared
Baar's as follows:

35.0g of NaCl lot #
947723 were added to
4L carboy. 171.5 mls
of Baar's p 164-165
of this notebook were
added. Prepared
$Fe(NH_4)_2(SO_4)_2$ as
follows: 5.0g of
lot # 951022 → $Fe(NH_4)_2(SO_4)_2$
were added to 100ml
aliquot volume was brought
up to 100 mls with
18.1 MΩH2O. added
3.5 mls of $Fe(NH_4)_2$
$(SO_4)_2$ to Baar's in
4L carboy brought
volume up to 3500 mls
with 18.1 MΩH2O.

all three sol'ns were
filter sterilized (0.2um)
on the day that they
were prepared. Filtered

into prestirilized 4L carboys autoclaved for 25 min @ 121°C, 14.7 psig.

MH
8/8/99

8/9/99 On 8/6/99 prepared Methanobacteria Medium for corrosion test as follows:

35.0 g of NaCl lot # 947723 was added to 4L carboy. 171.5 mls of MB p 167-168 of this notebook were added & the volume was brought up to 3500 mls with 18.1 MΩ H_2O. Autoclaved for 1 hr @ 121°C, 14.7 psig.

Prepared $FeSO_4$ as follows:

5.0 g of $FeSO_4$ lot # 946178 were added to 100 ml aliquot. Brought volume up to 100 mls with 18.1 MΩ H_2O.

Added 2% of $FeSO_4$ sol'n (p 170 of this notebook to 5 mls of Baar's p 164-165 of this notebook. But prior to adding $FeSO_4$ sol'n Baar's was autoclaved for 15 min @ 121°C, 14.7 psig.

Subcultured D. vulgaris C. acetobutylicum & V. natriegens as described on p 135 + 136 of this notebook.

Note $FeSO_4$ was not sterilized. Inoculated with D. vulgaris + incubated @ 31°C.

M Dill
8/9/99

8/10/99 Added 35.0 g of NaCl lot # 947723 to 4L carboy. Added 171.5 mls of Baar's p 164-165 of this notebook to carboy. Added 3.5 mls of $FeSO_4$ p 170 of this notebook. Brought volume up to 3500 mls with 18.1 MΩ H_2O. Filter sterilized (0.2 μm) into

4 L carboy previously sterilized by autoclaving for 25 min @ 121°C, 14.7psig.

Added 3.5 mls of L-cysteine ≠ Na_2S reducing agent p160 of this notebook to MB media prepared for abiotic corrosion test. M Hill 8/10/99

MH 8/14/99

8/14/99 Dispensed 5 mls Methanobacteria Medium p167-168 of this notebook into Hungate tubes. Sealed & purged with 80% hydrogen 20% CO_2 mixture. Autoclaved for 15 min @ 121°C, 14.7psig.

Collected sample of effluent from autoclave to check for sterility. Plated on nutrient agar & incubated @ 37°C. M Hill 8/14/99

It was noted that Vic's pump was not collecting set volume of nutrient. Test began on 8/9/99

with introduction of fluids on 8/10/99. M Hill 8/14/99

8/17/99 Performed routine subculture of *V. Netriegens*, *C. acetobutylicum* *D. vulgaris* & *M. formicicum*. MH 8/17/99

Also, sample of effluent collected from Vic's autoclave p172 of this notebook positive for bacterial growth. The contaminant did not resemble bacteria cultured for PRCI project. MH 8/17/99

*Note did not photograph contaminant because scanner is broken. MH 8/17/99 MH 8/18/99

8/18/99 Prepared Baar's ~~Amount~~ as follows for abiotic corrosion test.

35.0 g of NaCl lot # 947723 was added to 4L carboy. 171.5 mls of Baar's p

164-165 of this notebook. Added 3.5 mls of FeSO4 @ 170 of this notebook. Brought volume to 3,500 mls with 18.1 MΩ H_2O. Filter sterilized.

MH 8/18/99

8/20/99 Collected effluent from autoclave to check for contamination. Plated on nutrient agar. Incubated @ 31°C. MH 8/20/99

8/23/99 Prepared Biocoupon/Transport media as described below:

Biocoupon Preservative / Transport Media Type "A" Preparation

This will make 200 ml of solution

Ingredients:

200 ml filter sterile DI water
0.93 grams FTA Hemagglutination Buffer (in the small refrigerator)
0.28 ml Sodium Lactate (7 x 40uL) 280uL
2 ml Oxyrase enzyme system (freezer) = 60 units

Filter 200 ml of DI water through a 0.2 micron membrane filter. The filters and holder are located in the wall cabinet in the met lab. Dissolve the buffer in the sterile water. Add the Sodium Lactate using a graduated micro-pipette. Pour the solution into a clean flask and plug with cotton or cover with aluminum foil.

