DOE/ER/60991--T2

RECEIVED

JUN 18 1993

OSTI

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

PHYSIOLOGICALLY ANAEROBIC MICROORGANISMS OF THE DEEP SUBSURFACE

Progress Report

June 1, 1991 - May 31, 1992

S. E. Stevens, Jr. and K.-T. Chung

Department of Biology

Memphis State University

Memphis, Tennessee 38152

June 1992

PREPARED FOR THE U.S. DEPARTMENT OF ENERGY
UNDER GRANT NUMBER DE-FG05-90ER60991

MASTER

48
DISSEMINATION OF THIS DOCUMENT IS UNLIMITED

Herein, I summarize the progress made by the research team assembled at Memphis State University during the first 18 and 1/2 months of our ongoing DOE sponsored project titled "Physiologically Anaerobic Microorganisms of the Deep Subsurface" (DOE Contract No. DE-FG05-90ER60991).

SCHEDULE OF MAJOR RESEARCH ACTIVITIES

Year 1 (1990-91)

- Determine numbers, diversity, and morphology of anaerobic microorganisms in 15 samples of subsurface material from the Idaho National Engineering Laboratory (INEL), in 18 samples from the Hanford Reservation (HR) and in 1 rock sample from the Nevada Test Site (NTS); set up long term experiments on the chemical activities of anaerobic microorganisms based on these same samples; work to improve methods for the micro-scale determination of in situ anaerobic microbial activity; begin to isolate anaerobes from these samples into axenic culture; and, begin to identify the axenic isolates.

Year 2 (1991-92)

- Determine selected physiological characteristics of all isolates from INEL, HR, and NTS; after characterization of obligate anaerobes is well started, begin the characterization of facultative anaerobes; compare isolates from different depths at each site; publish fundamental results from the above work.

Year 3 (1992-93)

- Look for possible new anaerobic isolates from long term

enrichments in stored samples; characterize these as above, if found; begin studies leading to the long term preservation of each axenic culture; analyze data sets from each investigator and determine factors which influence community structure especially in regard to anaerobic microorganisms; transfer preserved isolates to the culture collection at Florida State University; publish additional results.

PROJECT OUTPUT: MAJOR ACCOMPLISHMENTS (6/90 - 12/91)

- We have completed plate counts, MPN's, spin-tube counts, acridine orange direct counts, 4'-6-diamidino-2-phenylindole dihydrochloride, and ATP estimates on 15 samples from the INEL site, 18 samples from the HR site, and 1 sample from the NTS site.

A flow chart summarizing sample manipulation is shown in Fig. 1. Medium 1 is an egg yolk emulsion medium recommended as a general medium for anaerobic bacteria. Medium 2 is a 1:50 dilution of medium 1. Medium 3 is full strength PTYG. Medium 4 is a 1:100 dilution of medium 3. The primary media, reducing agents, and redox indicators used in the project are described on pages 13-16 of this report. Both spread plates and spin-tubes with pre-reduced (bubbled with 4% H₂ balance CO₂) medium were used for counting purposes. The use of PTYG as a growth medium for anaerobic bacteria was validated by plating pure cultures of anaerobic bacteria obtained from the ATCC on both their ATCC

FIGURE 1

GRAM STAINING

CHARACTERIZATION

IDENTIFICATION: API INDEX

MEDIA FOR ANAEROBES

1. General Heterotrophs.

MEDIUM I.

COMPOUND	FINAL CONC. g/L medium	STOCK CONC. g/L	ml STOCK/ L medium
¹ Na ₂ EDTA	0.002	3.0	0.66
¹ A-5 METAL MIX	NA	NA	1.0
¹ Ferric ammonium citrate	0.012	6.0	2.0
K ₂ HPO ₄	0.065	65.0	1.0
KH ₂ PO ₄	0.035	35.0	1.0
Na ₂ CO ₃	0.025	25.0	1.0
NaCl	0.075	75.0	1.0
¹ MgSO ₄ -7H ₂ O	0.075	50.0	1.5
trypticase	15.0	NA	NA
glucose	5.0	NA	NA
yeast extract	1.0	NA	NA
chicken egg yolks	2	NA	NA
² BBL granulated agar	15	NA	NA

¹Stock solutions of these compounds are used in Medium B for cyanobacteria and need not be made up separately.

