IN SITU DETECTION OF MICROBIAL RESPIRATION IN SOILS AND SALT FLATS

INTERIM REPORT

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Department of Biological Sciences

University of Nevada, Las Vegas

Las Vegas, Nevada, 89109

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The report was written by the latter.

ABSTRACT

Increases in CO_2 partial pressures over a desert soil treated with casamino-acids glucose solution correlated with bacterial growth. Few or no increases in numbers of bacteria or CO_2 concentrations were noted in similar plots treated with water only or receiving no treatment. Growth in the soil appeared to be severely nutrient limited during the 10 day experiment.

Especially rapid growth took place between the third and fifth day, when temperatures ranged from 0° (night) to a maximum of 17.4° (day).

Under the conditions of the experiment, intermittent CO_2 assay was an insensitive indicator of growth, possibly because of restriction of gas escape by the desert pavement or solution, exchange, or precipitation of carbonate, but more likely because of inefficient sealing of hoods to and below the soil surface.

CO_2 assay was unable to detect microbial successions. The unpredictable course of these successions, plus unpredictable relative retentions mitigates against assay of organic gases as reliable in situ detection of microbial activity, except perhaps in very alkaline environments such as Owens Lake salts.

Introduction:

This report documents an in situ experiment on CO_2 production as the principle index of microbial metabolism in a creosote bush community soil, Clark County, Nevada, during January, 1973.

Site Location:

The experimental site was located on a relatively level, desert pavement covered "bench" in very rough lava boulder terrain north of the McCullough Range and was designated "Black Mountain #2". The exact location is shown in Figure 1.

Experimental:

Three hoods were placed as indicated in Figure 2, with Hood #1 on the left and #3 on the right.

The hoods were waste container lids drilled as shown in Figure 2 for addition of water or enrichment solutions, for obtaining 10 gram soil cores with 5/8" metal tubing, and for insertion of thermometers. The plastic was easily punctured by hypodermic needles for removal of gas samples by syringe.

The head space under each hood was approximately 8 liters. Each was "sealed" with soil as indicated in Figure 2. The soil under Hoods 2 and 3 were hydrated artificially with 4 liters distilled water and 4 liters of a solution containing 1.8% casamino acids and 1.8% glucose (CAG) respectively. Hood 1 was a control with no additions. The soil seals around the hoods seemed efficient retainers, although depth and lateral nutrient and water penetrance was not determined quantitatively.