Autoclave the solution and 25 vials and caps, for 35 minutes at 250°F, which is the lowest heat setting. To start the cycle, turn the Power and Slow Exhaust switches ON.

When sterilization is complete, remove the media from the autoclave and allow to cool. When the media (flask) is warm to the touch (<100°F), add the oxyrase using a sterile syringe or pipette. Agitate the flask to mix the oxyrase with the buffer (DO NOT SHAKE!). Fill the sample vials using aseptic technique, seal, label and store in the refrigerator.

Generally, the media can be stored for up to 30 days in the lab under constant refrigeration.

M Hill 8/23/99

used 18.1 MΩ H_2O FTA Hema. Buffer lot # 1000AZDJZN Sodium lactate lot # 943605 & oxyrase enzyme system PEC # 98713 CAB0019V.002.

M Hill 8/23/99

8/25/99 On 8/24/99 disassembled abiotic corrosion test I. Swabbed specimen # 5 to perform plate count. Vortexed swab in sterile PBS for 3 min. Shipped coupons to P. Angell. Performed routine sub-culture of C. aceto-butylicum + V. rotrigens.

M Hill 8/25/99

Also; effluent sample collected from Vic's autoclave p 174 of this notebook was + for contamination. Contaminant did not resemble bugs used in biotic test for PRCI project. M Hill 8/25/99

MA 9/2/99

MA 9/2/99

9/2/99 Plate Count ~~Abiotic~~ Control
Test I Corrosion Bld 90

date	time	cfu/ml
8/24/99	MA 9/24/99 ~~30~~	3.49×10^3

Subcultured D. vulgaris on 8/30/99. Subcultured C. acetobutylicum, V. natriegens & M. formicicum on 9/2/99.

MA 9/2/99

9/14/99 Subcultured D. vulgaris, C. acetobutylicum & V. natriegens. Incubated @ 31°C.

MA 9/14/99

9/17/99 Prepared Nutrient broth amended with 1% NaCl as follows:

5.0g of NaCl lot # 972274 were added to 500 mls of NB p 167 of this notebook. Chemostat vessel, lines & nutrient broth were autoclaved for 45 min @ 121°C 14.7 psig.

Prepared stock PYG broth as follows:

		lot #
peptone	20.0g	639285JB
glucose	10.0g	99915JA
yeast extract	10.0g	59695JB
L-cysteine HCl	0.50g	19H1152

added 40mls of VPI salt sol'n p91 of this notebook Brought volume up to 1000mls with 18.1MΩ H_2O.

Prepared PYG broth amended with 1% NaCl as follows:

5.0g of NaCl lot #972274 were added to 500ml aliquot. Brought volume up to 500mls with stock PYG broth p177 of this notebook. Autoclaved Chemostat vessel, lines & PYG nutrient for 45min @ 121°C, 14.7psig.

Prepared stock sol'n of Baar's as follows:

Component I	Lot # MA 9/17/99
$MgSO_4$ 2.0g	947250409

Sodium Citrate	5.0g	905357A
$CaSO_4$	1.0g	
NH_4Cl	1.0g	895752

into 400 mls of 18.1 MΩ H_2O.

9/21/99

Component II

		Lot #
K_2HPO_4	0.5g	950082

into 200mls of 18.1 MΩ H_2O.

Component III

		Lot #
Sodium lactate	3.5g	943605
Yeast Extract	1.0g	59695JB

into 400 mls 18.1 MΩ H_2O.

MA 9/17/99

9/20/99 Prepared media for batch reactor (Methanogens) biotic Corrosion test I:

Added 20.0g of NaCl lot # 972274 to 1 L aliquot. Added 343 mls of Methanobacteria Medium (MB) p 167-168 of this notebook. Added 7000mls of 18.1 MΩ H_2O.

Purged with 80% hydrogen 20% CO_2 mixture for 30 min.

MHill 9/20/99

Autoclaved media prepared for batch reactor (Methanogens) biotic corrosion test I (p 178 of this notebook) for 2 hrs @ 121°C, 14.7 psig.