²The agar is added if appropriate.

NA = not applicable.

Milli-Q water should be used in preparing this medium. Measure out about 900 ml of water, add the above listed ingredients, pH the solution to 6.9 as indicated below, and then make to 1L with the required amount of water. If agar is to be added, it is added after the solution is made to 1L. Likewise with reducing agents and redox indicators.

The pH of the medium should be adjusted to 6.9 prior to autoclaving.

Reducing agents and Redox indicators are prepared separately and added to the medium just prior to use.

Medium II.

Medium II is a 1:50 dilution of Medium I in Milli-Q water.

Medium III.

Medium III is full-strength PTYG medium.

COMPOUND	FINAL CONC. g/L medium
Peptone	5.0
Tryptone	5.0
Yeast extract	10.0
Glucose	10.0
MgSO ₄ -7H ₂ O	0.6
CaCl ₂ -2H ₂ O	0.07
BBL granulated agar	15.0

MEDIUM IV.

Medium IV is a 1:1000 dilution of the first four ingredients of Medium III.

COMPOUND	FINAL CONC. g/L medium
Peptone	0.05
Tryptone	0.05
Yeast extract	0.1
Glucose	0.1
MgSO ₄ -7H ₂ O	0.6
CaCl ₂ -2H ₂ O	0.07
BBL granulated agar	15.0

2. Specific anaerobes

MEDIUM V, for enumeration and isolation of sulfate reducing bacteria.

COMPOUND	FINAL CONC. g/L medium	STOCK CONC. g/L	ml STOCK/ L medium
*KH ₂ PO ₄	0.5	50.0	10.0
NH ₄ Cl	1.0	none	weigh
CaSO ₄ -2H ₂ O	1.26	none	weigh
MgSO ₄ -7H ₂ O	2.0	none	weigh
#Sodium lactate	3.5	60% syrup	4.7
Yeast extract	1.0	none	weigh
Ascorbic acid	0.1	none	weigh
Thioglycollic acid	0.1	none	weigh
FeSO ₄ -7H ₂ O	0.5	none	weigh
BBL granulated agar	15.0	none	weigh

Use building distilled water. Adjust to pH 7.5 with NaOH after boiling to dissolve agar.

*Use ml indicated in column 3 from medium A stocks in Rm 521.

#Use Sigma DL-Lactic Acid, catalog number L-1375.

REDUCING AGENTS AND REDOX INDICATORS FOR ANAEROBIC MEDIA

The following reducing agents will be used as appropriate in Media I - IV for the viable cell counts of general heterotrophic anaerobic bacteria.

Reducing agent	E_o' [mV]	Preparation of stock solution	Final conc. in medium
Sodium thioglycollate	<-100	1% (w/v) in MQ ¹ H ₂ O, autoclaved and stored under O ₂ free argon.	0.05%
Na ₂ S-9H ₂ O	-225	1.2% (w/v) Na ₂ S-9H ₂ O in MQ H ₂ O, autoclaved and stored under O ₂ free argon.	0.025%
Cysteine	-340	1% (w/v) in MQ H ₂ O, autoclaved and stored under O ₂ free argon.	0.025%
Titanium (III) citrate	-480	5 ml of 15% (w/v) solution of TiCl ₃ added to 50 ml of 2.36% (w/v) sodium citrate dihydrate and neutralized by addition of saturated Na ₂ CO ₃ . Sterilized with 0.22μm filters. <u>NOTE: must be used within 1 day at a concentration of 30ml/L medium!</u>	0.5-2mM

REDOX INDICATOR.

Sterilize a 0.1% aqueous stock solution of resazurin by passing it through a 0.22μm filter, and add to the growth medium to give a final concentration of 0.0003%. NOTE: resazurin is not needed when titanium (III) citrate is used as reducing agent!

