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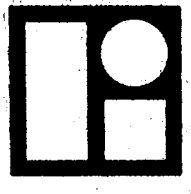

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MARS BIOLOGICAL SAMPLE COLLECTION AND PROCESSING STUDY PROGRAM

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Prepared by:

D. A. Lundgren
V. W. Greene
M. J. Grundtner

Submitted by: L. W. Rees

L. W. Rees
Manager, Aerosol Physics

Approved by: for

A. A. Anderson
Director, Applied Research

APPLIED SCIENCE DIVISION
Litton Systems, Inc.
2295 Walnut Street
St. Paul, Minnesota 55113

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MARS BIOLOGICAL SAMPLE COLLECTION AND PROCESSING STUDY

I. INTRODUCTION

This report presents the results of the thirteen-month study, Mars Biological Sample Collection and Processing Study Program, which has been carried out by the Applied Science Division of Litton Systems, Inc. under Contract No. 950771 with Jet Propulsion Laboratory. The period of performance extended from November 15, 1963 to December 31, 1964 with one additional month for the final report preparation.

The intent of this project has been 1) to provide information that will lead to an understanding of the problems, and possible solution thereto, of obtaining a sample of material from the surface of Mars for biological detection experiments. 2) Develop, improve and evaluate an aerosol method of collection, transport and recovery of a sample of material from the Mars surface, 3) Study and develop methods of separating and processing Mars surface samples for the biological fraction.

At the initiation of this program the work was divided into two basic areas: 1) Sample Acquisition Studies and 2) Biological Support Studies. For clarity this Report is divided into these two sections with specific aims and results reported therein.

II. SAMPLE ACQUISITION STUDIES

A. Introduction

In the sample acquisition studies the aims were:

1) To investigate the mechanics of aerodynamically and mechanically dislodging particles from a surface with emphasis on the processes applicable to biological collection.

2) To study the effects of environmental conditions upon the dislodging and collection of sample material, particularly with reference to the ambient air pressure, temperature and humidity.

3) To investigate the mechanics of pneumatically transporting particulate material.

4) To determine or establish the design parameter limits of a pneumatic particulate recovery process as affected by environmental conditions.

5) To assess an air ejector pump at Martian atmospheric pressures and temperatures with emphasis upon collection and power requirements.

6) To construct and test elementary devices representing the most promising techniques developed from these studies.

During the first two months of the project, preparations were made for an adequate investigation of the problems. Undisturbed soil samples were obtained and an environmental test chamber was designed and built. A program was devised to provide selected soil samples for biological assay. Testing of the Sample Collection Unit built on Contract No. JPL 950123 was also started during this time. A major portion of the contract was then devoted to pneumatic transport and collection studies. An investigation of an air ejector pump suitable for use in a Martian atmosphere was conducted in the latter phase of the project.

B. Laboratory Soil Samples

To conduct a realistic testing program undisturbed soil samples were required for use in the laboratory. These samples were obtained by sawing the frozen native soil of Minnesota into blocks to fit large rectangular pans. The pans of soil were then placed in a cabinet in the laboratory to thaw, and dry air was continuously circulated in the cabinet to simulate the dry conditions of Mars. Other types of bulk soil samples were also obtained from various parts of the world, but they were not obtained in an undisturbed condition. Figure 2-1 shows the representative soil samples. A complete list and description of the soils is contained in the Biological Support Studies, Section III-B.

C. Environmental Test Chamber

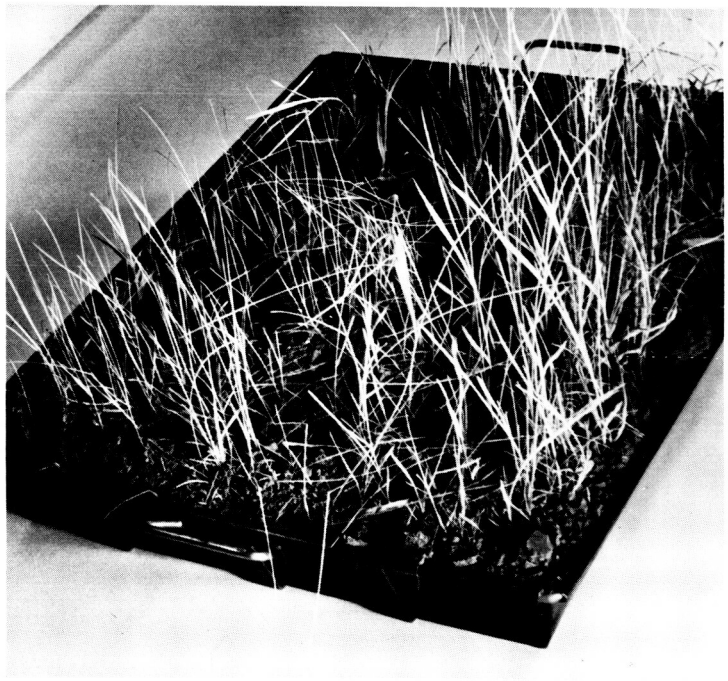
To evaluate sampling concepts at Martian atmospheric conditions, a suitable environmental chamber was designed and built. The aluminum chamber is 5 feet long and 2 feet in diameter with 8 plexiglass viewing ports. Suitable electrical feed-throughs were installed to provide control from outside the chamber for the sampler, lights, etc. Atmospheric pressures in the range of 5 mb to 100 mb can be obtained rapidly and maintained easily. The chamber is designed so that the various pans of bulk soil (Figure 2-1) can be placed on the side rails and held at a convenient view height. Figure 2-2 shows the exterior and interior views of the chamber.

The chamber was fitted with two simple manipulators to permit objects to be moved manually from the outside of the chamber. Each manipulator consisted of a 1/4 inch diameter rod, which passed through a vacuum seal into the chamber, with a 2 inch diameter spool on the end of the rod that is inside the chamber. A cord was stretched between the two spools and wound around them. The manipulation was accomplished by attaching objects, such as the aerosolizer, to the cord

Gravel

Sandy Soil

Rocks



Rich Garden Soil

Figure 2-1. Bulk Samples of Four Soil Types

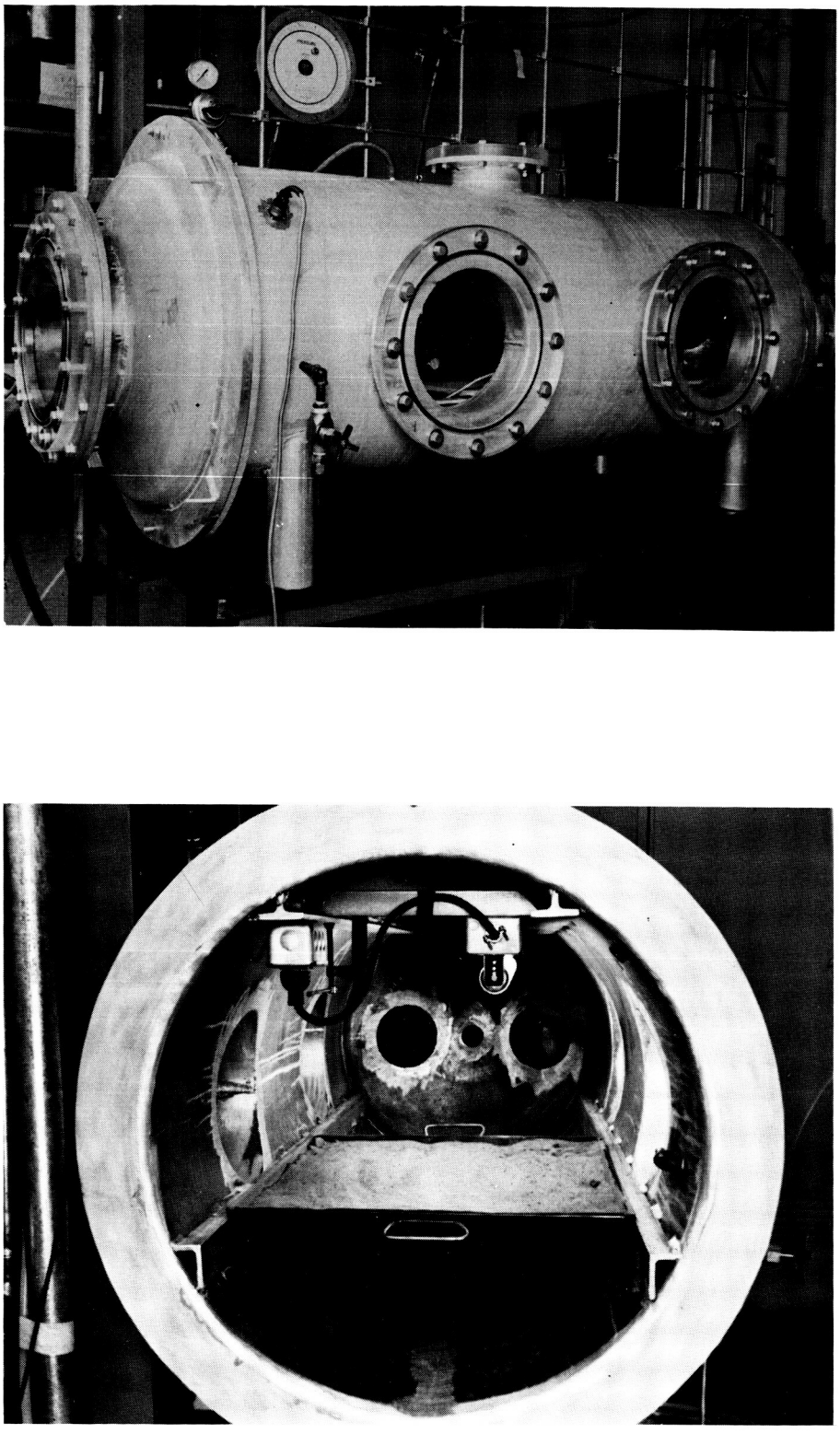


Figure 2-2. Exterior and Interior Views of Environmental Test Chamber

and winding the cord alternately on each of the spools with knobs on the outside ends of the rods. See Figure 2-3.

To evaluate the effects of temperature on the pneumatic transport and collection of particulate matter the environmental test chamber was modified for cooling. This was done by wrapping 200 ft of 1/2-inch diameter aluminum tubing around the outside of the chamber. The tubing was bonded to the walls of the chamber with a mixture of 80 percent aluminum powder and 20 percent epoxy resin. A plywood box was built around the chamber to contain vermiculite insulation to a minimum thickness of 4 inches. The viewing ports were fitted with additional plexiglass windows to provide an insulating air space; the system is shown schematically in Figure 2-4.

To cool the chamber, it is evacuated and alcohol cooled with solid CO_2 to a temperature of approximately -100°F is then pumped through the cooling coils on the outside of the chamber. By regulating the flow of alcohol, the chamber can be maintained at the proper temperature. The chamber has been tested at various low pressures and at temperatures below -75°F.

D. Evaluating Previously Designed Sampler

To better understand and appreciate problems of soil sampling at very low pressures (5 to 35 mb) tests were run at atmospheric pressure and then at reduced pressures using the sampler designed and built on Contract No. JPL 950123. The complete unit is shown in Figure 2-5.

The sample collection unit was tested over a sandy soil, with the sampler inlet held at various heights above the soil surface to determine the effect of height on sample collection rate. These data were needed for a comparison with performance data to be obtained later at low pressures. The sampler cyclone section was maintained at 12 inches above the soil surface. In normal operation the wheels held