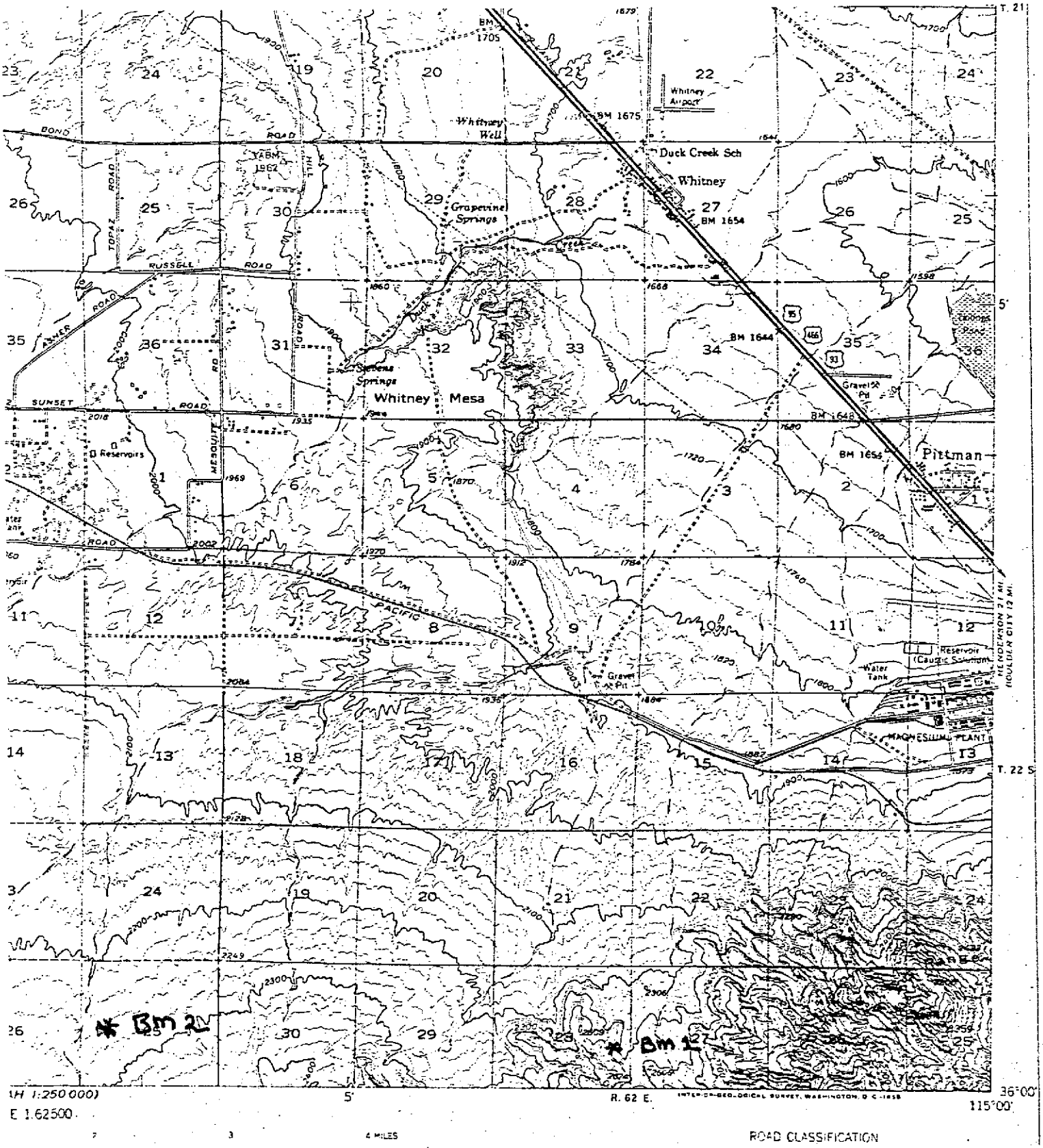

Figure 1: Location of Sites

Figure 2: Hoods in Place

The entire site was hydrated naturally by snow on 9 January and by rain on the 17th.

Microbiology:

Total bacteria were determined by surface spread plate counts. These counts were done only to show that increases in numbers had or had not occurred and that successions had taken place, and are not to be construed as a representation of all the microorganisms present.

Glass slides were driven into the soil before emplacement, removed carefully, and fixed and stained according to reference 1.

A chromosorb 101 column, 1/4" x 72", was used for CO_2 determinations.

Results:

The rate and extent of bacterial growth (Table 1) under Hood 3 (CAG treatment) seemed rather remarkable in view of temperatures prevailing (Tables 2 and 3) and the observation that no colonies appeared on plates incubated 10 days at 11° C.

The morning-afternoon temperature profile for 18 and 19 January (Table 3) implies that only brief periods above 11° pertained at the surface when rapid growth was taking place (12-15 January), and even less time at 15° where previously reported work indicated that most isolates from a similar site could grow. Unfortunately, a continuous record of temperature changes at and below the surface was not obtained.

Growth in the particular soil was apparently severely nutrient limited, although, admittedly, the normal nutrient flow through the system is unknown. Mere addition of water brought about a relatively minor increase in numbers of bacteria detected.

All but 1 of 63 isolates were capable of growth on prototrophic media, thus, a simpler defined enrichment solution than CAG might produce equivalent results.

Under the conditions of the experiment, headspace CO_2 did not accumulate in proportion to the count. Unless concentrated continuously to produce favorable alteration of the soil-headspace-outside air equilibrium and to avoid transients, CO_2 assay may be a rather insensitive indicator of metabolism. Obviously, interaction with the atmosphere outside the hood should be minimized in both cases.

On the 18th (Table 3), apparently anomalous transient decreases in CO_2 partial pressures occurred counter to the more general trend of increase with temperature. A 5 mph northwesterly wind in the morning and a 10 - 20 mph southwesterly wind starting just after 1200 may have been contributing factors.

The effect of the desert pavement on CO_2 release from the soil and on promotion of facultative growth was not investigated, nor were CO_2 - soil relations.

Sampling and qualitative testing for ammonia on 15 January revealed more in the outside air than the Hood 3, although the soil under Hood 3 was greatly enriched.

Enough of the data from work on identification of isolates has been presented in Table 4 to convey an idea of the impact of enrichment on the microbial climax population of 1/9 and the production of successions. (Hood 3). The normal population distribution seemed little disturbed by hydration (Hood 1 by natural and Hood 2 by natural and artificial means, Table 2). However, the population under Hood 3 went through two stages of massive succession during the experimental period.*

Comments such as those of Cameron (reference 2) on the stimulatory effect of water on soil microflora and Johnson's (reference 3) on the bacterial growth supporting potential of barren soils should perhaps be interpreted in terms of possible successsions, in which the "normal" flora is a terminal result.

Examination of buried slides revealed a more prolific and diverse flora under Hood 3 than under Hoods 1 and 2.

Conclusions and Recommendations:

Three general conclusions are readily apparent from the previous discussion.

First, factors influencing hood design and performance need to be evaluated. Among these are 1) an efficient seal, 2) the spatial fate of water and nutrients, and 3) means of altering gas-soil interactions in a favorable direction.

Second, variables such as temperature, soil, water, and gas production rates should be monitored continuously.

Third, upon enrichment, organic gas production will be a function of the organisms in successions and not of those

* Unless a morphological change of the dominant had occurred.

in a climax. Unless some way can be found to direct successions uniformly to a, for example, slightly soluble and negligibly absorbed gas, detection of organics appears to be a waste of time.

Literature cited:

1. Allen, O. N. "Experiments in Soil Bacteriology" Burgess Publishing Co., Minneapolis, 1949.

2. Cameron, R. E. "Soil Sampling Parameters for Extraterrestrial Life Detection". J. Arizona Acad. Sci. 4, 3-27, (1966)

3. Johnson, Roy M. "Growth of Indigenous Bacteria in Desert Soil". J. Arizona Acad. Sci. 5, 240-242, (1969)

Table 1. Carbon dioxide production and microbial growth in a creosote bush community soil, January, 1973.