Diluent or medium is useful only if colorless!

recommended medium and on PTYG. The results support the use of PTYG as a plating medium for anaerobes (see Tables 1~~7~~³). In addition, we used Postgate's medium to determine the presence of sulfate reducing bacteria by MPN, and found none. We essentially made one change in this medium (the revised recipe is given on page 15) and used it to successfully culture sulfate reducing bacteria from several core subsamples from INEL and HA. We also used 2 media, CO₂/H₂ and acetate/methylamine to determine the presence of methanogens and acetogens in INEL, HR, and NTS samples. Plate count data, MPN data, and spin-tube counts for 15 samples from INEL and 18 samples from HR are given in Tables 2² and 3³. ATP estimates for these same samples are not given because none of them, with the exception of the spiked sample from INEL, indicated a reliably significant concentration of ATP.

The NTS sample provided by Dr. Penny Amy did not show any anaerobic growth.

Methane production occurred in two HR samples and gas was taken up in an additional HR sample. These results indicate methanogenic bacteria is samples 0007b and 0307 and a probable acetogenic bacterium in sample 0407 from HR.

AODC data are plotted in Figs. 2 and 3 as number of cells versus depth. The highest numbers of cells were between 200 and 300 feet below the land surface at both INEL and HR.

- We have recorded the colonial morphology, cellular morphology, and Gram reaction on each colony type observed

TABLE 1

MEDIUM	CULTURE COUNT (PER ML)
REINFORCED CLOSTRIDIAL MEDIUM (OXOID)	9.5 X 10 ⁸ 6.8 X 10 ⁸ 1.3 X 10 ⁹
TPYG	1.2 X 10 ⁹ 1.1 X 10 ⁹ 1.1 X 10 ⁹
NUTIENT AGAR	<10 ⁵
LACTOBACILLUS AGAR	1.1 X 10 ⁹ 7.8 X 10 ⁸ 7.7 X 10 ⁸
MEDIUM 1	NO GROWTH

**BACTEROIDES FRAGILIS ATCC 23745
GROWN ON BHI FOR 48 HOURS.**

TABLE 2

MEDIUM	CULTURE COUNT (PER ML)
REINFORCED CLOSTRIDIAL MEDIUM (OXOID)	1.1×10^7
	1.1×10^7
	2.9×10^7
TPYG	1.1×10^7
	8.8×10^6
	7.5×10^6
NUTRIENT AGAR	$<10^5$
LACTOBACILLUS AGAR	8.0×10^5
	4.5×10^5
	3.5×10^5

**CLOSTRIDIUM PERFRINGEN ATCC 3624
GROWN ON BHI FOR 24 HOURS.**

TABLE 3

MEDIUM	CULTURE COUNT (PER ML)
LACTOBACILLUS AGAR*	1.5x10 ⁵ 3.2x10 ⁶ 4.7x10 ⁶
TYPG*	5.5x10 ⁴ 6.0x10 ⁴ 6.0x10 ⁴
MEDIUM I**	4.4x10 ⁵ 9.9x10 ⁵ 7.9x10 ⁵
MEDIUM II**	2.7X10 ⁵ 6.5X10 ⁵

LACTOBACILLUS BULGARICUS ATCC 27558
GROWN ON BHI FOR 24 HOURS

TABLE 4.

INEL

Sample #	PC-OXY	PC-OXY	PC-OXY	PC-OXY	PC-OXY	SUL-RED	PR-OXY	PR-OXY	PR-OXY
	I	II	III	IV	MPN	MPN	MPN	III*	IV*
207	<0.5	NG	<0.5	<0.5	<0.5	<0.5	>7	NG	<0.5
240/5	<0.5	<0.5	<0.5	<0.5	<0.5	NG	>2	NG	NG
394/1-7	<0.5	<0.5	<0.5	<0.5	<0.5	NG	>2	NG	NG
395/6-8	<0.5	<0.5	<0.5	<0.5	<0.5	NG	>2	NG	NG
400/9-11	<0.5	NG	<0.5	NG	NG	NG	>2	NG	NG
443/8-11	NG	<0.5	<0.5	<0.5	<0.5	NG	>2	<0.5	NG
448/0-3	NG	NG	NG	NG	NG	NG	NG	NL	ND
451/10-452/10	NG	NG	<0.5	NG	NG	NG	>2	NG	NG
452/1-453/1	4.8	<0.5	<0.5	<0.5	<0.5	NG	ND	<0.5	NG
452/11-453/1	<0.5	NG	<0.5	NG	NG	NG	>7	NG	<0.5
455/10-456/5	<0.5	<0.5	NG	<0.5	<0.5	NG	>7	<0.5	NG
458/6-459	<0.5	<0.5	<0.5	<0.5	<0.5	NG	>7	<0.5	NG
520/2	NG	NG	NG	NG	NG	NG	NG	ND	ND
560-564	<0.5	<0.5	<0.5	<0.5	<0.5	NG	>7	NG	NG
0-4-6	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	>7	3.2	<0.5