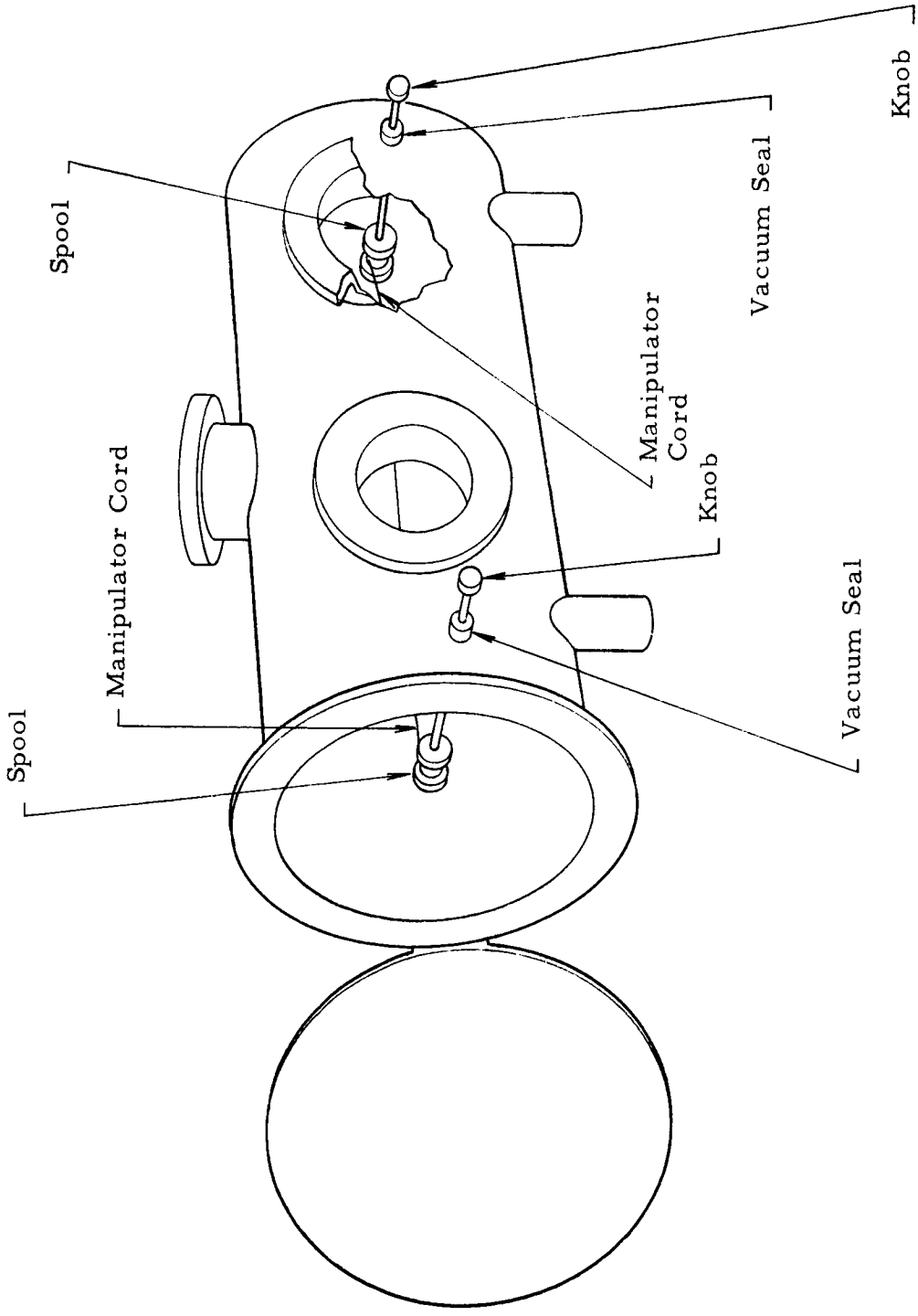


Figure 2-3. Schematic Showing Manual Manipulators in Environmental Test Chamber

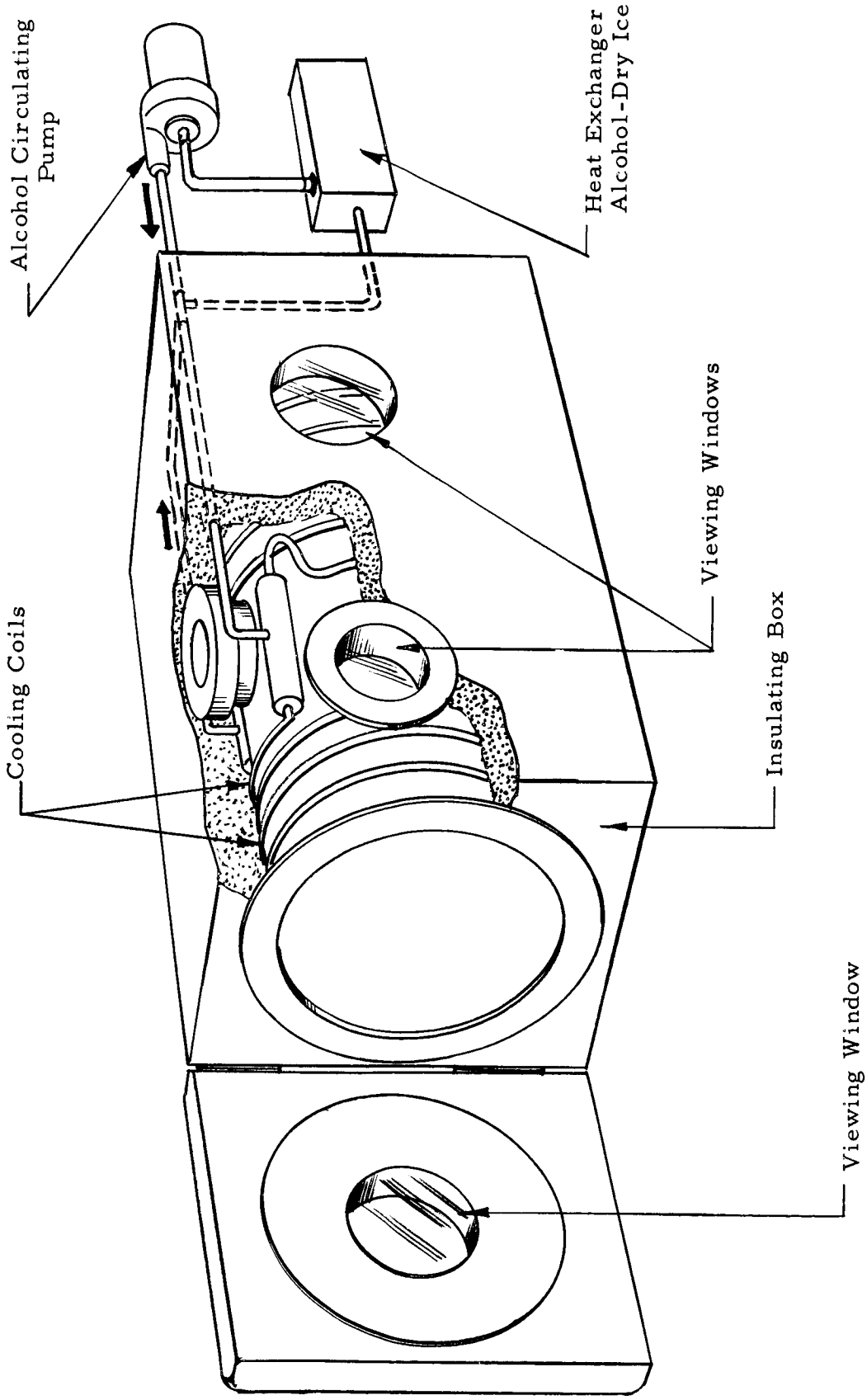


Figure 2-4. Schematic Showing Cooling System for the Environmental Test Chamber

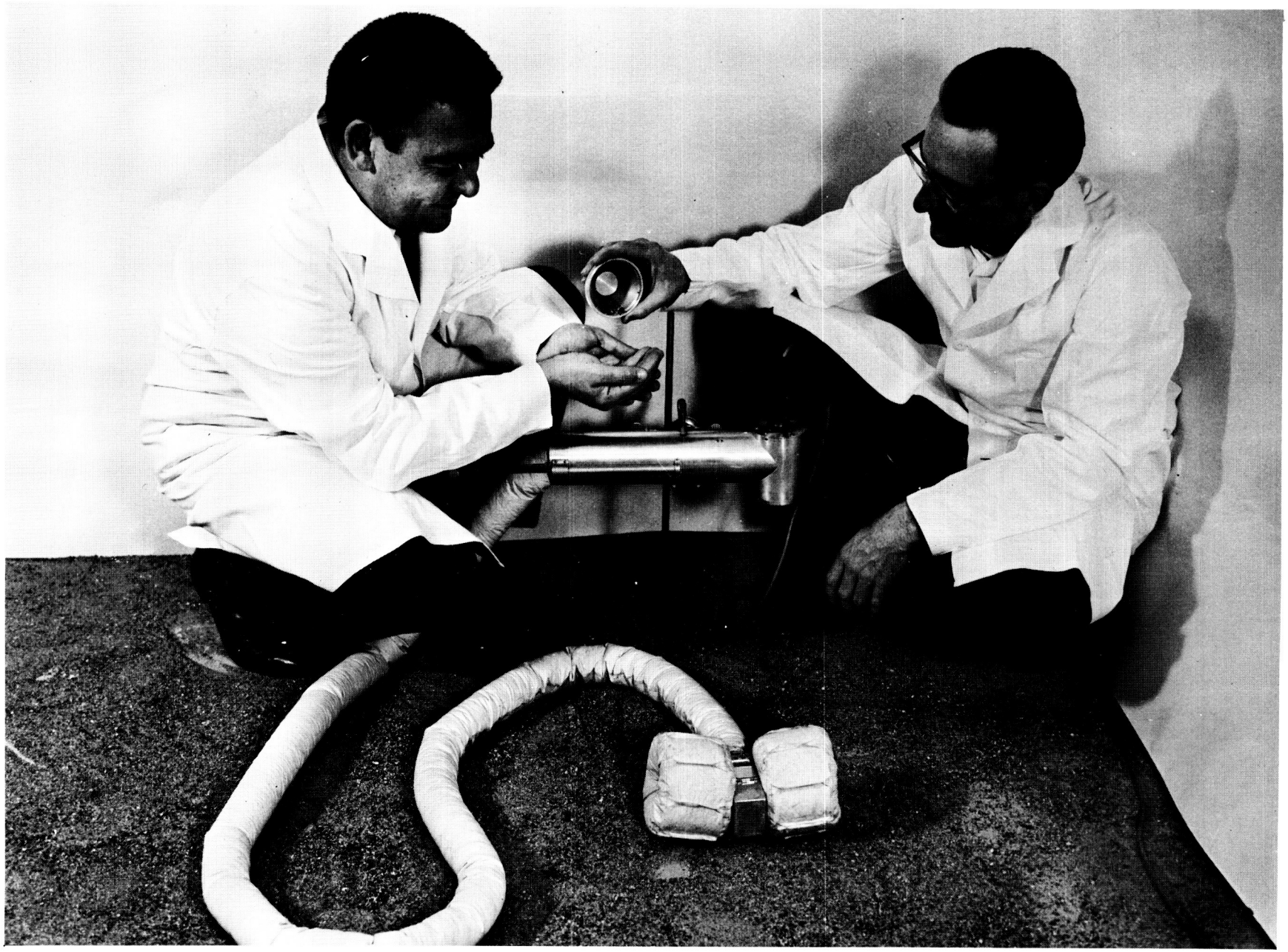


Figure 2-5. Sample Collection Unit

the sampler approximately 1 inch above the soil surface; therefore, to operate at different heights the sampler had to be clamped to a ring-stand and moved manually over the soil surface at approximately 3 ft/min. These data are presented in Figures 2-6 and 2-7.

It was visually apparent that with increasing sampler height the particles collected were of decreasing size. A sieve analysis of the samples obtained with the sampler inlet 1 inch above the surface and 1-1/4 inches above the surface is presented in Table 2-1. It is important that the sampler be maintained at a given height during operation if a valid comparison is to be made of sampler performance at reduced pressures.

Table 2-1. Sieve Analysis of Two Sand Samples

Sampler Inlet at 1"		Sampler Inlet at 1-1/4"	
Particle Dia. microns	Fraction Contained % by weight	Particle Dia. microns	Fraction Contained % by weight
$210 < D_p$	0	$210 < D_p$	0
$88 < D_p < 210$	70.5	$88 < D_p < 210$	41.0
$62 < D_p < 88$	15.0	$62 < D_p < 88$	25.0
$D_p < 62$	14.2	$D_p < 62$	33.9

The sampler had previously been tested and found to work at the then-estimated Martian atmospheric pressure of about 100 mb. However, the new estimated pressure range of about 5 to 35 mb required additional testing of the pneumatic transport principle, as well as the actual sampler.

Therefore, the complete sampler was tested at the new estimated Martian pressure. It was apparent, as the pressure was reduced below 80 mb, that sufficient air pressure could not be attained with the existing blower to inflate the tires for operation. Also the air bleed aerosolizing

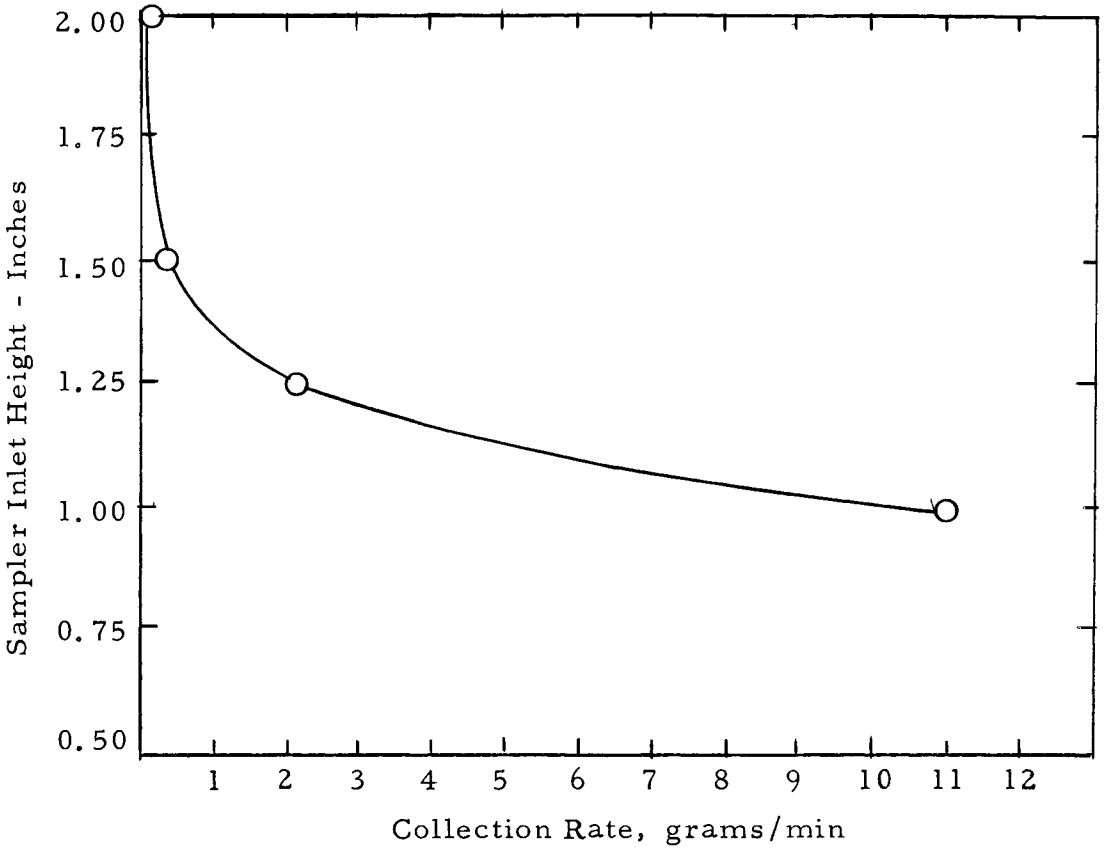


Figure 2-6. Collection Rate vs Sampler Inlet Height

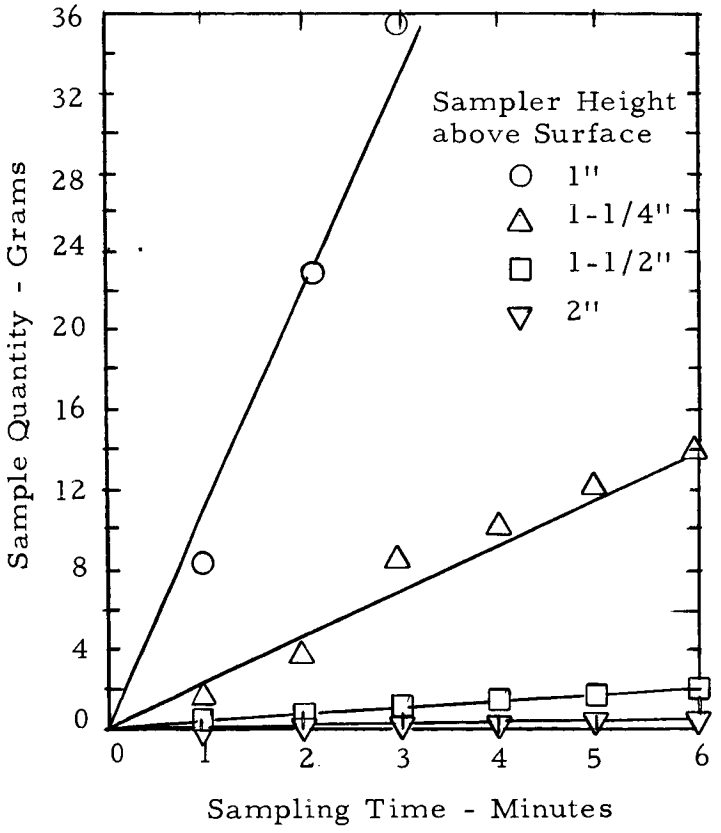


Figure 2-7. Sample Quantity vs Sampling Time

jet from the blower was not satisfactory for aerosolization of the sand particles. The tire problem was corrected by filling the tires with polyurethane foam to keep them expanded. An aerosolizer, utilizing a high velocity jet of air, was attached to the sampler unit and used to aerosolize the sand. The single jet, however, had a tendency to disperse the soil rather than just aerosolize it. A twin jet aerosolizer, which had a jet emanating from each end of the sampler and converging directly under the transport tube inlet, was then built and tried out. This device imparted an upward velocity to much of the sand and thereby directed aerosol particles into the sampler inlet.

With the above modifications, the sampler was again tested at reduced pressures. The unit performed very inefficiently at low pressure even though the aerosolizer worked well and raised a very adequate cloud of dust. Tests showed that most sand particles settled out in the horizontal sections of tubing. Sampler testing was then temporarily discontinued and a particle transport study begun.

E. Horizontal Pneumatic Transport of Particulates

1. Particle Transport at Reduced Pressure

A simple test set-up was devised to give a qualitative analysis of pneumatic transport of particles in the pressure range of 5 mb to 80 mb. To the inlet of a vane-axial blower was attached a 1-inch diameter clear Mylar tube supported by a coil spring held inside for its entire length. The tube was directed vertically upward from the inlet for approximately 6 inches, directed horizontally for an additional 12 inches and then turned into the blower. With this configuration, it was possible visually to evaluate the horizontal and vertical transport of particles in the stated pressure range. No attempt was made to collect the transported particles. Soil particles were aerosolized with a high velocity jet of air from a small nozzle located near the transport tube inlet and directed at a tray of soil.

This device was placed in the environmental chamber, and the chamber evacuated until desired test pressure was obtained. With the aerosolizer and blower operating, sand particles could be seen moving up the vertical section of the tube. As the direction of flow changed from vertical to horizontal, the larger particles began to precipitate from the air stream and slide down the wall of the vertical tube. Additional settling occurred in the horizontal section. Nonetheless, a considerable amount of smaller particulates were successfully transported completely through the blower and ejected out the end. It was concluded that this method of particle transport at Martian atmospheric pressures as low as 5 mb is entirely possible even though some of the aerosolized dust may settle out in the horizontal section of the transport tube. Studies were then undertaken to determine the effects of changes in various parameters on pneumatic transport efficiency.

2. Experimental Equipment

Investigation of the penumatic transport efficiency required a system whereby the quantities of particulate matter transported could be reasonably accurately determined. Therefore, an aerosolizing-transport-collection apparatus was constructed to evaluate the particulate transport at low pressures.

The aerosolizer section consisted of a Syntrol vibratory feeder for feeding particles directly into the transport tube inlet at a desired rate. At the feeder discharge end, a high-velocity jet from a small nozzle aerosolized the particles. The combination of the air jet and feeder vibration produced excellent aerosolization (essentially complete breakup of the feed particulate material). This apparatus was mounted in a clear plastic box, which was then sealed and positioned inside the environmental test chamber as shown in Figure 2-8. Air could then be fed into the box and controlled from outside the chamber at a measured flow rate, or by opening a port, the blower on the cyclone could draw the air through the box.

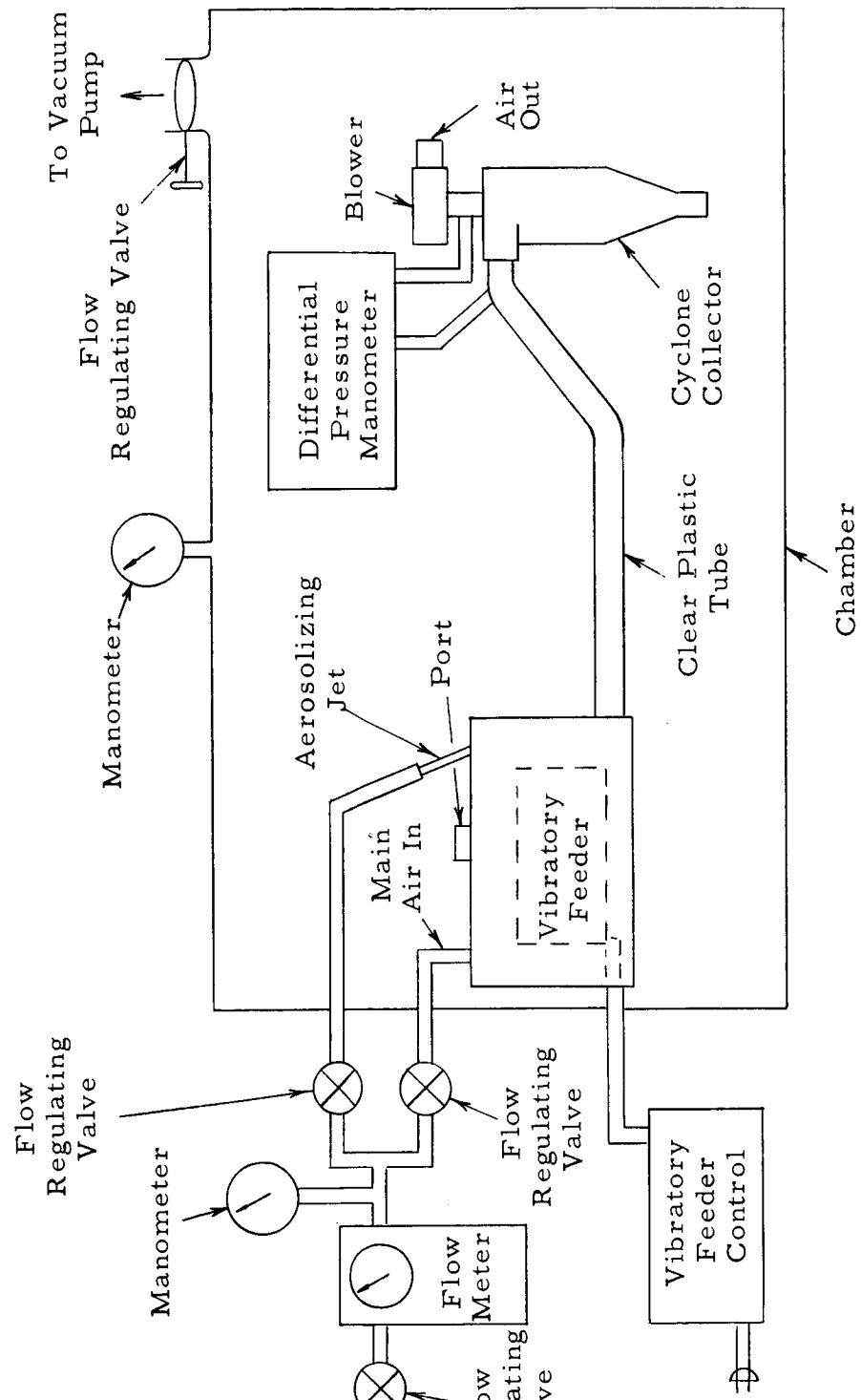


Figure 2-8. Schematic Representation of Aerosol Transport Apparatus

A flexible, clear plastic tube connected the aerosolizer section and the cyclone section and served as the particle transport path (see Figure 2-8). This smooth-walled tube was 5 ft long and 3/4-inch inside diameter.

The collector section utilized a very efficient and convenient cyclone separator to collect out the transported sample. The cyclone was designed with a removable sample cup at the bottom, and a mounting flange at the top for the attachment of various blowers (see Figure 2-9).

To measure and control flow rate through the experimental system, the cyclone separator was fitted with pressure taps at its inlet and outlet. A flow calibration was then obtained by measuring the pressure drop across the cyclone at various flow rates for various atmospheric pressures. At very low pressures and low flow rates the calibration was not sufficiently accurate; during these tests, therefore, the flow was measured with a gas meter.

To evaluate fully the performance of air movers at reduced pressures, we must know the actual flow rates obtained. The instrument that was used is an iron-case American gas meter, designed to be used at atmospheric pressure or above. To determine its accuracy at reduced pressure, this meter was placed in the environmental chamber and operated at 5 mb pressure. Air at 970 mb was metered through a Brooks Rotometer into the American gas meter located in the chamber. The flow rate, measured by the Brooks Rotometer at 970 mb pressure, was converted to the actual flow rate at 5 mb pressure and compared with the American gas meter reading. The flow rate indications of the American meter were less than 10 percent lower than the Brooks Rotometer, which is within the accuracy of the latter. (See Figure 2-10).