MHill 9/21/99

9/23/99 Prepared nutrient broth amended with 1.5% NaCl as follows:

8.0g of Difco nutrient broth lot # 79113JF & 15.0g of NaCl lot # 97 2274 were placed in 1000 ml aliquot. Brought volume up to 1000 mls with 18.1 MΩ H_2O. Dispensed ~9mls into screw-top tubes. Autoclaved for 20 min @ 121°C, 14.7 psig.

Prepared L-cysteine reducing agent as

follows:

0.3 g of L-cysteine lot # 94H1152 were added to Balch-Hungate tubes. Dispensed 10 mls of 18.1 MΩ H_2O capped & placed in autoclave (fast exhaust) for 15 min.

$Na_2S \cdot 9H_2O$ reducing agent prepared as follows:

0.3 g of $Na_2S \cdot 9H_2O$ lot # 990385 were added to Balch-Hungate tube. Dispensed 10 mls of 18.1 MΩ H_2O. capped & autoclaved for 15 min (fast exhaust) at 121°C, 14.7 psig.

Prepared chemostat reactor biotic comparison I as follows:

autoclaved 500 mls of Baar's p177-178 of this notebook, lines & reactor for 45 min @ 121°C, 14.7 psig.

MKull 9/23/99

9/24/99 Dispensed 9mls of PYG broth into screw-top tubes & autoclaved for 20 min @ 121°C, 14.7 psig. PYG broth prepared on 9/17/99 p177 of this notebook.

Prepared 100mls of $FeSO_4$ as follows:

5.0 g of $FeSO_4$ lot # 946178 were placed in a 100ml aliquot. Brought volume up to 100mls with 18.1 MΩ H_2O.

Prepared 50 mls of L-cysteine reducing agent as follows:

1.5 g of L-cysteine lot # 94H1152 was added to Balch-Hungate tube. Brought volume up to 50mls with 18.1 MΩ H_2O.

Prepared 50 mls of $Na_2S \cdot 9H_2O$ as follows:

1.5 g of $Na_2S \cdot 9H_2O$ lot # 990385 was

added to Balch-Hungate tube. Brought volume up to 50 mls with 18.1 MΩ H_2O.

Both L-cystine & $Na_2S \cdot 9H_2O$ reducing agents were autoclaved for 15 min (fast exhaust) @ 121°C, (14.7 psig).

Subcultured *V. natriegens*, *D. vulgaris* & *C. acetobutylicum*. Incubated @ 31°C. MB 9/24/99

Inoculated 3 chemostat reactors PYG broth, Nutrient Broth, + Baar's with 1000 m 1 ml of culture, respectively. MB 9/24/99

Filter sterilized FeSO4 p 181 using Sartorius 0.2μm filter & added 10mls to Baar's prior to inoculating with *D. vulgaris*. MB 9/24/99

9/27/99 On 9/25/99 subcultured *M. formicicum*. Inoculated 1 ml of *M. formicicum* into batch reactor for biotic

corrosion test I after adding reducing agents. On 9/27/99 Inoculated batch reactor with another 1 ml of *M. formicicum*.

Autoclaved nutrient reservoirs (PYG + nutrient broth) which will be used in biotic corrosion test I for 45 min @ 121°C, 14.7 psig.

MDill 9/27/99

9/28/99 Prepared PYG broth as follows:

		Lot #
peptone	20 g	63928 JB
dextrose	10 g	99915 JA
yeast extract	10 g	59695 JB
L-cysteine HCl	0.5 g	19H1152

Added 40mls of VPI salt sol'n p 91 of this notebook & brought volume up to 1000 mls with 18.1 MΩ H_2O.

C. acetobutylicum chemostat knocked over in bldg 90 (Steve Clay) so reassembled a new chemostat. Prepared PYG broth amended with 1% NaCl as

follows:

Full strength → so bacteria grow quickly

5.0 g of NaCl lot # 972274 were added to 500 mls of PYG broth p 183 of this notebook. Autoclaved nutrient (PYG) chemostat vessel, lines @ 121°C, 14.7 psig for 45 min.

Dilute to enhance biofilm growth

Prepared nutrient broth for biotic corrosion test I following the directions on p 168 of this notebook.

Prepared PYG broth for biotic corrosion test I following the directions on p 168-169 of this notebook.

NaCl lot # 972274
PYG p 177 of this notebook
Nutrient broth p 179 of this notebook.

M Hill 9/28/99

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

N. Sundlass 6/8/2010