TABLE 5.

YAKIMA

Sample #	PC-OXY	PC-OXY	PC-OXY	PC-OXY	PC-OXY	SUL-RED	PR-OXY	PR-OXY	PR-OXY
	I	II	III	IV	MPN	MPN	MPN	III*	IV*
HMB-1	<0.5	<0.5	<0.5	3.6	<0.5	<0.5	<1.0	<1.0	<1.0
0007A	NG	4.1	<0.5	4.2	<0.5	<0.5	>7	4.2	<1.0
0007B	NG	<0.5	NG	4.5	<0.5	<0.5	8.1	2.6	<1.0
107	<0.5	<0.5	<0.5	<0.5	NG	NG	>7	NG	NG
207	NG	<1.0	NG	5.9	<0.5	<0.5	>7	NG	NG
307	<0.5	<0.5	<0.5	<0.5	NG	NG	4.4	NG	NG
407	NG	NG	NG	NG	NG	NG	4.3	NG	NG
507	NG	NG	NG	NG	NG	NG	<2.0	NG	NG
537	NG	NG	NG	NG	NG	NG	4.7	NG	NG
607	4.1	4.9	5.0	4.3	NG	NG	3.8	NG	NG
707	NG	<0.5	<1.0	2.5	NG	NG	<2.0	NG	NG
807	NG	NG	NG	NG	NG	NG	<2.0	NG	NG
907	NG	NG	NG	NG	NG	NG	<2.0	NG	NG
1007	NG	NG	<0.5	<0.5	NG	NG	ND	NG	NG
1107	4.7	NG	NG	NG	NG	NG	ND	NG	NG
1207	5.5	5.6	5.3	5.6	NG	NG	ND	NG	NG
1307	NG	4	<1.0	<1.0	NG	NG	ND	NG	NG
1407	NG	5.1	<1.0	4.8	NG	NG	ND	<1.0	NG

FIGURE 2

FIGURE 3

on plates and/or spin-tubes. These results are tabulated in Tables ⁴6 and ⁵7. The Gram reaction and morphological results also give a preliminary notion of diversity. Pie charts summarize these results and are presented in Figs. 4 and 5. About 85% of the isolates from INEL were Gram + while only about 67% of those from HA were Gram +. Several of these Gram + isolates were isolated from long term enrichments and were very slow in appearing. Coccobacilli were the predominant morphological type at both INEL and HA. Gram - bacteria and those with a rod-type cellular morphology were minority representatives at both INEL and HA.

- So far, we have isolated into pure culture 64 apparently distinct anaerobes from all the samples examined. Of the 65 pure cultures obtained, 44 are facultative anaerobes and 21 are obligate anaerobes. In addition, we have evidence for sulfate reducing bacteria (positives for 6 different samples), methanogens, and an acetogen although we do not yet have axenic cultures of these. (Figs. 6 & 7)
- We have begun to obtain physiological/biochemical profiles of our axenic cultures. The results of these tests are tabulated in Tables ⁶8 and ⁷9. The physiological tests performed to date afford no unequivocal identifications. Further characterizations are planned.
- ~~As an indication of future plans~~ we compiled the data on aerobic chemoheterotrophs from HA obtained by 4 laboratories and tabulated it according to the specific depth at which the sample was prepared. The tabulation is presented in

TABLE 6.