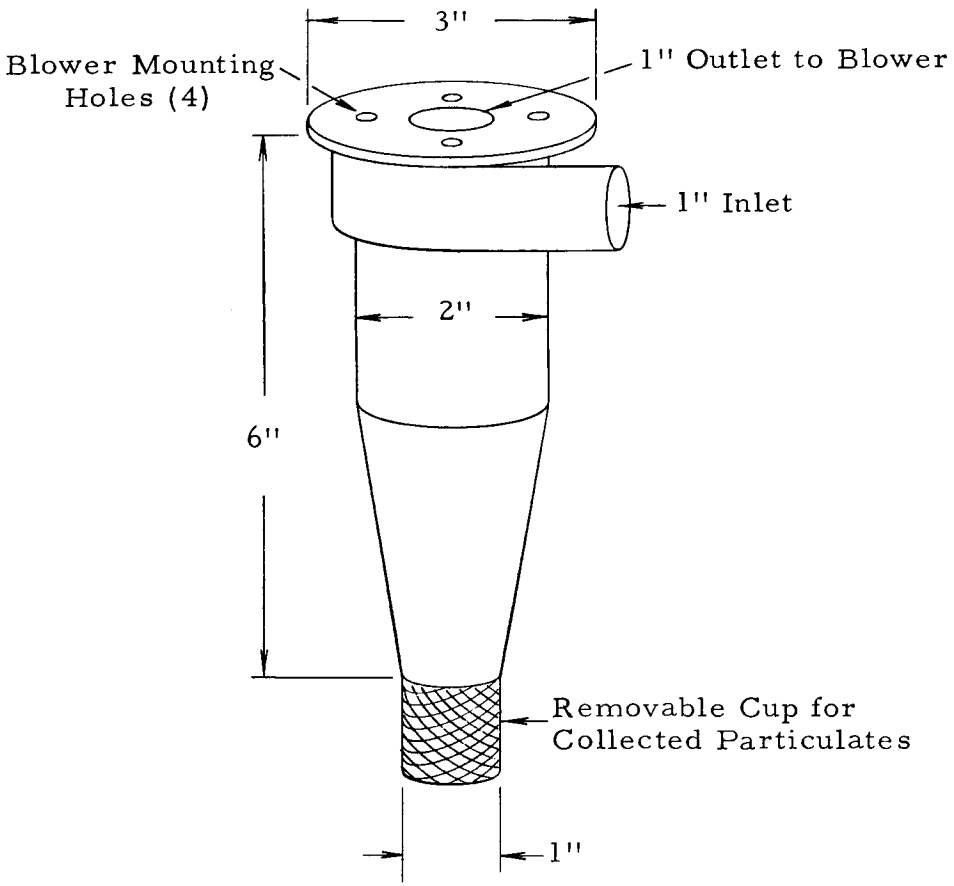


Figure 2-9. Cyclone Separator

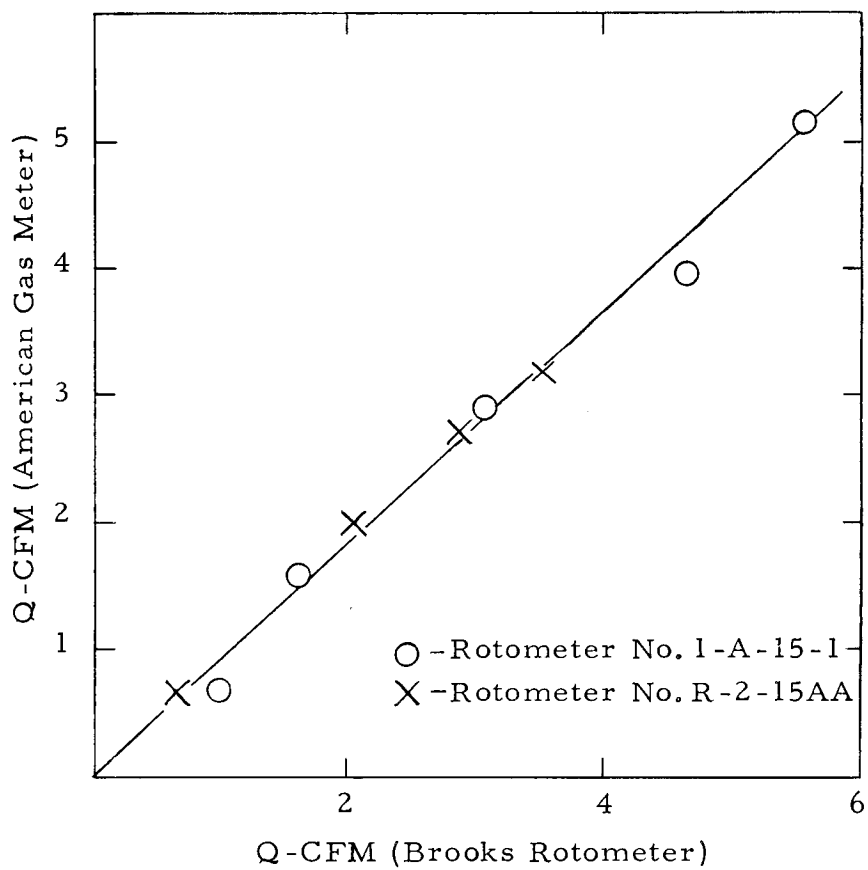


Figure 2-10. Brooks Flow Rate vs American Gas Meter Flow Rate at 5 mb Pressure

3. Transport of Glass Beads

Size-classified glass beads were used in the initial transport studies; they were available in the desired graded sizes and they deagglomerate readily to effectively produce homogeneous, spherical, monodispersed particles. (These beads were purchased from Minnesota Mining and Manufacturing Company in ten sizes of the Class B Narrow Size Distribution). The median bead diameter of the ten sizes used ranged from 29 microns to 390 microns. The glass bead density is 2.5g/cc - approximating that of sand.

Theoretically, pneumatic particle transport should become increasingly difficult with decreasing atmospheric pressure. Studies were directed, therefore, toward determining empirically the minimum pressure requirements that must be met under optimal transport conditions. With these data, a sampler can be evaluated to determine how much its performance could be improved.

All data reported in this section were obtained under the following conditions:

Constants:

1. Particulate feed rate (vibratory feeder setting)
2. Aerosolizer operation (position and air flow)
3. Transport tube (3/4-inch ID Tygon Tube 5 ft long)
4. Inlet and outlet of transport tube (cyclone inlet positioned 4 inches above the feeder outlet)
5. Aerosolized particulates (glass beads of density 2.5 g/cc)

Variables:

1. Transport tube air velocity (from about 300 to 5000 fpm)
2. Air flow rate (from 1 to 15 cfm)
3. Glass bead diameter (from 29 to 290 microns)
4. Test chamber pressure (from 5 to 973 millibar)

Initial testing was done with particles having an average diameter of 100 microns. At normal atmospheric pressure of 973 mb, virtually all particles fed into the transport tube were collected in the cyclone when the tube air velocity was above 800 ft/min. The collection dropped near zero at 300 ft/min. At the velocities between 300-800 ft/min some particles settled out of the air stream and remained on the bottom of the tube. Thus, the transition range for particle transport through a smooth-walled tube is between 300 and 800 fpm for spherical particles of 100 micron diameter, 2.5 g/cc density, at an atmospheric pressure of 973 mb.

Similar tests were conducted by varying only the atmospheric pressure. These results are shown in Figure 2-11. It can be seen that pneumatic transport of 100-micron particles is possible at any estimated Martian atmospheric pressure above 5-mb without resorting to excessive air velocities. With a 3/4-inch diameter transport tube, this required a 5-cfm flow rate which is not difficult to obtain. A smaller diameter tube would, of course, require even a lower volumetric flow rate.

Tests were next conducted under the above test conditions using the aerosols of different median particle diameter. Figure 2-12 shows how the air velocity, required to transport a given particle size, increases as the pressure decreases. It can be seen that particles much larger than 300-microns in diameter would become somewhat difficult to transport pneumatically at atmospheric pressures of less than 20-mb. Figure 2-13 presents these data plotted differently; it is seen that as particle size increases, the velocity required for transport increases much more rapidly at the lower air density.

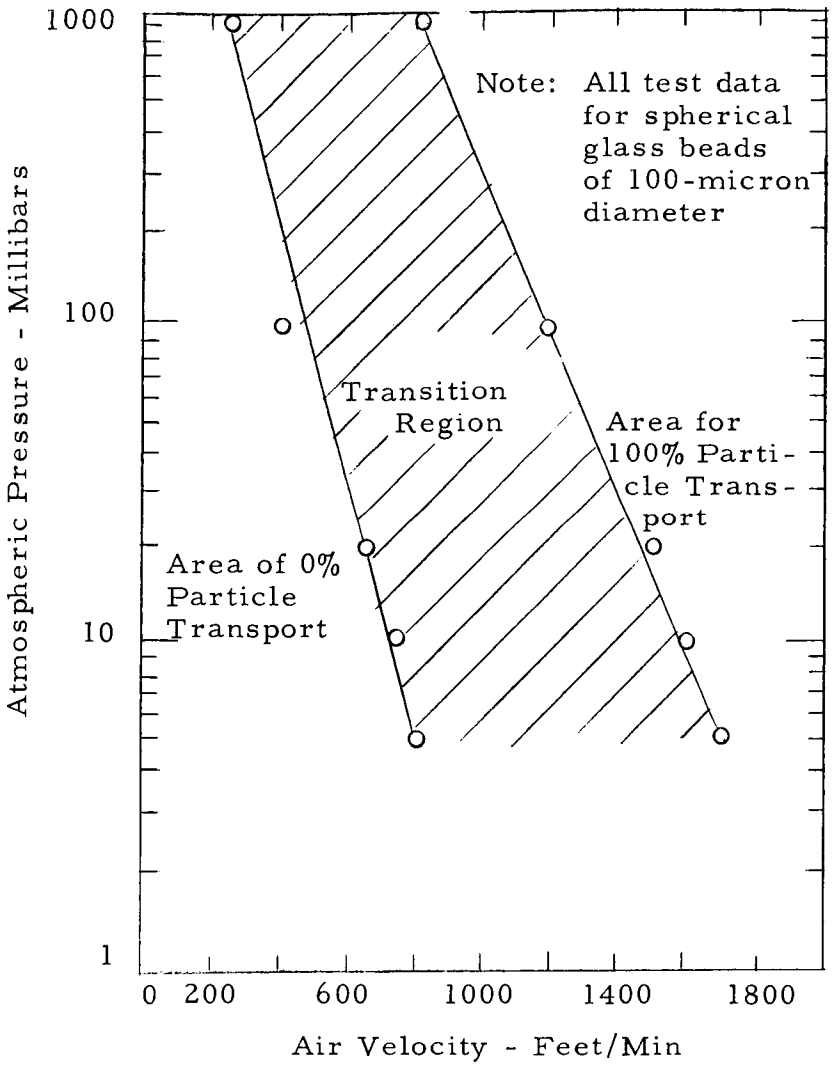


Figure 2-11. Air Velocity - Atmospheric Pressure Relationship for Transport of 100-Micron Particles

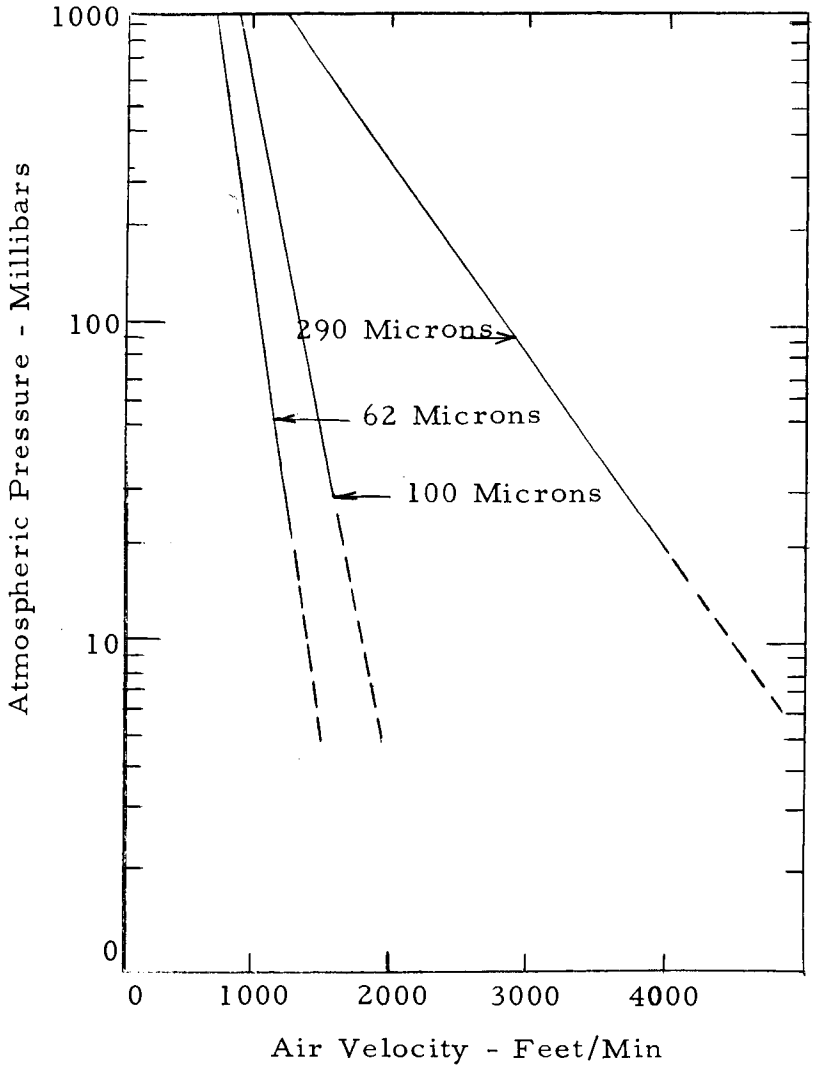


Figure 2-12. Air Velocity - Atmospheric Pressure Relationship for Several Size Particles

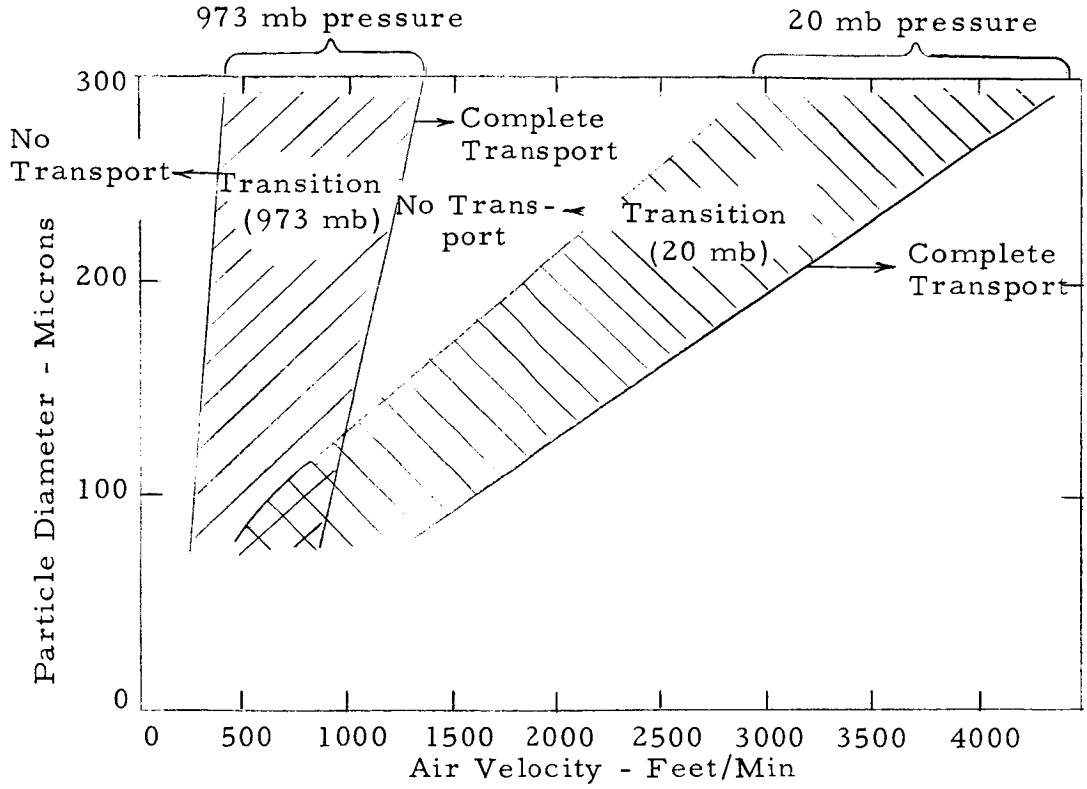


Figure 2-13. Air Velocity - Particle Diameter Relationship for Particle Transport at 2 Pressures

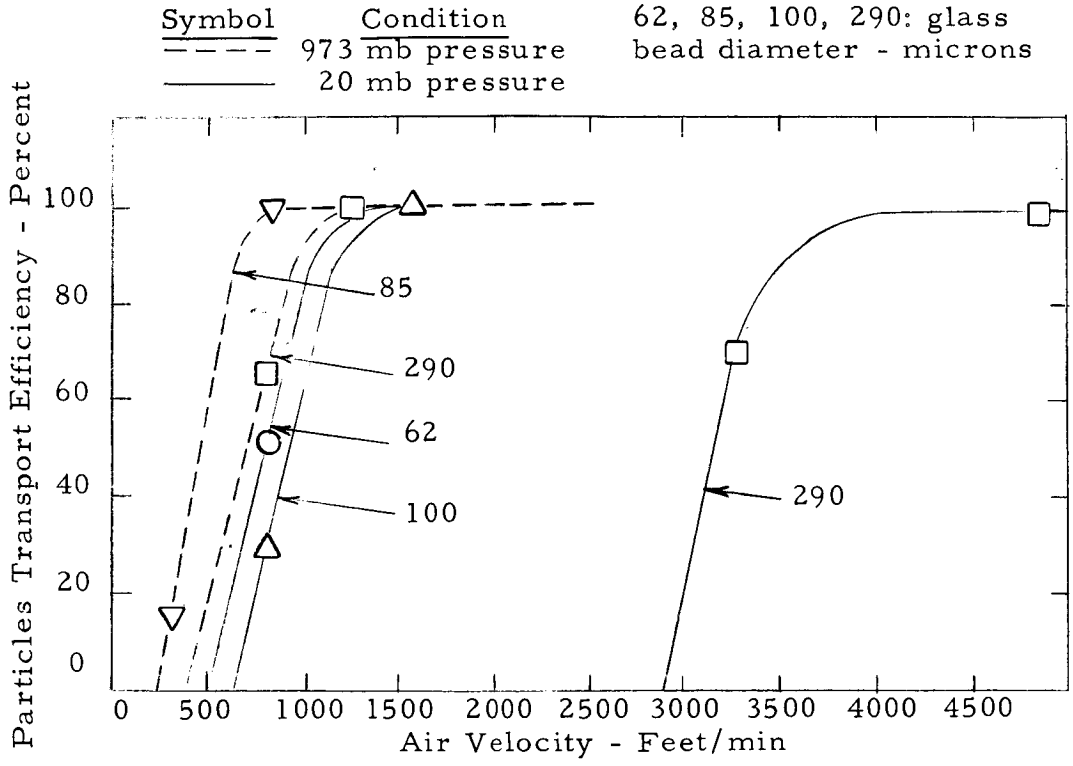


Figure 2-14. Air Velocity - Percent Transported Relationship for Various Particle Sizes and Pressures

Figure 2-14 is a plot of data in the transition range of transport at two different pressures and several particle diameters. At a given pressure and transport air velocity, the approximate efficiency of transport can be determined for a given particle diameter.

These data were obtained under optimal conditions (use of spherical beads and smooth-walled transport tube) to show what can be expected under the best conditions. Further studies were conducted with various soils and rough-walled tubing to determine the effects of each of these variables upon the efficiency of particle transport.