Date	Hood 1 CO_2	Hood 1 Count x 10^6 $\bar{X}$	Hood 1 Count x 10^6 σ	Hood 2 CO_2	Hood 2 Count x 10^6 $\bar{X}$	Hood 2 Count x 10^6 σ	Hood 3 CO_2	Hood 3 Count x 10^6 $\bar{X}$	Hood 3 Count x 10^6 σ
9 Jan.	3.5	.65	.07	3.5	.67	.04	4.7	.60	.03
11	3			3.9			4.8		
12	2.8	.57	.07	3.1	1.30	.05	6.1	2.70	.09
15	3	.52	.03	4.3	1.85	.05	15.5	1200	100
16	2.4			3.3			23		
17	2.6			2.6			22		
18	2.8	.66	.09	2.8	2.10	.38	24	1100	70
19	3.0			2.5			14		

Hood 1: No treatment

Hood 2: 4 liters distilled water

Hood 3: 4 liters 1.8% casamino acids and 1.8% glucose

CO_2: Ratio of height of CO_2 peak, attenuation 16, to height of air peak, attenuation 1024, in percent. Two milliliter injections.

n-1 = 4

Incubation Temperature: 28° C

Table 2. Temperature and soil water data, January, 1973.

Date	Temperature, °C High*	Low*	Collection	Percent Soil Water Hood 1	Hood 2	Hood 3
8 January Snow	6.1	5				
9	3.3	0	5	4.4 **	3.6 **	8.8 **
10	10.5	.5	12			
11	11.1	0	13			
12	11.7	0	14	12	13	10
13	14.4	1.1				
14	17.2	1.7				
15	15	2.8	14	7	8	8
16	13.3	2.1	16			
17 Rain	14.4	4.4				
18	15.6	3.3	20	7	7	7
19	11.1	4.4				

* Weather Bureau

** Before Hydration

Table 3. Variations in headspace CO_2, January 18 and 19, 1973.

Time	Headspace Temperature, °C Hood 1	Hood 2	Hood 3	Headspace CO_2 Hood 1	Hood 2	Hood 3
18 Jan.						
0700	4	3	4	1.8	1.8	17.2
0730	5	6	5	1.8	2.1	18.2
0800	6	7	6	1.8	1.7	16.5
0830	7	8	7	2.5	2.2	14.7
0900	9	9	9	2.1	2.8	16.4
0930	12	12	13	2.5	4.7	16.1
1000	14	14	16	1.7	2.4	20.3
1040	16	15	18	2.8	3.6	23.1
1100	17	17	19	2.5	2.9	24.3
1130	19	19	20	2.5	2.7	24.2
1200	19	19	20	2.3	2.8	21.3
1300	19	19	19	---	2.8	21.6
1400	16	16	16	1.7	2.8	16.5
1500	15	15	15	1.8	2.6	16.7
1545	--	--	14	---	---	16.7
19 Jan.						
1130	22	22	22	4.7	2.5	12.8
1200	22.2	22.2	22.2	2.2	2.5	14.0
1230	22.2	22.2	22.2	2.0	2.5	14.3

Table 4. Percent Streptomyces, bacilli, and cocci in soil samples, January, 1973.

	Hood 1			Hood 2			Hood 3		
	1/9	1/15	1/18	1/9	1/15	1/18	1/9	1/15	1/18
Streptomyces	7	12*	6	32	16	14	18	--	--
bacilli	81	80	77	14	65	64	57	95	--
cocci	12	8	15	23	19	12	25	--	100
Accounted For	100	100	98	69	100	90	100	95	100

* 6% probably Nocardia.

Table ____. Carbon Dioxide Production and Microbial Growth in a Creosote Bush Community Soil.

Date	Hood 1 CO_2*	Hood 1 Count** $\bar{X}$	Hood 1 Count** σ	Hood 2 CO_2	Hood 2 Count $\bar{X}$	Hood 2 Count σ	Hood 3 CO_2	Hood 3 Count $\bar{X}$	Hood 3 Count σ
9 Jan. 73	3.5	655	70	3.5	665	40	4.7	600	30
11 Jan. 73	3			3.9			4.8		
12 Jan. 73	2.8	565	65	3.1	1,300	50	6.6	2,700	85
15 Jan. 73	3	520	25	4.3	1,850	45	15.5	1,200,000	100,000
16 Jan. 73	2.4			3.3			23		
17 Jan. 73	2.6			2.6			22		
18 Jan. 73	2.8	660	85	2.8	2,100	380	24	1,100,000	70,000
19 Jan. 73	3			2.5			14		

Hood 1: No treatment.

Hood 2: 4 liters distilled water

Hood 3: 4 liters 1.8% casamino acids and 1.8% glucose

* Ratio of height of CO_2 peak, attenuation 16, to height of air peak, attenuation 1024, in percent. Two milliliter injections.

** Thousands, surface spread plates.