INEL

Sample #	Gram Stain	Cell Type	Cell Type	Colony Shape	Colony Color	Colony Elev	Colony Size
207							
207A (I) S	+	cb	pairs	spindle	wo	dbl con	small
207A (II) S	+	cb	pairs	?	?	?	?
207A L	+	cb	pairs	?	?	?	?
207AB L	-	cb	pairs	spindle	wo	dbl con	small
207 INEL S	-	rd	single	circ	w t	con	large
207 INEL L	-	rd	single	circ	w t	con	large
240/5							
240/5A	-	rd	single	circ	w t	con	large
240/5B S	+	cb	pairs	spindle	wo	dbl con	small
240/5B L	+	cb	pairs	spindle	wo	dbl con	small
240/5C S	+	cb	single	?	?	?	?
240/5C L	+	cb	single	?	?	?	?
394//1-7							
394/1-7A S	+	cb	single	spindle	wo	dbl con	small
394/1-7A L	+	cb	single	spindle	wo	dbl con	small
394/1-7B S	+	rd	single	circ	w t	con	large
394/1-7B L	+	rd	single	circ	w t	con	large
395/6-8							
395/6-8A	+	rd	single	spreading	w t	raised	large
395/6-8B S	+	cb	single	spindle	wo	dbl con	small
395/6-8B L	+	cb	single	spindle	wo	dbl con	small
400/9-11							
400/9-11A S	+	rd sh	pairs	circ	w t	con	large
400/9-11A L	+	rd sh	pairs	circ	w t	con	large
400/9-11B	+	cb	chains	spindle	wo	dbl con	small
443/8-11							
443/8-11A	-	rd	single	circ	wo	con	large
443/8-11B	-	rd	single	spindle	wo	dbl con	small
448/0-3	No data - sample autoclaved						
451/10-452/10							
451/10-452/10A S	+	cb	pairs	circ	w t	con	large
451/10-452/10B L	+	cb	pairs	circ	w t	con	large
451/10-452/10B S	+	cb	pairs	spindle	wo	dbl con	small
451/10-452/10B L	+	cb	pairs	spindle	wo	dbl con	small

S=Solidified medium   B=Broth medium

INEL

S=Solidified medium   B=Broth medium

TABLE 7.

YAKIMA

Sample #	Gram Stain	Cell Type	Cell Type	Colony Shape	Colony Color	Colony Elev	Colony Size
HMB-1							
HMB-1A S	+	cb	pairs	spindle	wo	dbl con	small
HMB-1A L	+	cb	pairs	spindle	wo	dbl con	small
HMB-1B	-	rods	single	circ	w t	con	large
0007A							
0007A-A S	-	rods	single	circ	w t	con	large
0007A-A L	-	rods	single	circ	w t	con	large
0007A-B S	+	cb	pairs	spindle	wo	dbl con	small
0007A-B L	+	cb	pairs	spindle	wo	dbl con	small
0007B							
0007B-A S	-	cb	pairs	circ	w t	con	large
0007B-A L	-	cb	pairs	circ	w t	con	large
0007B-B S	+	cb	pairs	spindle	wo	dbl con	small
0007B-B L	+	cb	pairs	spindle	wo	dbl con	small
0007B-C S	-	cb	pairs	?	?	?	?
0007B-C L	-	cb	pairs	?	?	?	?
0007B-D S	-	cb	pairs	?	?	?	?
0007B-D L	-	cb	pairs	?	?	?	?
107							
107A S	+	cb	pairs	circ	w t	con	large
107A L	+	cb	pairs	circ	w t	con	large
107B S	+	cb	pairs	spindle	wo	dbl con	small
107B L	+	cb	pairs	spindle	wo	dbl con	small
107C S	+	cb	pairs	irr und	wo	flat	tiny
107C L	+	cb	pairs	irr und	?	flat	tiny
107D S	+	cb	pairs	?	?	?	?
107D L	+	cb	pairs	?	?	?	?

S=Solidified medium

L=Broth medium

TABLE 7 continued.