4. Horizontal Transport of Various Soils

Glass beads are somewhat easier to pneumatically transport because of their spherical shape. Tests were, therefore, conducted under the same conditions using various soils. The test apparatus was the same as reported above, i.e., an enclosed feeder-aerosolizer section, 3/4-inch diameter, clear plastic transport tube, and a cyclone collector. The soil samples were fractionated by sieving; a fraction size comparable to the glass bead size of 100 microns was used. Test data were obtained under the following conditions:

Constants:

1. Particulate feed rate (vibratory feeder setting)
2. Aerosolizer operation (position and air flow)
3. Transport tube (3/4-inch ID Tygon Tube 5 ft long)
4. Inlet and outlet of transport tube (cyclone inlet positioned 4 inches above the feeder outlet).
5. Particle diameter (average diameter of 100 microns).

Variables:

1. Particle diameter (average diameter of 100 microns)
2. Air flow rate (from 1 to 15 cfm)
3. Aerosolized particulates (various soils of density 2.2 gm/cc to 2.6 gm/cc).
4. Test chamber pressure (from 5 to 970 millibars).

Fine sand (soil #1), which approximates the glass beads' density of 2.5gm/cc,was studied first. Pneumatic transport efficiency data for the sand particles were similar but of lower efficiency to those obtained using particulates of glass beads. Figure 2-15 shows the characteristic decrease in transport efficiency with a decrease in atmospheric pressure over the air velocity range from about 500 ft/min to 3500 ft/min.

Similar tests were conducted using soils 5 and 6. Soil 5 is brown clay loam with a density of 2.5 gm/cc, and soil 6 is silty clay loam with a density of 2.2 gm/cc. The results of these tests are shown in Figure 2-16 and 2-17 for soils 5 and 6 respectively.

Soil transport data obtained at 20 mb pressure were plotted on a graph with the data for glass beads of similar size. Figure 2-17 shows that the transport efficiency is a function of particle shape, particle density, etc., for the various soils tested. Spherical glass beads were transported with somewhat greater ease over this velocity range than were any of the soils tested. Figure 2-19 gives a comaprison of the transition ranges for horizontal transport of glass beads and soil 5, both with a density of 2.5 gm/cc. Soil 5 required somewhat higher air velocity for equal transport efficiency; however, the transport was still accomplished very effectively at reduced atmospheric pressures with reasonable air velocities.

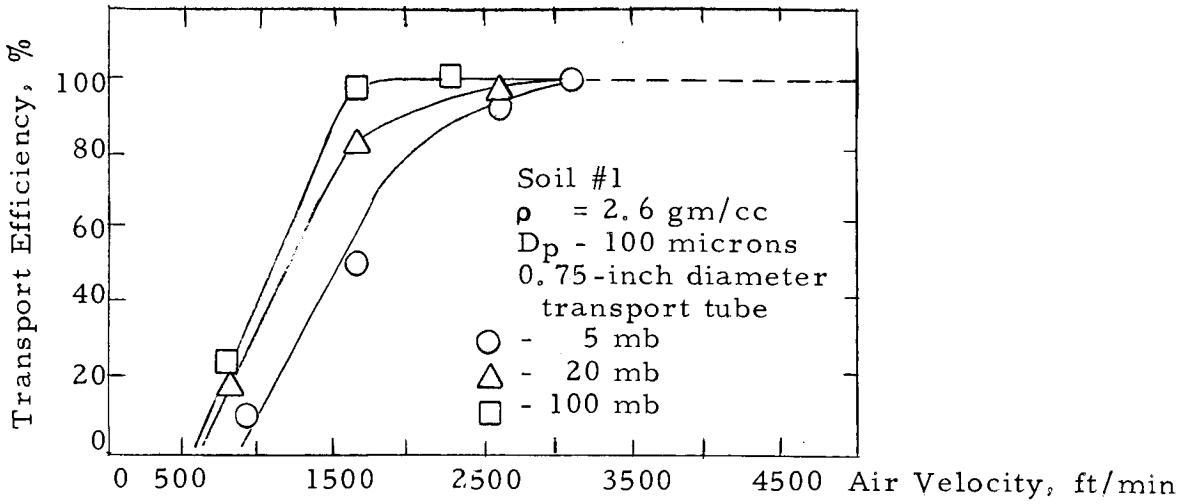


Figure 2-15. Air Velocity-Horizontal Transport Efficiency Relationship for Fine Sand

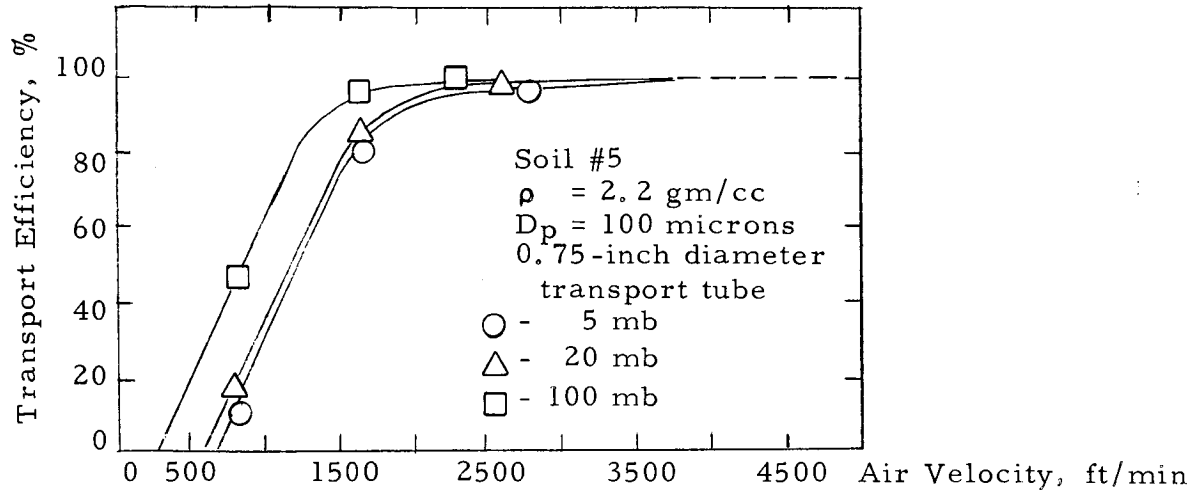


Figure 2-16. Air Velocity-Horizontal Transport Efficiency Relationship for Brown Clay Loam Soil

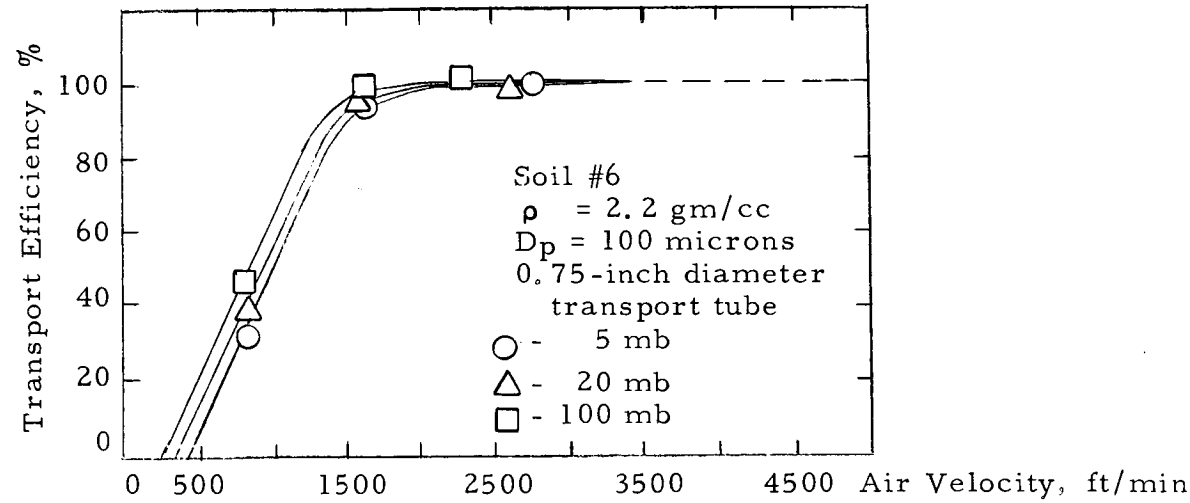


Figure 2-17. Air Velocity-Horizontal Transport Efficiency Relationship for Silty Clay Loam

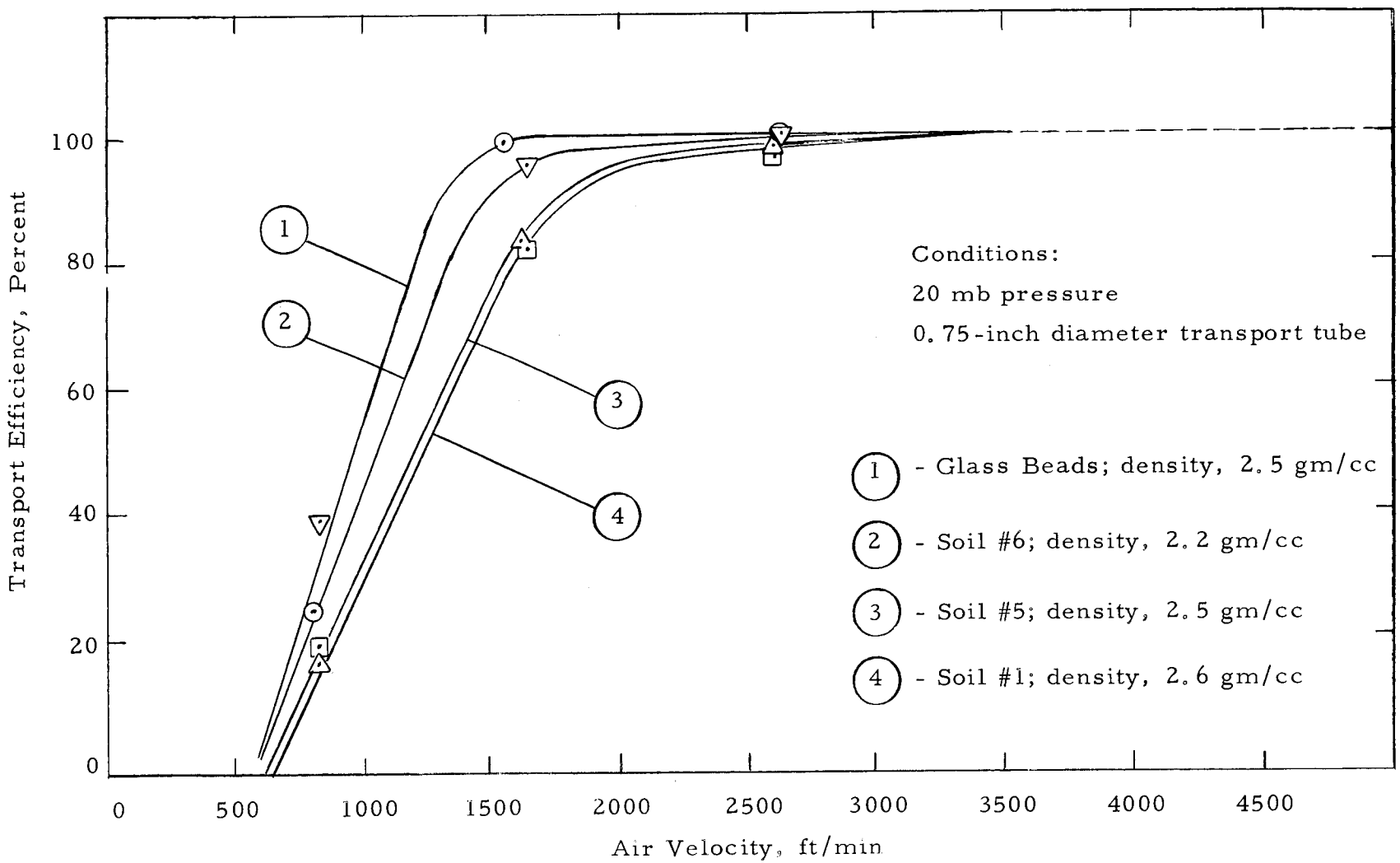


Figure 2-18. Air Velocity-Horizontal Transport Efficiency Relationship for Soil Particles and Glass Beads

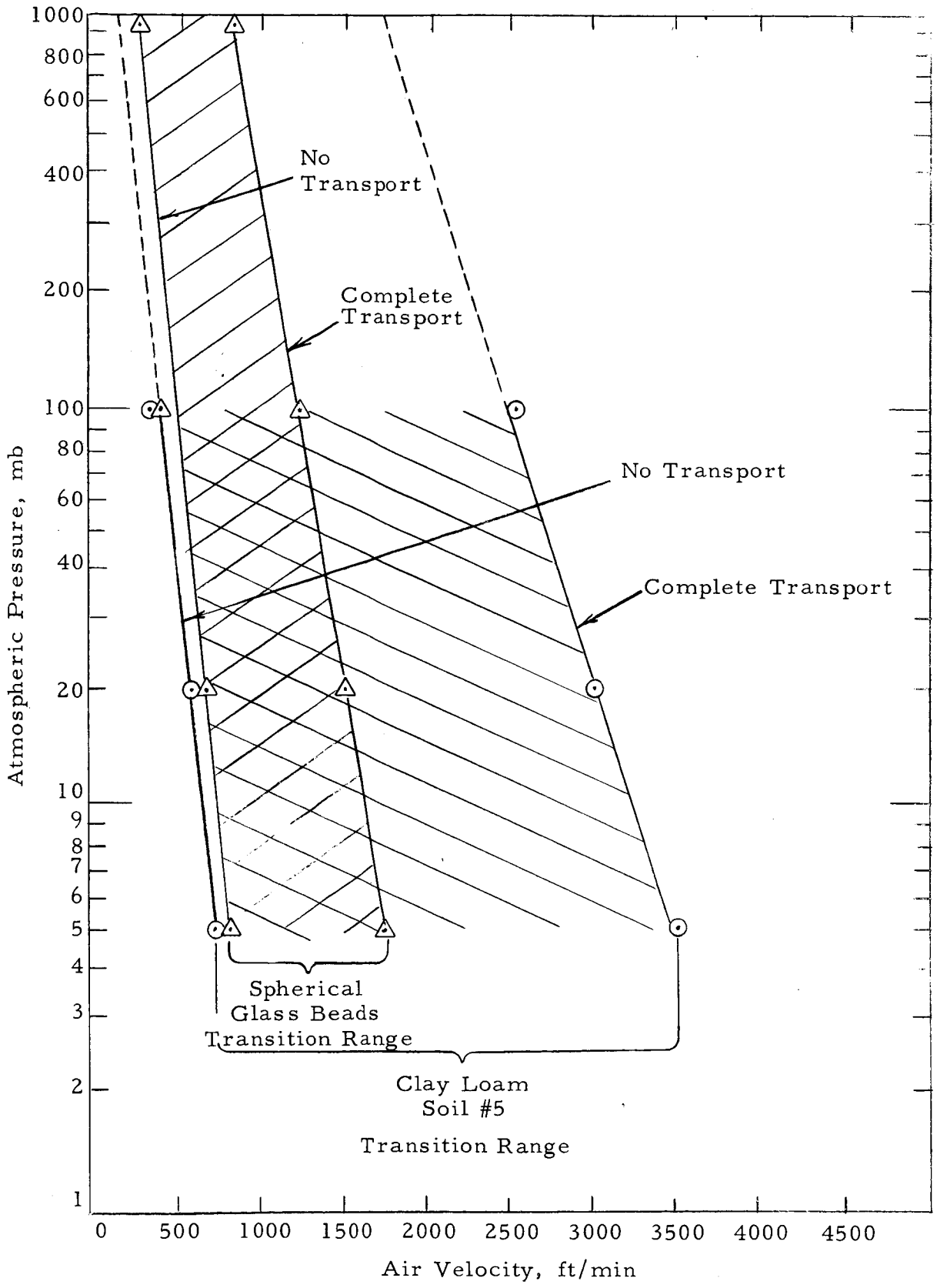


Figure 2-19. Air Velocity-Atmospheric Pressure Relationship for Horizontal Transport of 100 Micron Particles

5. Horizontal Transport Through Various Diameter Tubes

The horizontal transport studies had all been conducted thus far with a 3/4-inch I.D. transport tube. Tests were then conducted with 1/4 inch and 1/2-inch I.D. tubes. The apparatus used was the same as that reported previously for the horizontal transport studies. The length of each tube was 5 ft. Spherical glass beads with an average diameter of 100 microns were used for the particulate matter.

Tests were conducted at an atmospheric pressure of 20 mb. Figure 2-20 presents these data along with those data obtained previously for the 3/4 inch I.D. transport tube. It can be seen that the transition range of the 1/4 inch diameter transport tube covers lower absolute air velocities than the larger tubes, similarly with the 1/2 inch diameter tube. A small tube has an advantage in that the absolute air velocity required for complete transport is lower; therefore, the volumetric flow rate required for transport is reduced even more than the area ratio predicts. It should be noted, however, that this is true for smooth walled tubing only.

6. Horizontal Particle Transport in Rough-Walled Tubing

Particulate transport studies were then conducted with rough-walled tubing. The identical test apparatus and conditions previously reported for smooth-walled tubing were used. The rough-walled tube was fabricated with a coil spring covered with Mylar film. Two tube diameters were tested: 3/4 inch I.D. and 1/4 inch I.D. —both wound from 0.030-inch diameter music wire to a 1/4-inch pitch. All testing was done at 20 mb pressure.

Test results indicated the relative undesirability of rough-walled tubing for particle transport owing to the relative high loss of particulate matter at the tubing surface. For example, it can be seen in Figure 2-21 that an air velocity of 1,000 fpm is as efficient for particle transport

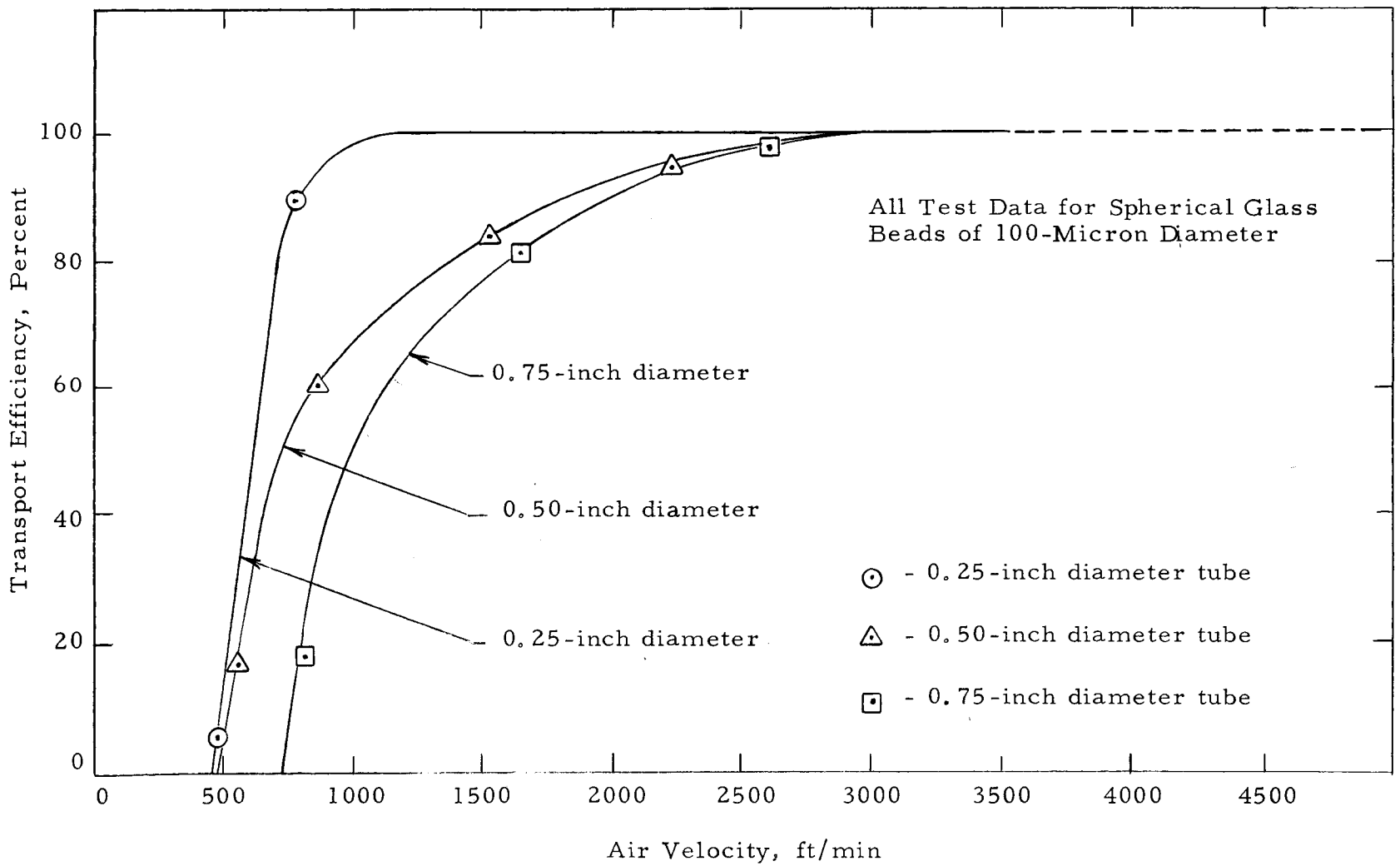


Figure 2-20. Air Velocity-Horizontal Transport Efficiency Relationship for Various Transport Tube Diameters

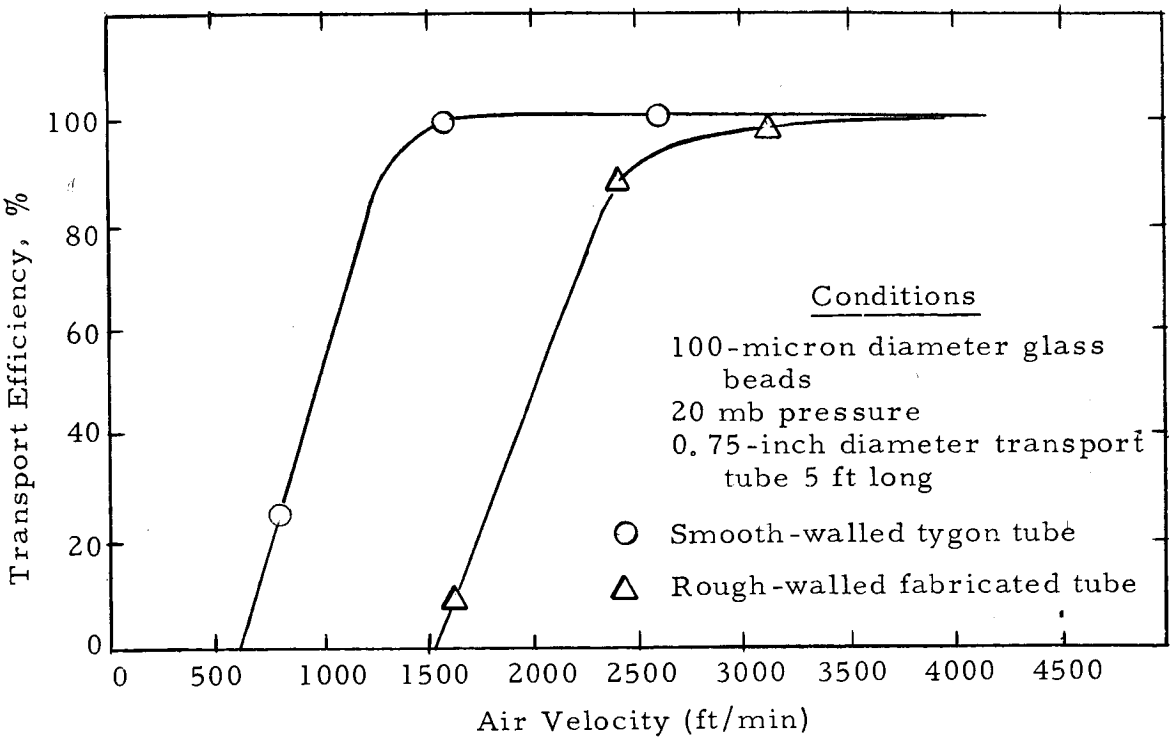


Figure 2-21. Air Velocity-Horizontal Transport Efficiency Relationship for Smooth and Rough-Walled Tube

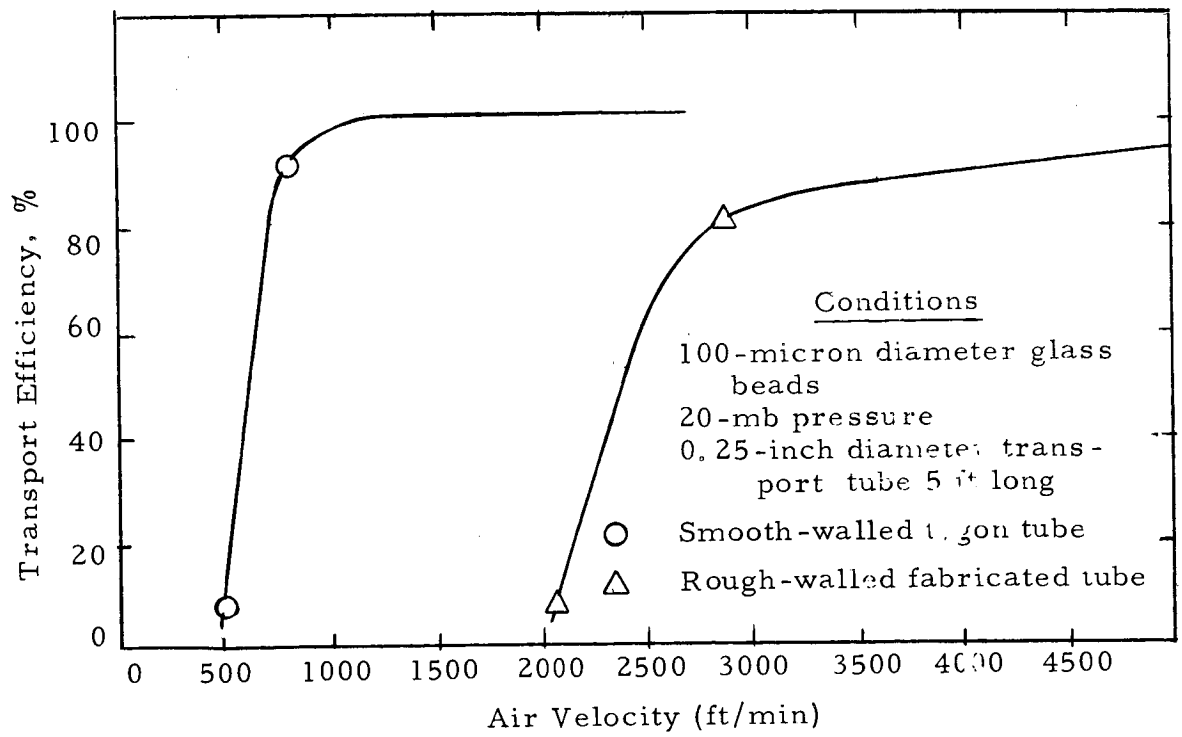


Figure 2-22. Air Velocity-Horizontal Transport Efficiency Relationship for Smooth and Rough-Walled Tube

through smooth-walled tubing as an air velocity of 2,000 fpm is for transport through rough-walled tuging. These data were obtained by feeding approximately 12 g of 100-micron glass beads into a 5-ft long tube. Figure 2-22 shows the comparison of the smooth-walled and rough-walled tubing of 1/4-inch diameter. It is apparent from these data that considerably higher air velocities are required for complete transport of particulate matter through fabricated rough-walled tubing.

F. Vertical Pneumatic Transport of Particulates

The theoretical movement of particles vertically in an air stream can be determined from Stokes law. However, such things as wall effects are not accounted for by this method. Therefore, an investigation was carried out on the vertical pneumatic particulate transport at reduced pressure in tubing. The test apparatus consisted of an aerosolizer, transport tube, and collector to visually determine the vertical transport efficiency.

The aerosolizer section was simply a glass jar fitted with a rubber stopper through which was inserted a small tube to serve as the aerosolizer and input air flow, and a large glass tube to serve as the aerosol transport tube. The jar was partially filled with spherical glass beads with the aerosolizer jet placed just above them. The input air flow was controlled by a flow-regulating valve on the outside of the chamber. A flowmeter was placed in the chamber, between the regulating valve and the aerosolizing jet; the apparatus is shown in Figure 2-23.

A clear glass tube, 1/2 inch I. D. and 18 inches long, was used for the vertical pneumatic transport section. The lower end of the glass tube was placed several inches above the surface of the glass beads. The upper end was connected to a 1/2 inch I. D. clear plastic tube which conducted the air to the collector section.

The cyclone collector used in the horizontal testing was also utilized for the vertical transport; however, the sample collection cup was replaced with a glass flask to permit visual observation of the collection.

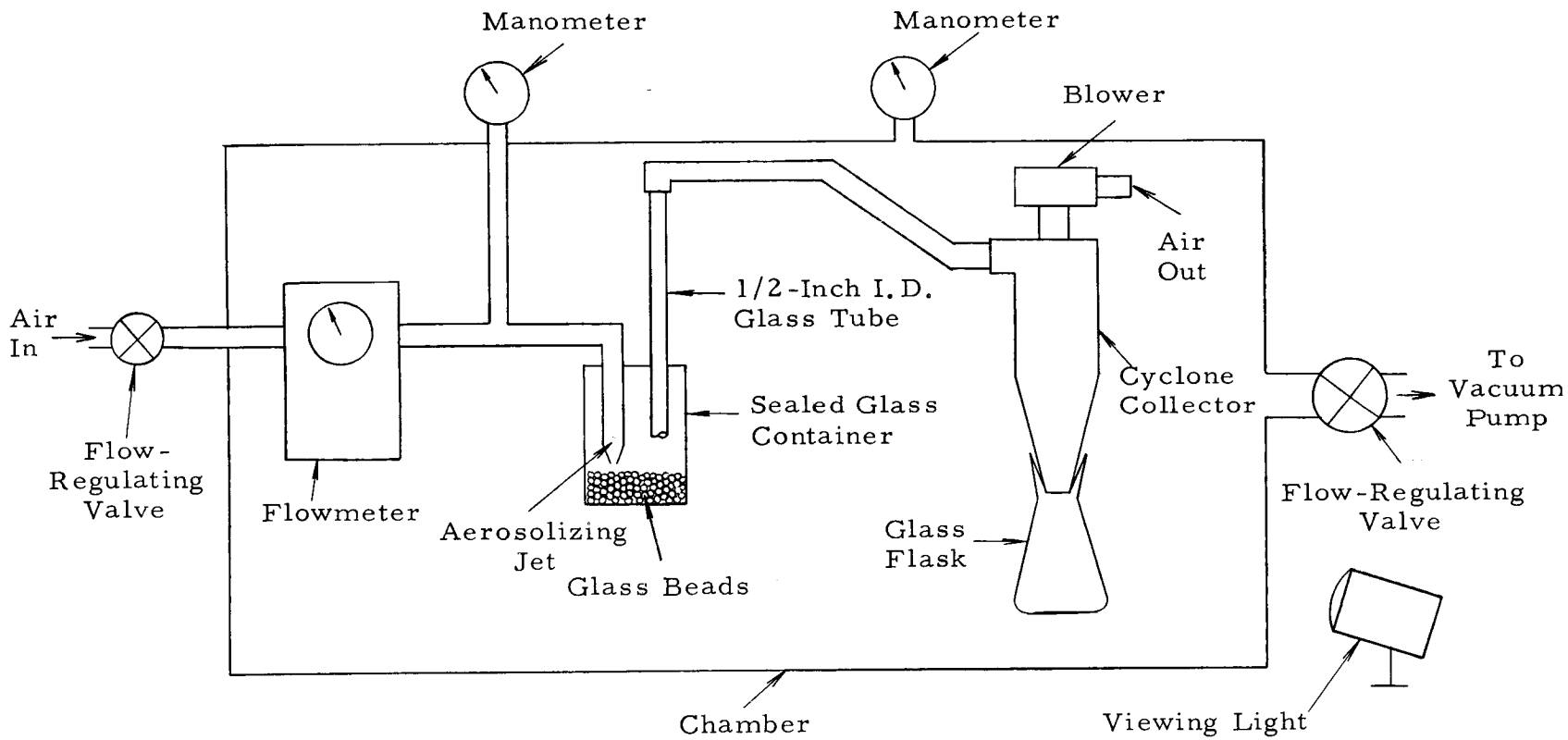


Figure 2-23. Schematic Representation of Vertical Aerosol Transport Apparatus

It was observed that the air velocity near the transport tube wall was relatively low, resulting in greater settling of the particles near the wall than in the tube center. In horizontal transport the particles would simply settle out in the transport tube, while in vertical transport the settling particles would drop down the tube wall toward the inlet or be re-entrained when they drifted outwards toward the tube center. Movement of 100-micron diameter glass beads in the glass vertical transport tube could easily be observed visually by using strong side lighting.

The object of this vertical transport study was to determine the velocity range for complete particle transport and little or no particle transport. This was done by slowly increasing the flow rate until particles were seen being transported up the vertical tube and collected out by the cyclone collector. (The cyclone is essentially 100 percent efficient at the test conditions used.) Air flow rate was then reduced slowly until it could be observed that the collection had just ceased. This constitutes the threshold air velocity for the start of vertical pneumatic particulate transport. The air flow rate was then increased until no particles could be observed falling back toward the vertical transport tube inlet. This was considered the velocity above which there would be complete vertical pneumatic transport.

These studies were conducted with spherical glass beads having an average diameter of 100 microns. These data were plotted showing the transition range from no transport to complete transport over the pressure range from 5 to 970 mb. On the same graph was plotted the data obtained previously for the horizontal transition range. Figure 2-24 shows that for all atmospheric pressures the vertical transition range is lower than the horizontal transition range. Even though there is some overlapping of the ranges at the higher atmospheric pressures, it can be concluded that horizontal transport is the most important consideration. Settling of particles in the horizontal tube is the limiting factor in pneumatic transport.

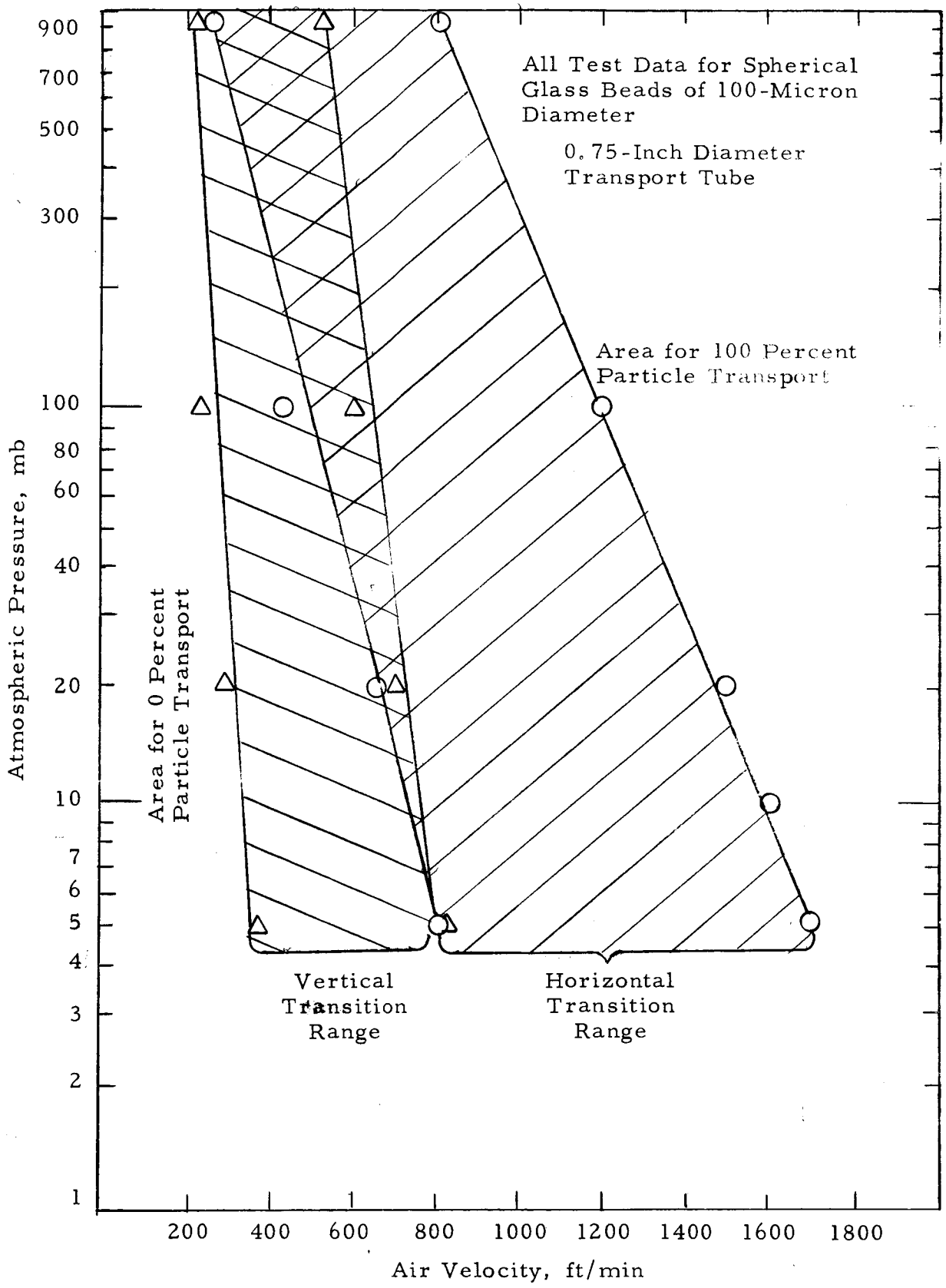


Figure 2-24. Velocity-Atmospheric Pressure Relationship for Transport of 100-Micron Particles

G. Aerosolization Studies

Soil aerosolization is an important part of any pneumatic sampling device, since small particulates are necessary for ease of transport. Usually there are small particles available on almost any soil surface; however, they must be dislodged and made air-borne near the transport tube inlet. Studies were conducted to determine the requirements for aerosolization of various soils and rock formations.