YAKIMA

Sample #	Gram Stain	Cell Type	Cell Type	Colony Shape	Colony Color	Colony Elev	Colony Size
207 Yakima L		rd/cb	sin/pairs	?	?	?	?
307							
307A	-	rd long	single	circ	w t	con	large
307B S	+	rd sh	single	circ	wo	flat	tiny
307B L	+	rd sh	single	circ	wo	flat	tiny
307C	+	rd long	single	spindle	wo	dbl con	small
407							
407A S	-	cb?	pairs?	circ	w t	con	large
407A L	-	cb?	pairs?	circ	w t	con	large
407B S	-	cb?	pairs?	circ	w t	con	large
407B L	-	cb?	pairs?	circ	w t	con	large
507							
507A S	+	cb	pairs	circ	w t	con	large
507B-(I) S	+	cb	pairs	?	?	?	?
507B-(II) S	+	cb	pairs	?	?	?	?
507B L	+	cb	pairs	?	?	?	?
507A L	+	cb	pairs	circ	w t	con	large
507A B L	+	cb	pairs	?	?	?	?
537							
537A S	+	cb	pairs	irr und	wo	dbl con	medium
537A L	+	cb	pairs	irr und	wo	dbl con	medium
537B S	+	cb?	pairs?	circ	w t	con	large
537B L	+	cb?	pairs?	circ	w t	con	large
607							
607A S	-	cb?	pairs?	circ	w t	con	large
607A L	-	cb?	pairs?	circ	w t	con	large
607B S	+	cb	pairs	spindle	wo	dbl con	small
607B L	+	cb	pairs	spindle	wo	dbl con	small
607C	-	cb	pairs	filamen	w t	flat	exlarge

S=Solidified medium

L=Broth medium

TABLE 7 continued.

YAKIMA

Sample #	Gram Stain	Cell Type	Cell Type	Colony Shape	Colony Color	Colony Elev	Colony Size
707							
707A S	+	cb	pairs	circ	w t	con	large
707A L	+	cb	pairs	circ	w t	con	large
707B S	+	cb	pairs	spindle	wo	dbl con	small
707B L	+	cb	pairs	spindle	wo	dbl con	small
807	could not culture						
907	could not culture						
1007							
1007 S	-	cb	pairs	?	?	?	?
1007 L	-	cb	pairs	?	?	?	?
1007(II) L	?	?	?	?	?	?	?
1007A S	-	cb	pairs	circ	w t	con	large
1007A L	-	cb	pairs	circ	w t	con	large
1007B S	+	sh rod?	single	irr und	wo	flat	small
1007B L	+	sh rod?	single	irr und	wo	flat	small
1107	could not culture						
1207	could not culture						
1307							
1307A	+	cb	pairs	circ	w t	con	large
1307B	-	rods	single	spindle	wp	dbl con	small
1407				circ	wo	con	small

S=Solidified medium

L=Broth medium

FIG. 4.

INEL - Morphological Types

FIG. 5.

Yakima - Morphological Types

TABLE 8.

INEL

Sample #	IND	UPE	GEL	ESC	CAT	GLJ	MAN	LAC	SAC	MAL	SAL	XYL	ARA	GLY	CEL	MNE	MLZ	RAF	SOR	RHA	TRE
207																					
207A (I) S	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
207A (II) S	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
207A L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
207AB L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
207 INEL S	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+
207 INEL L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+
240/5																					
240/5A		No API strip																			
240/5B S	-	-	-	+	-	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+
240/5B L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
240/5C S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
240/5C L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
394/1-7																					
394/1-7A S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
394/1-7A L		No API strip																			
394/1-7B S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
394/1-7B L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
395/6-8																					
395/6-8A		No API strip																			
395/6-8B S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
395/6-8B L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400/9-11																					
400/9-11A S	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400/9-11A L	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400/9-11L		No API strip																			

TABLE 8 continued.