1. Field Sampling

Because soil samples available in the laboratory were not sufficiently large for complete analysis, we devised a sampling apparatus for field use. This device consisted of an air ejector pump (made of standard copper tubing fittings); it replaced the motor-blower and was mounted on top of the cyclone separator. An aerosolizing unit was in the form of a bottomless box having a 2 inch by 4 inch top, and three sides, 3/8 inch high. On the open side, we mounted a small steel wire brush. This unit simply was dragged over the soil surface, and soil particles became dislodged as the housing and brush moved over the surface. A schematic of the apparatus is shown in Figure 2-25.

The following procedure was used in tesing on all soils

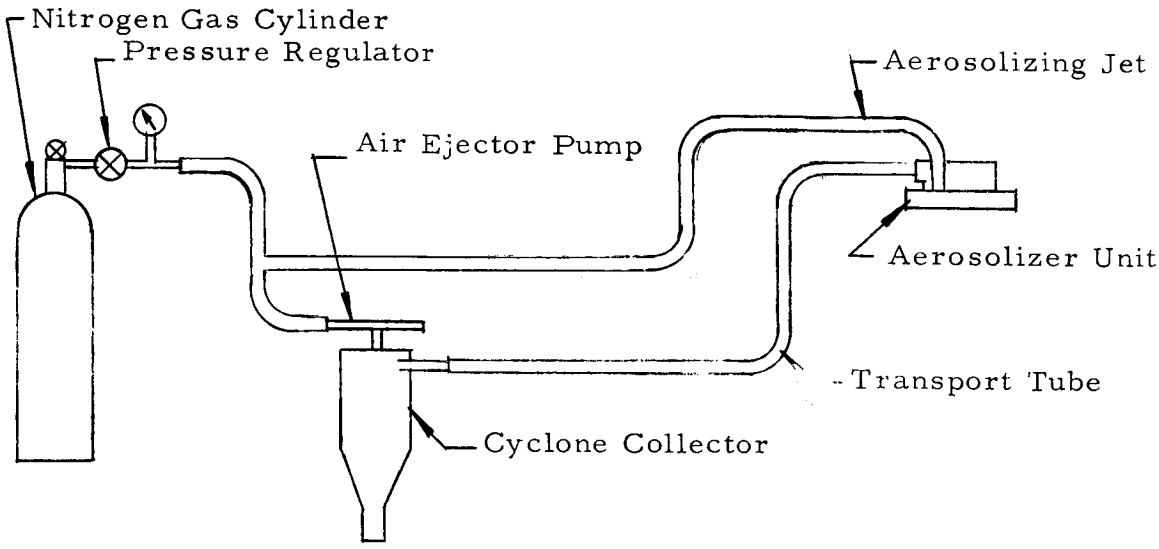
1) Natural soil, use of wire brush and aerosolizing jet

2) Natural soil, use of wire brush only

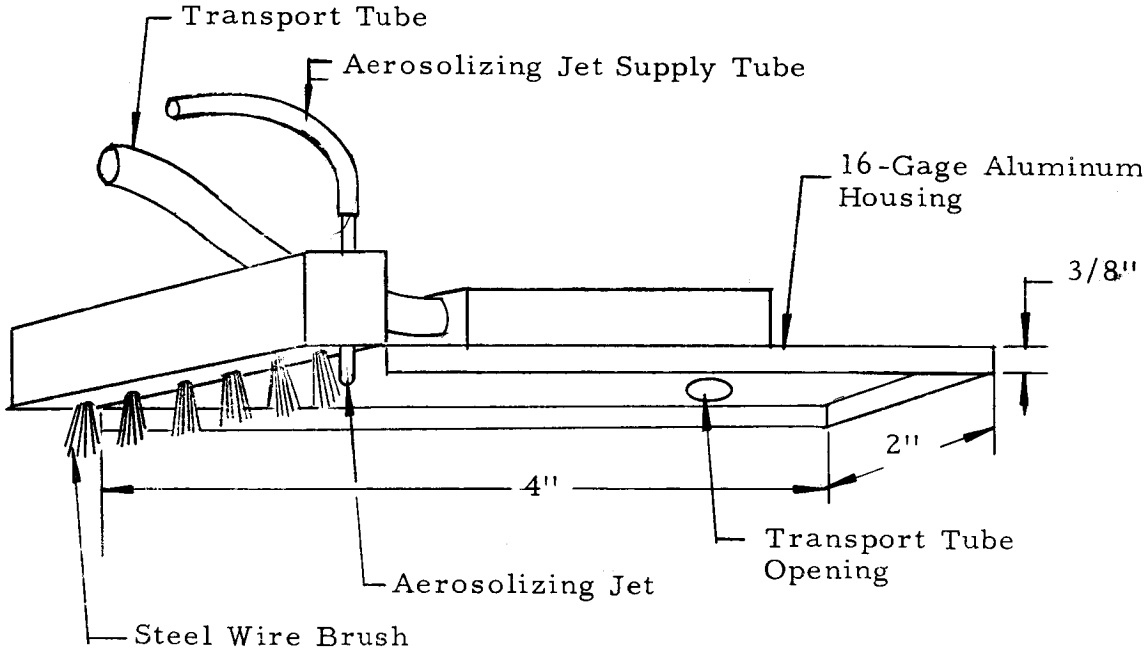
3) Surface blown clean, use of brush and jet

4) Surface blown clean, use of brush only

Any vegetation on the surface was removed to a depth of about 1/2 inch using a shovel. Various types of natural soils were utilized in the testing: clay, black dirt, gravel-clay mixture, rocks, and sandy types (See Figure 2-26). Table 2-2 presents data for 1-min sampling of some of these soils with the aerosolizer unit moved at approximately 15 ft/min.



A. Complete Apparatus



B. Aerosolizer Unit

Figure 2-25. Aerosol Soil Sampling Apparatus

(a) Rocks

(b) Black Dirt

(c) Clay

(d) Gravel-Clay

Figure 2-26. Soils Used in Aerosolization Sutdies

Table 2-2. Aerosolization Tests of Various Soils

Test (above)	Clay (gm)	Black Dirt (gm)	Clay-Gravel (gm)	Sand (gm)
1	27.4	27.2	4.1	35.6
2	23.1	11.7	0.5	30.2
3	3.9	2.2	2.0	33.7
4	2.4	1.0	0.4	30.1

From Table 2-2 we see that use of the aerosolizing jet provides more soil from the undisturbed surface than does the brush alone in a given length of time (see Tests 1 and 2).

A jet of nitrogen was directed at the surface (comparable to a strong wind) until no dust could be observed coming from the soil. Then the use of the aerosolizing jet—along with the mechanical scraping by the brush and housing—provided a considerable increase in the amount of soil collected over that obtained without the use of the aerosolizing jet when sampling from a hard surface. This test was not meaningful on sandy soils, for any attempt to blow the surface clean merely resulted in the new surface being similar to the original (see Tests 3 and 4).

It was apparent that on a windswept surface, mechanical agitation of the soil would help the aerosolization. Therefore, it was decided that a more vigorous mechanical agitation of the soil might be required. To accomplish this, another aerosolizing unit was built with a small rotary wire brush driven by an air turbine. The turbine was mounted in a bottomless aluminum box 4-1/2 inches long, 2-3/8 inches wide, and 1-3/4 inches high. The effluent air from the turbine was used as the aerosolizing jet. The wire brush was removable from the turbine. Figure 2-27 presents a drawing of this unit that was used in the field for sampling various soil surfaces. The same sampling apparatus was used as shown in Figure 2-25A.

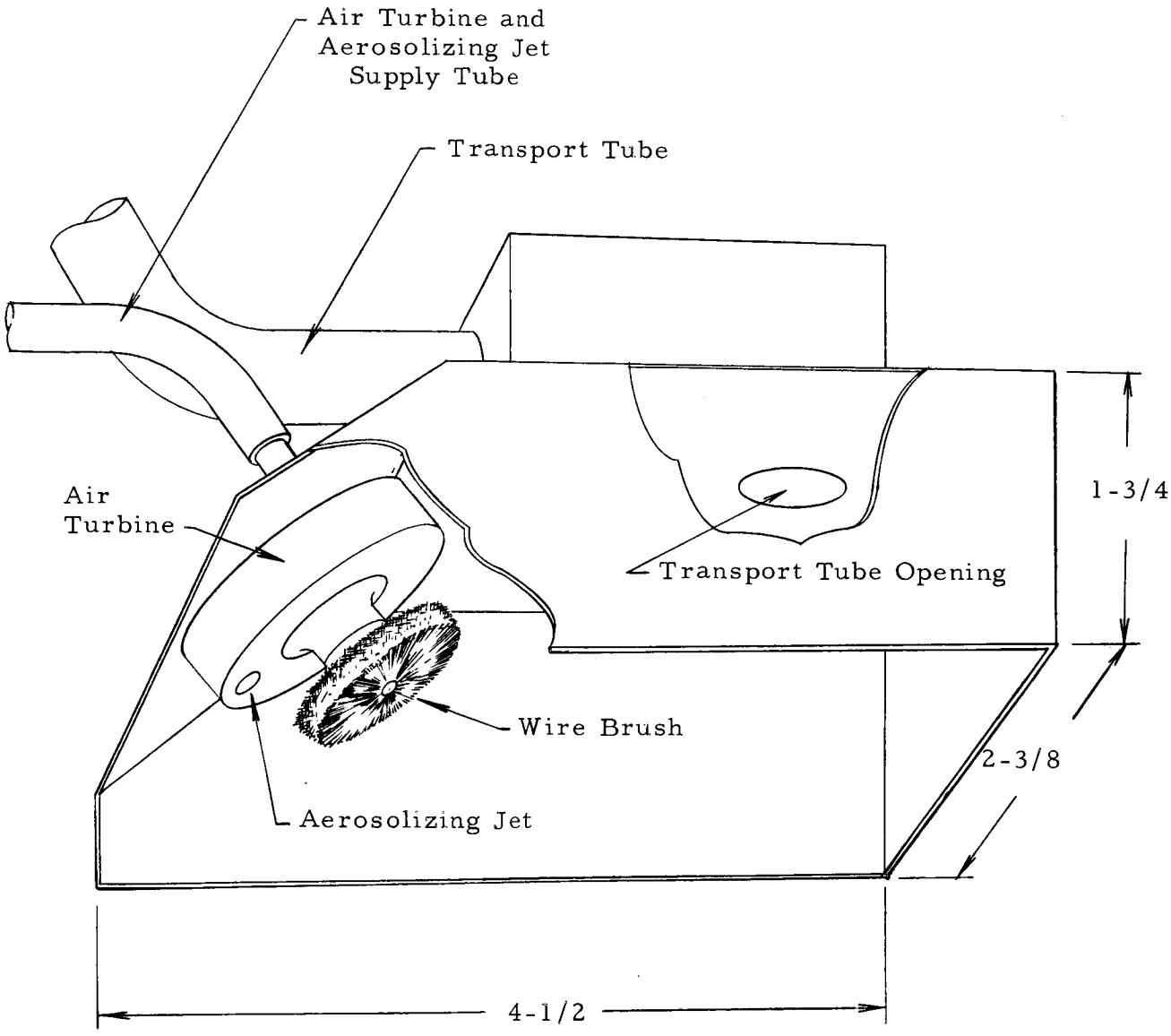


Figure 2-27. Aerosolizing Unit

A series of sampling tests was conducted on each soil surface, using the following procedure:

Test	Description
A	Natural soil, use of vacuum only
B	Natural soil, use of vacuum and aerosolizing jet
C	Natural soil, use of vacuum, aerosolizing jet and brush
D	Surface blown clean, use of vacuum only
E	Surface blown clean, use of vacuum and aerosolizing jet
F	Surface blown clean, use of vacuum, aerosolizing jet and brush

Surfaces were selected that were relatively free of vegetation. Tests D, E, and F were conducted to simulate high wind action on a surface. These data are presented in Table 2-3.

Table 2-3. Aerosolization Tests of Various Terrestial Surfaces

Soil	Test A	Test B	Test C	Test D	Test E	Test F
			(gm/min)			
#40 Clay-gravel	0.1	1.2	1.6	0.05	0.5	1.4
#41 Clay	0.3	4.7	4.9	0.2	1.5	4.0
#42 Black Dirt	0.2	10.1	11.2	0.1	1.6	2.8
#43 Soft Sandstone	0.2	7.9	12.2	0.1	2.5	6.3
#44 Hard Sandstone	0.03	4.8	5.2	0.04	0.15	0.4
#45 Fine Sand	0.2	18.1	19.2	0.2	17.5	19.0
#46 Granite	Trace	0.01	0.07	0.0	0.0	0.06
#47 Limestone	Trace	0.02	0.09	0.0	0.0	0.08

These data show that the samples collected from most of the natural, undisturbed surfaces (Tests A, B, C) were only moderatly increased by the use of the mechanical brushing along with the aerosolizing jet, as compared with use of the aerosolizing jet alone. On the surfaces that

were blown free of loose soil before testing (Tests D, E, F), however, the additional use of the brush with the aerosolizing jet improved the collection rate considerably. On very loose soils such as sand, the aerosolizing jet alone is sufficient for sampling.

2. Aerosolization at Reduced Pressure

Various types of soil were then tested in the chamber at reduced pressure using the same procedure as reported for the atmospheric testing, that is:

Test	Description
A	Natural soil, use of vacuum only
B	Natural soil, use of vacuum and aerosolizing jet
C	Natural soil, use of vacuum, aerosolizing jet, and brush
D	Surface blown clean, use of vacuum only
E	Surface blown clean, use of vacuum and aerosolizing jet
F	Surface blown clean, use of vacuum, aerosolizing jet, and brush

The results of these tests were relatively the same as for tests at atmospheric pressure; that is, the mechanical agitation of the surface increased the collection rate in all cases. These data are presented in Table 2-4.

No attempt was made to optimize the size, position, etc., of the brush or aerosolizing jet, and only a flow rate of 4 cfm through the 1/2-inch diameter tube was employed in all testing.

Table 2-4. Aerosolization Tests of Various Terrestial Surfaces (At 5 mb Pressure)

Soil	Test A	Test B	Test C	Test D	Test E	Test F
			(gm/min)			
#1 Sand	0.06	5.91	9.42	0.05	5.80	9.10
#5 Clay	0.04	0.86	1.04	0.10	0.12	0.13
#6 Black Dirt	0.02	0.29	0.50	0.02	0.09	0.21
#50 Sand Stone	0.00	0.01	0.06	0.00	0.01	0.03

H. Sampling at Low Temperatures

The possibility exists that sample collection may be required at low temperatures in a Martian atmosphere. To determine the feasibility of such sampling, tests were run in the environmental test chamber with several gas compositions at temperatures in the range of +25°C to -45°C. The sampling system described in the Aerosolization Studies (Section G) was used.

The sampler was tested over the above range of conditions on a dry sandy soil. Equal collection efficiency was obtained over this entire range. Tests at 20 millibars pressure gave a collection rate of 6 grams per minute ± 1 gm for air. Several tests run at various low temperatures and atmospheric pressure produced collection rates from about 6 to 12 grams per minute over the sandy soil. This variation could possibly be attributed to the formation of frost on the soil surface when the chamber was opened to obtain the collected sample. Subsequent tests could then have been affected by this frost. An interesting point is that the soil did not appear frozen even at temperatures of -45°C. This soil was not specially dried or prepared but is a natural soil which has been kept in a laboratory storage cabinet for approximately nine months. The sample

has long ago reached a temperature-moisture equilibrium with the laboratory air. This moisture level is undoubtedly higher than that which would be expected on the surface of Mars.

I. Air Ejector

In any pneumatic transport device an air mover is a prime requisite. We had used motor-blowers in the past; however, experience gained by Litton personnel in the field of high altitude air sampling indicated that the use of an air ejector pump for Martian sampling would be very advantageous. In a sample collection system utilizing an air ejector pump, the ejector would replace a motor-blower, and a small tank of compressed gas would replace the power supply or battery required for the blower. Such a system would have two important advantages:

1) Increased reliability. The only moving part would be a valve to turn on the air ejector. There would be no problems of wearing or binding of parts and, since there is no electric motor or electric energy required, there would be no problem of heat dissipation.

2) Lighter weight. A simple air ejector will weigh less than an equivalent motor-blower system and will require less duplication because of the increased reliability. The high pressure gas supply for an air ejector will be as light as or lighter than the battery supply required for an equivalent motor-blower system. The weight advantage of an air ejector increases as the pressure decreases because the required mass flow rate to be pumped by the air ejector decreases with pressure.

Basically, the operating principle of the air ejector is simple. A high velocity jet of primary air is injected into a mixing tube from a nozzle. This primary jet, on expanding, entrains the surrounding secondary air and, by a turbulent exchange of momentum, creates a region of low pressure and causes the secondary air to flow.

There are two basic types of air ejectors described in the literature: a constant pressure mixing system and a constant area mixing system. The constant pressure ejector is more difficult to design for

optimum performance and is more sensitive to changes in primary, secondary, and ambient pressures and flow rates. The constant area ejector, therefore, was chosen for use in this application because a versatile system is required. In a constant area ejector most of the pressure rise is produced in the constant area section; therefore, the performance is not strongly dependent upon the performance of the diffuser section as it is in the constant pressure ejector system. Low air densities tend to be detrimental to the performance of a diffuser; hence, problems associated with the constant pressure ejector become even more severe when the low air density of a Martian environment is considered.