INEL

Sample #	IND	URE	GEL	ESC	CAT	GLU	MAN	LAC	SAC	MAL	SAL	XYL	ARA	GLY	CEL	MNE	MLZ	RAF	SOR	RHA	TRE
443/8-11																					
443/8-11A		No API strip																			
443/8-11B		No API strip																			
448/0-3		No API strip																			
451/10-452/10																					
451/10-452/10-A S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
451/10-452/10-A L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
451/10-452/10-B S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
451/10-452/10-B L	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
452/1-453/1																					
452/1-453/1-A		No API strip																			
452/1-453/1-B		No API strip																			
452/11-453/1																					
452/11-453/1-A S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
452/11-453/1-A L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+
452/11-453/1B		No API strip																			
455/10-456/5																					
455/10-456/5-A		No API strip																			
455/10-456/5-B S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
455/10-456/5-B L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
458/6-459																					
458/6-459-A S	-	-	-	-	+	+	+	+	+	-	+	-	-	-	+	-	+	-	+	-	+
458/6-459-A L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
458/6-459-B		No API strip																			
520/2		No API strip																			

TABLE 8 continued.

INEL

Sample #	IND	URE	GEL	ESC	CAT	GLU	MAN	LAC	SAC	MAL	SAL	XYL	ARA	GLY	CEL	MNE	MLZ	RAF	SOR	RHA	TRE
560-564																					
560-564A S	-	-	-	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+	-	+	+
560-564A L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
560-564B S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
560-564B L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0-4-6																					
0-4-6A S	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0-4-6A L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0-4-6B S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0-4-6B L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0-4-6C			No API strip																		

YAKIMA

TABLE 9.

Sample #	IND	URE	GEL	ESC	CAT	GLU	MAN	LAC	SAC	MAL	SAL	XYL	ARA	GLY	CEL	MNE	MLZ	RAF	SOR	RHA	TRE
HMB-1																					
HMB-1A S	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
HMB-1A L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HMB-1B					No API strip run																
0007A																					
0007A-A S	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
0007A-A L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0007A-B S	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0007A-B L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
007B																					
0007B-A S	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0007B-A L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0007B-B S	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0007B-B L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0007B-C S	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0007B-C L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0007B-D S	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0007B-D L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
107																					
107A S	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
107A L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
107B S	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
107B L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
107C S	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
107C L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
107D S	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
107D L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

S= Solidified medium B= Broth medium

TABLE 9 continued.

YAKIMA

Sample #	IND	URE	GEL	ESC	CAT	CLU	MAN	LAC	SAC	MAL	SAL	XYL	ARA	GLY	CEL	MNE	MLZ	RAF	SOR	RHA	TRE
207 Yakima L	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
307																					
307A							No API strip run														
307B S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
307B L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
307C	No API strip run																				
407																					
407A S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
407A L	-	-	-	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
407B S	-	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+
407B L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
507																					
507A S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
507B (I) S	-	+	-	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
507B (II) S	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
507B L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
507A L	No API strip run																				
507AB L	No API strip run																				
537																					
537A S	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
537A L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
537B S	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
537B L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

S= Solidified medium   B= Broth medium

TABLE 9 continued.

YAKIMA

Sample #	IND	URE	GEL	ESC	CAT	GLU	MAN	LAC	SAC	MAL	SAL	XYL	ARA	GLY	CEL	MNE	MLZ	RAF	SOR	RHA	TRE
607A S	+	-	-	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+
607A L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
607B S	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
607B L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
607C				No API strip run																	
707																					
707A S	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
707A L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
707B S	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
707B L	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
807	No API strip run																				
907	No API strip run																				
1007																					
1007 S	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+
1007 L	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+
1007 (II) L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
1007A S	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1007A L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+
1007B S	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+
1007B L	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
1107	No API strip run																				
1207	No API strip run																				
1307																					
1307A	API strip	No results, all negative																			
1307B	No API strip run																				
1407	No API strip run																				

S= Solidified medium   B= Broth medium

our DATA FROM JUNE 1988 PRESENTED (FIGURE 7b)
Table 10--and a sequential graphic presentation of Log₁₀CFU versus depth is presented in Fig. 6. During the 3rd year of our study we plan to add each primary data set to this base and analyze for controlling/influential datums.