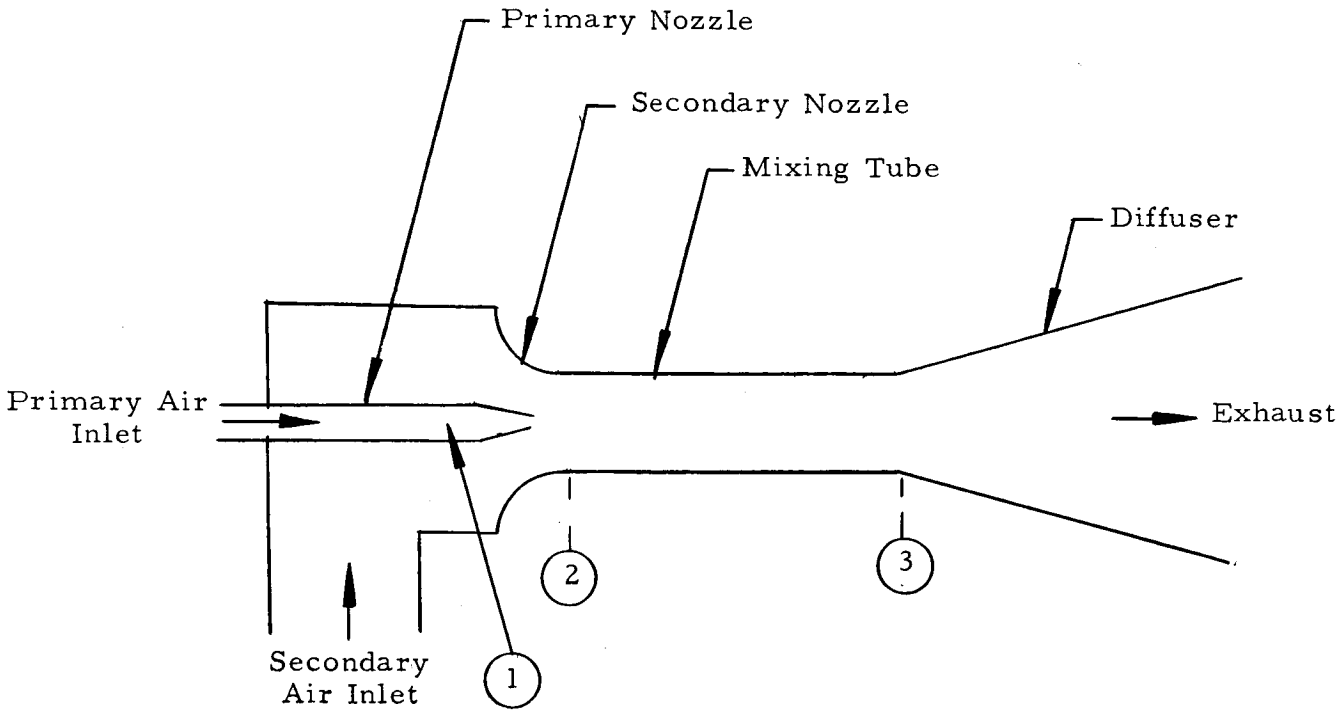
1. Theoretical Analysis of an Air Ejector

The following equations can be used to describe performance of the constant area air ejector if the flow is considered to be one-dimensional, steady, frictionless, adiabatic, and to undergo neither phase nor chemical changes. In the following analysis, the primary and secondary streams are assumed to be of the same gas and at the same total temperature. (See Figure 2-28 and nomenclature for description of states). Performance of the air ejector is determined from the continuity equation:

$$\rho_1 A_1 V_1 + \rho_2 A_2 V_2 = \rho_3 A_3 V_3$$

and the momentum equation:

$$P_1 A_1 + \rho_1 A_1 V_1^2 + P_2 A_2 + \rho_2 A_2 V_2^2 = P_3 A_3 + \rho_3 A_3 V_3^2.$$



Nomenclature

A - area

M - Mach number

P - pressure

V - velocity

γ - ratio of heat capacity at constant pressure to that at constant volume

η_d - diffuser efficiency

μ - mass augmentation

ρ - density

a - ambient conditions

1 - conditions at primary nozzle exit

2 - conditions at secondary nozzle exit

3 - conditions at mixing tube outlet

Figure 2-28. Schematic of a Constant Area Air Ejector

Using the energy equation, equation of state, definition of the Mach number, and the isentropic relationship for the total states in combination with a temperature relationship from the energy equation, the continuity equation and momentum equation may be expressed by the following thermodynamic notation:

1. Continuity Equation

$$\frac{P_{o1}A_1}{P_aA_3}\left(\frac{2}{\gamma+1}\right)^{\frac{\gamma+1}{2(\gamma-1)}} + \frac{P_{o2}A_2M_2}{P_aA_3}\left(\frac{2+(\gamma-1)M_2^2}{2}\right)^{\frac{-(\gamma+1)}{2(\gamma-1)}} = \frac{M_3\left(1+\frac{\gamma-1}{2}M_3^2\right)^{1/2}}{\left(1+\frac{\gamma}{2}\eta_d M_3^2\right)}$$

2. Momentum Equation

$$\frac{P_{o1}}{P_a}\frac{A_1}{A_3}\frac{2^{\frac{\gamma}{\gamma-1}}}{\left(1+\gamma\right)^{\frac{1}{\gamma-1}}} + \frac{P_{o2}}{P_a}\frac{A_2}{A_3}\frac{\left(1+\gamma M_2^2\right)}{\left(1+\frac{\gamma-1}{2}M_2^2\right)^{\frac{\gamma}{\gamma-1}}} = \frac{1+\gamma M_3^2}{1+\frac{\gamma}{2}\eta_d M_3^2}$$

Solution of the continuity and momentum equations defines the operation of an air ejector pump for fixed values of the area ratio, diffuser efficiency, and primary and secondary pressure ratios.

Performance of an air ejector is often illustrated by plotting the mass augmentation as a function of the back pressure ratio. Mass augmentation is defined as follows:

$$\mu = \frac{\rho_1A_1V_1 + \rho_2A_2V_2}{\rho_1A_1V_1} = \frac{m_1 + m_2}{m_1} .$$

Where m_1 is the primary mass flow rate and m_2 is the secondary mass flow rate. The back pressure ratio is the ratio of the ambient air pressure to the secondary air inlet total pressure.

It is therefore, desirable to obtain a maximum mass augmentation to permit use of a minimum mass of primary gas to obtain the required secondary flow. For a given flow rate and back pressure, mass augmentation is affected by

1) Mixing the tube length,

2) Ratio of primary nozzle area to the secondary nozzle area,

3) Ratio or primary air pressure to ambient air pressure, and

4) Diffuser length and angle.

Test were run with an air ejector to determine the effects of each of the above on the performance.

2. Design of an Air Ejector

Using the estimated Martian atmospheric pressure of 20 millibars and a desired secondary air flow rate of 5 cfm, an air ejector was designed and built for use with a 1/2-inch I.D. transport tube. A compatible cyclone separator was also built. These units are shown in Figures 2-29 and 2-30.

The air ejector was designed so that each component could be changed easily for testing purposes. Each unit was fabricated from aluminum and the entire system (air ejector and cyclone separator) weighs less than 4 ounces. The weight and size of the primary gas supply would be dependent upon the secondary flow rate required and the length of time of operation.

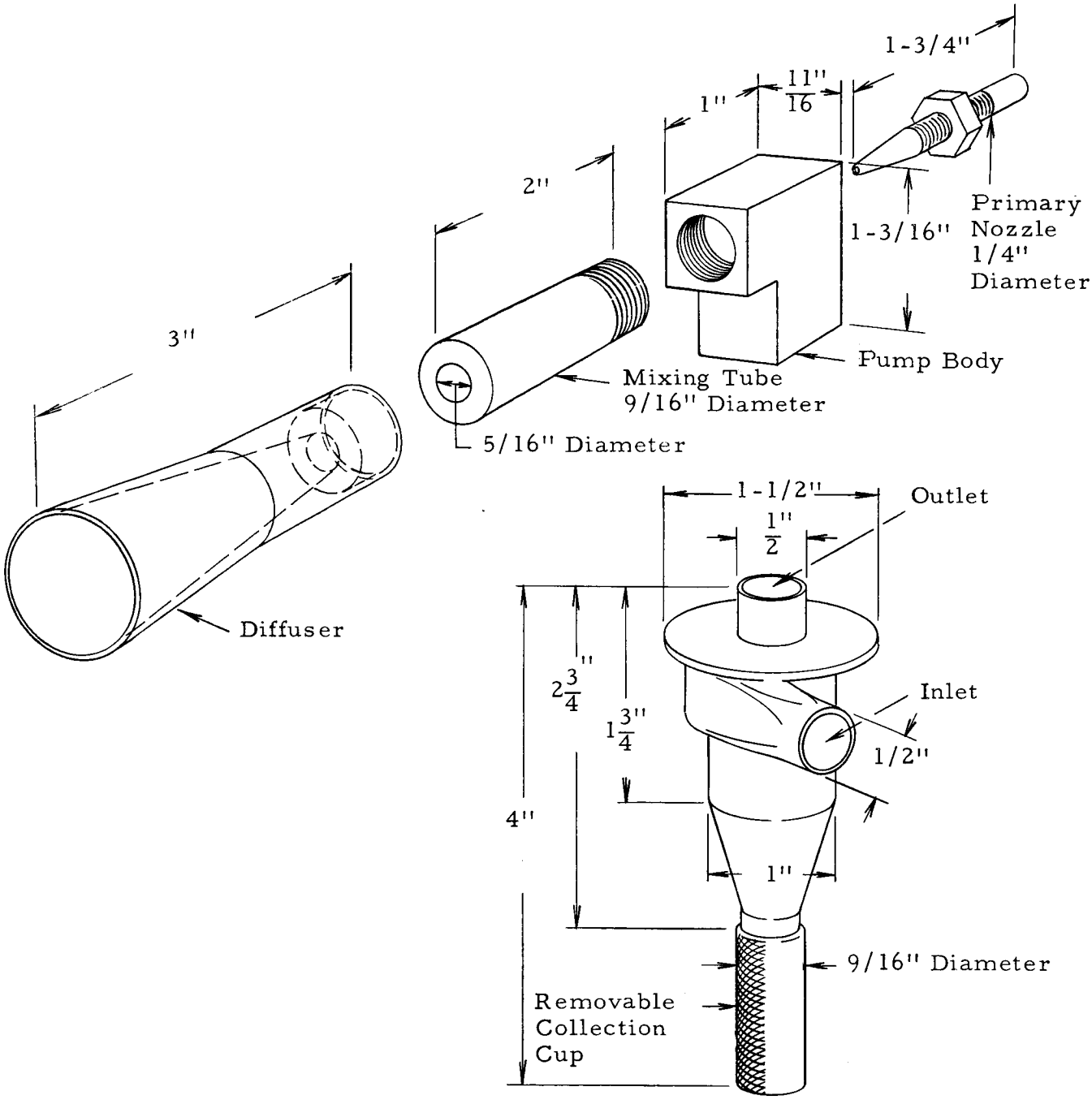


Figure 2-29. Air Ejector Pump and Cyclone Separator

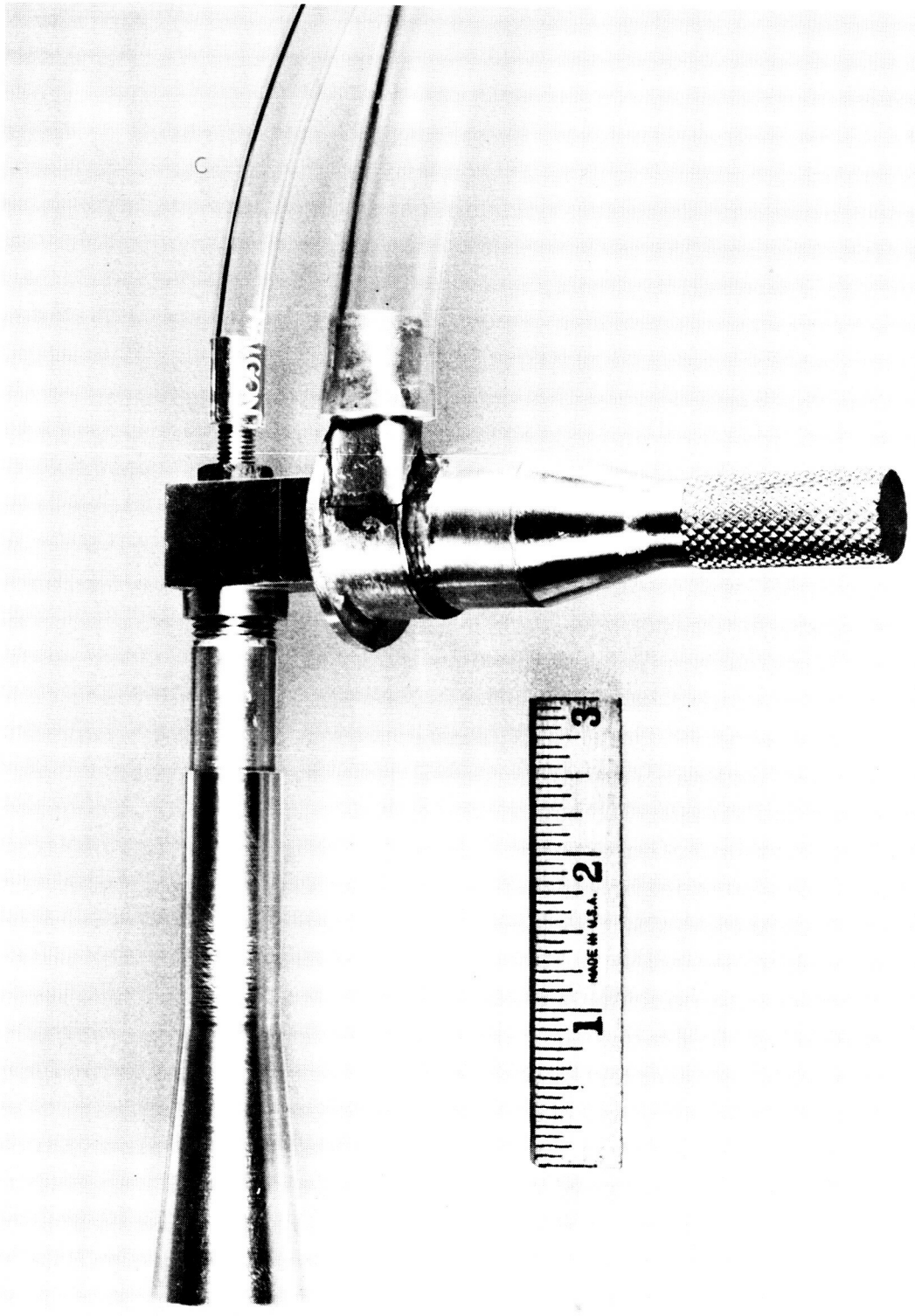


Figure 2-30. Air Ejector and Cyclone Separator

3. Experimental Procedure

The purpose of this investigation was to determine the combination of parameter values that will give optimum performance at Martian pressures with a sampling system. Therefore, the testing was carried out to cover the parameter ranges that could be encountered in such a system. Table 2-5 lists the parameters investigated.

Table 2-5. Air Ejector Investigation Parameters

Variable Investigated	Parameter Range
Primary Nozzle Diameter	0.020 - 0.052 inch
Mixing Tube Diameter	0.186 - 0.375 inch
Mixing Tube Length	1.5 - 3.0 inch
Primary Nozzle Pressure	50 - 1,000 millibars
Ambient Pressure	5 - 1,000 millibars
Secondary Flow Rate	0.4 - 7 cfm
Ambient-to-Back Pressure Ratio	1.02 - 2.3

All tests were run at room temperature using dry nitrogen as the primary gas source and using the ambient air as the secondary gas. Gas meters were used to measure both the primary and secondary air volumetric flow rates. Pressure measurements were taken at all points in the system and appropriate corrections applied to the volumetric flow rate readings. The test setup is shown in Figure 2-31.

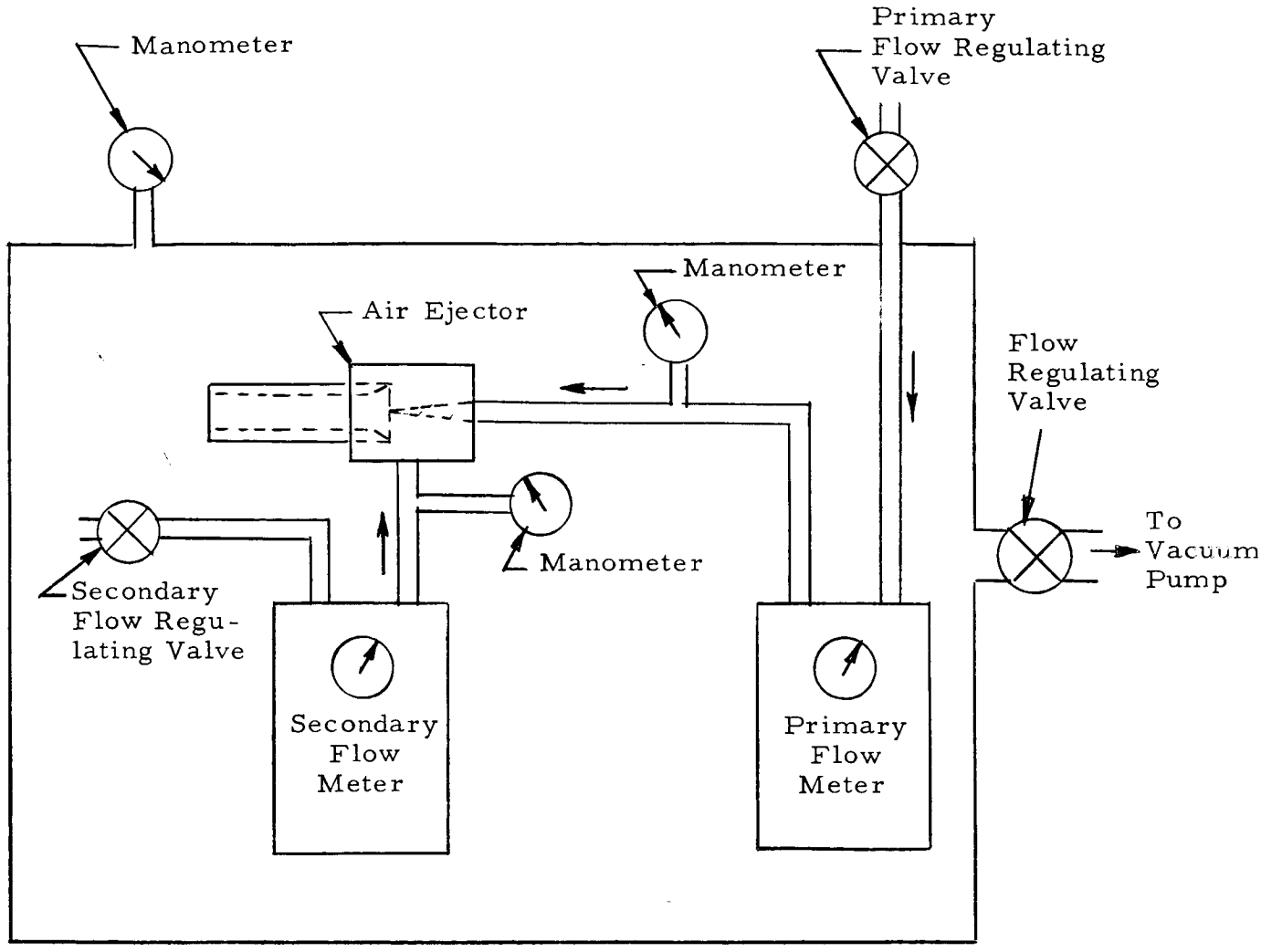


Figure 2-31. Schematic Representation of Air Ejector Performance Apparatus

4. Effect of Mixing Tube Length

A properly designed air ejector should have a mixing tube length of approximately 6 to 9 tube diameters. This range was covered by testing mixing tube lengths of 5, 7, and 10 tube diameters, or 1.5 inches, 2.0 inches, and 3.0 inches respectively for the tube diameter of 0.312 inches.

Tests were first run without a diffuser to determine if adequate performance could be obtained with the mixing tube alone. It was found that the 2.0 inch long tube performance was better than either the 1.5 inch tube or the 3.0 inch tube. This testing was done to cover the range of secondary flow rates of 0.4 to 7.0 cfm and back pressure ratios of 1.02 to 1.5. Figure 2-32 shows a typical graph of the performance of the air ejector with different tube lengths as expressed in terms of the mass augmentation and back pressure ratio.

Tests were run also with a 7-degree included-angle diffuser with the 1.5 inch tube, 2.0 inch tube, and 3.0 inch tube. It was found that the 1.5 inch tube length gave better performance than the 2.0 inch tube and the 3.0 inch tube over the range of testing conditions noted above. Figure 2-33 shows the results obtained at 20 mb ambient air pressure.