- We initiated microelectrode experiments of selected samples to see if microbial activity is detectable with time. This approach apparently will not work given the low numbers of bacteria present in the core subsamples.
- Presentations on this work:
  - (i) Poster presentation by E.B. Sullivan, T.T. Hendrix, K.-T. Chung, and S.E. Stevens, "Isolation and characterization of anaerobic microorganisms from the deep subsurface." Third Annual Graduate Research Forum, Memphis State University, March 26, 1991.
  - (ii) Poster presentation as above at the annual meeting of the Western Collegiate Division of the Tennessee Academy of Science, Rhodes College, April 13, 1991.
  - (iii) Abstract ^{POSTER PRESENTATION AT} submitted to the 1992 ASM General Meeting. Rothschild, M., S. E. Stevens, Jr., and K.-T. Chung. "Facultatively and obligatively anaerobic bacteria from the deep subsurface." Copy attached.

TABLE 10. YAKIMA

Sample #	Depth	BALKWILL Log ₁₀ CFU	FREDERICKS Log ₁₀ CFU	STEVENS Log ₁₀ CFU	GHIORSE Log ₁₀ CFU
0001a	0	6.6			
0001b	0	6.4			
?	0		8.2		
0007a	0			5.0	
0007b	0			4.4	
?	0				~7
137a	101.5				
106	103.7		0		
126	104				bd
126a					bd
101	104.2	-			
107	104.5			<0.5	
206	150.3		0		
240	154.1	NG			
207	154.3			<1.0	
201	154.7	NG			
226	155.3				1.2
301	173.9	NG			
306	174.2		0		
307	175.7			NG	
326	176.8				bd
401	205.3	NG			
406	205.4		0		
407	205.8/205.9			NG	
426	205.8/205.9				bd
431	206.1/207.5		0		
437	207.4	NG			
501	235.2	NG			
506	235.2/235.4		0		

TABLE 10 continued.

Sample #	Depth	BALKWILL Log ₁₀ CFU	FREDERICKS Log ₁₀ CFU	STEVENS Log ₁₀ CF	GHIORSE Log ₁₀ CFU
526	235.2/235.4				bd
507	235.4			NG	
531	240.0/240.7		?		
535	240.6	2.5			
537	240.9			1.3	
539	240.9				1.5
607*	292.6			>7.0	
606*	292.8		4.03		
626*	292.8				5.9
601*	292.9	>7.0			
726	294.5				3.1
706	294.8		1.82		
707	294.8			2.0	
701	294.9	1.9			
807a	315.7			1.2	
826a	315.7				1.3
806a	315.8		?		
801a	316	1.5			
801b	316.9	<0.5			
806b	317		?		
807b	317.1		<0.5		
826b	317.2				bd
901a	323.7/323.9	NG			
907a	323.8/324.9			NG	
906a	323.9/324.3		NG		
926	325.9				1.0
1007	327.5/327.7			NG	
1006a	327.7/328		NG		
1001	328.5	NG			

Sample #	Depth	BALKWILL Log ₁₀ CFU	FREDERICKS Log ₁₀ CFU	STEVENS Log ₁₀ CFU	GHIORSE Log ₁₀ CFU
1026	328.5				bd
1006b	328.6		[NG]		
1126	330				bd
1107	330.1			NG	
1426	332				6.5
1407	332.2/332.4			6.0	
1406	332.3/332.6		6.57		
1201	332.8	4.3			
1226	332.8/334.1				6.9
1401	333.1	1.4			
1206	333.8		3.09		
1207	333.4			5.6	
1326	338.2				4.0
1307	338.3			5.5	
1306	338.4		3.87		
1301	339.1	2.1			
SI-107	144.7			NG	
SI-101	145.4	NG			
SI-207	146.7/147.3			<0.5	
SI-201	147.4	NG			

FIG. 6.

Yakima - surface to 60 feet subsurface
Average CFU Values

Yakima - 61 to 120 foot subsurface Average CFU Values

Yakima - 121 to 180 feet subsurface Average CFU Value

Yakima - 181 to 240 feet subsurface Average CFU Values

Average CFU Value

Yakima - 241 to 300 feet subsurface
Average CFU VALUES

Yakima - 301 to 350 feet subsurface Average CFU Values

END

**DATE
FILMED
7/13/93**