5. Effect of Primary Nozzle Area Ratio

A good ratio of primary nozzle area to secondary nozzle area has been reported to be about 0.01.* Tests were run using nozzle area ratios of 0.005, 0.008, 0.015, 0.028 utilizing primary nozzle diameters 0f 0.022 inch, 0.028 inch, 0.038 inch, 0.052 inch respectively in a fixed mixing tube diameter of 0.312 inch.

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* General Mills, Inc. Electronics Division. Report No. 2277. Investigation or an air ejector pump for high altitude sampling systems, by A. R. McFarland. Contract AT(11-1)-401. Final Report (May 15, 1962). (This contract has been novated, naming Applied Science Division Litton Systems, Inc., as successor in interest to GMI).

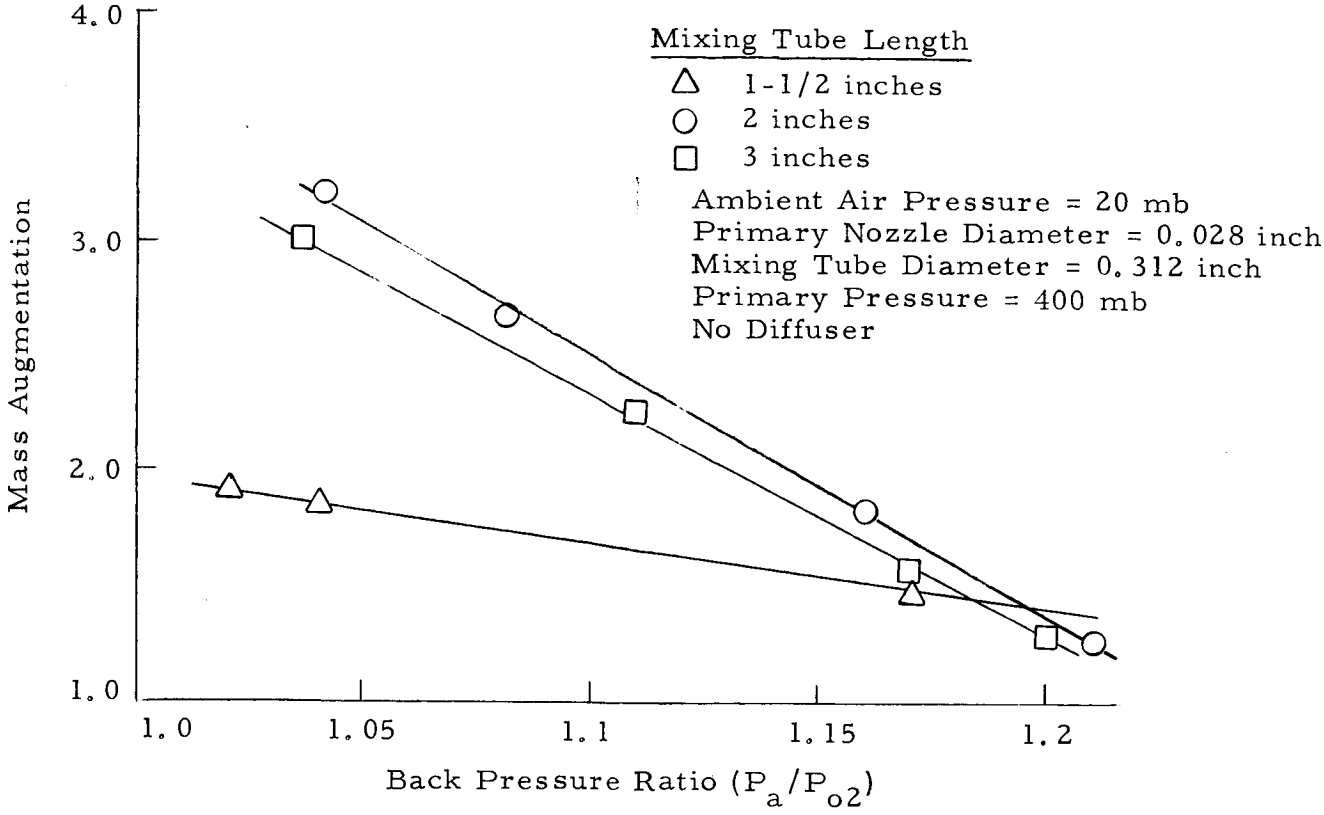


Figure 2-32. Performance of an Air Ejector with Various Mixing Tube Lengths and No Diffuser

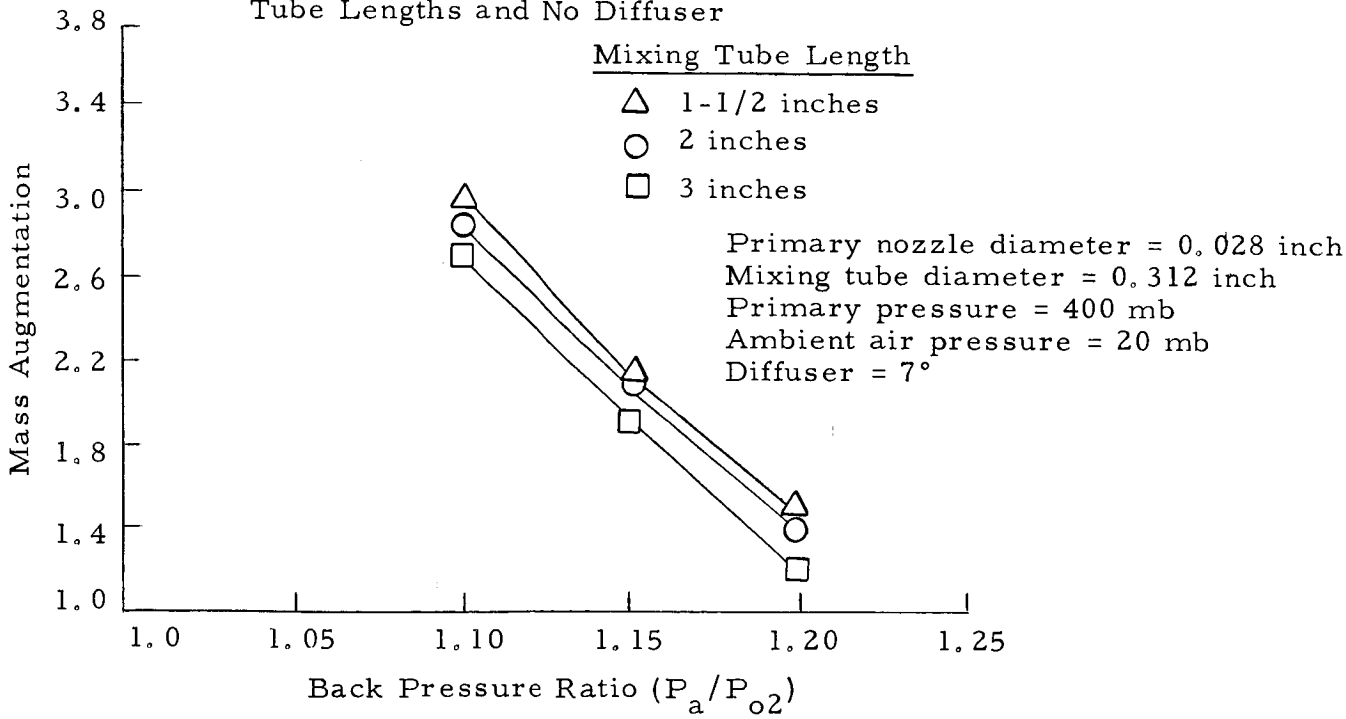


Figure 2-33. Performance of an Air Ejector with Various Mixing Tube Lengths and a 7° Diffuser

The primary air pressure was varied and the secondary flow rate was fixed at 3 cfm by adjusting the secondary flow regulating valve, and the mass augmentations were compared for the various primary nozzle ratios. Figure 2-34 presents the data obtained at 20 mb ambient air pressure. It can be seen that at back pressure ratios less than 1.07 there is not an appreciable difference in the efficiency with the various primary area ratios. However, at the higher back pressure ratios, the 0.008 primary area ratio appears to be somewhat more efficient.

The optimum primary area ratio is dependent on the air ejector parameters and on the pneumatic transport system operating conditions. The pneumatic transport system being tested required about 3 cfm flow rate with a back pressure ratio of 1.15 to transport soil at 20 mb pressure. Thus, the 0.008 primary area ratio appears to be the most efficient for this system without the use of a diffuser.

Similar testing with a 7 degree diffuser added, gave the same results, in that the 0.008 primary area ratio gives better performance for the air ejector configuration tested.

6. Effect of Primary Pressure Ratio

The optimum primary air pressure to ambient air pressure ratio is determined from the operating conditions of the pneumatic system. Typical data obtained at several ambient air pressures is presented with mass augmentation given as a function of back pressure ratio for constant primary pressure ratio. Figures 2-35 through 2-39 also show an envelope generated by the constant primary pressure ratio curves. This envelope defines the mass augmentation obtainable with optimum primary pressure ratio and back pressure ratio.

For a given set of conditions, the envelope curves generated are almost identical for each of the ambient air pressures. Figure 2-40 shows that only at 5 mb ambient pressure is there an appreciable deviation from the other data, and this may be attributable to other effects.

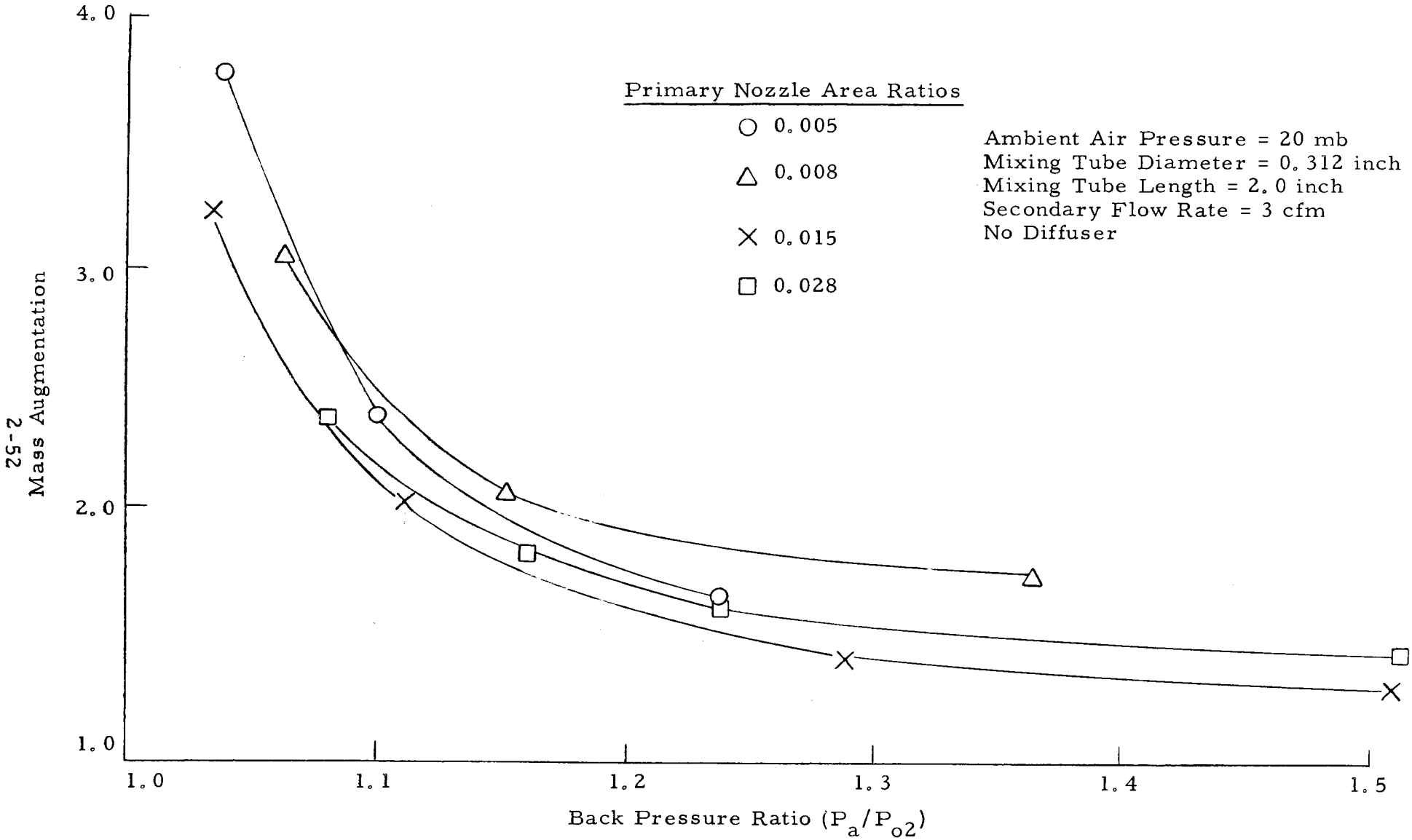


Figure 2-34. Mass Augmentation as a Function of Back Pressure Ratio

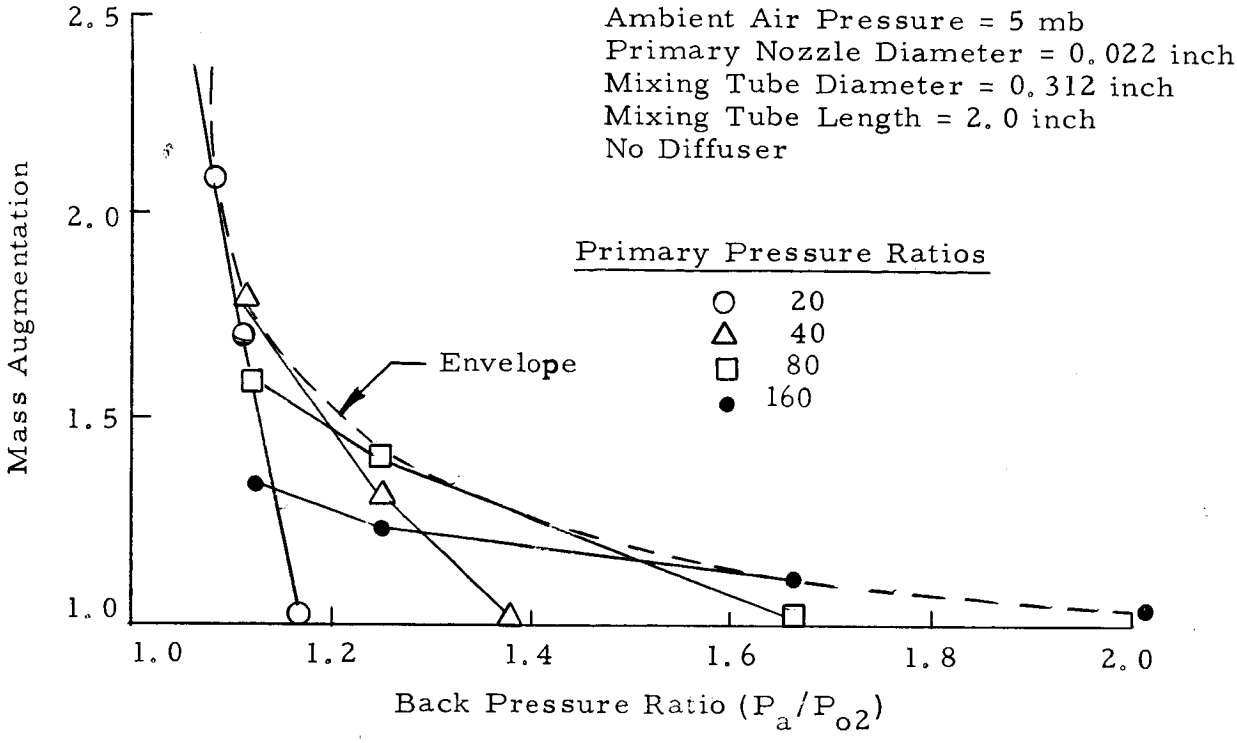


Figure 2-35. Mass Augmentation as a Function of Back Pressure Ratio

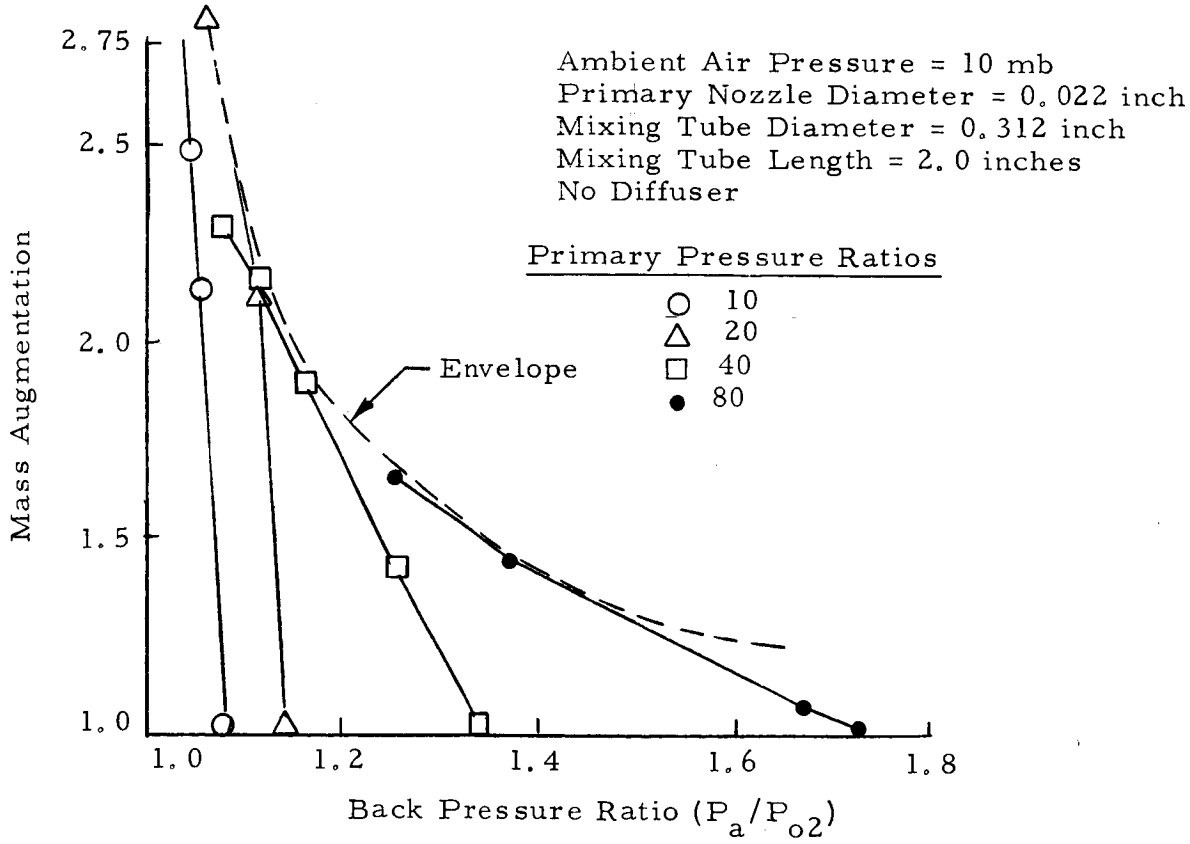


Figure 2-36. Mass Augmentation as a Function of Back Pressure Ratio

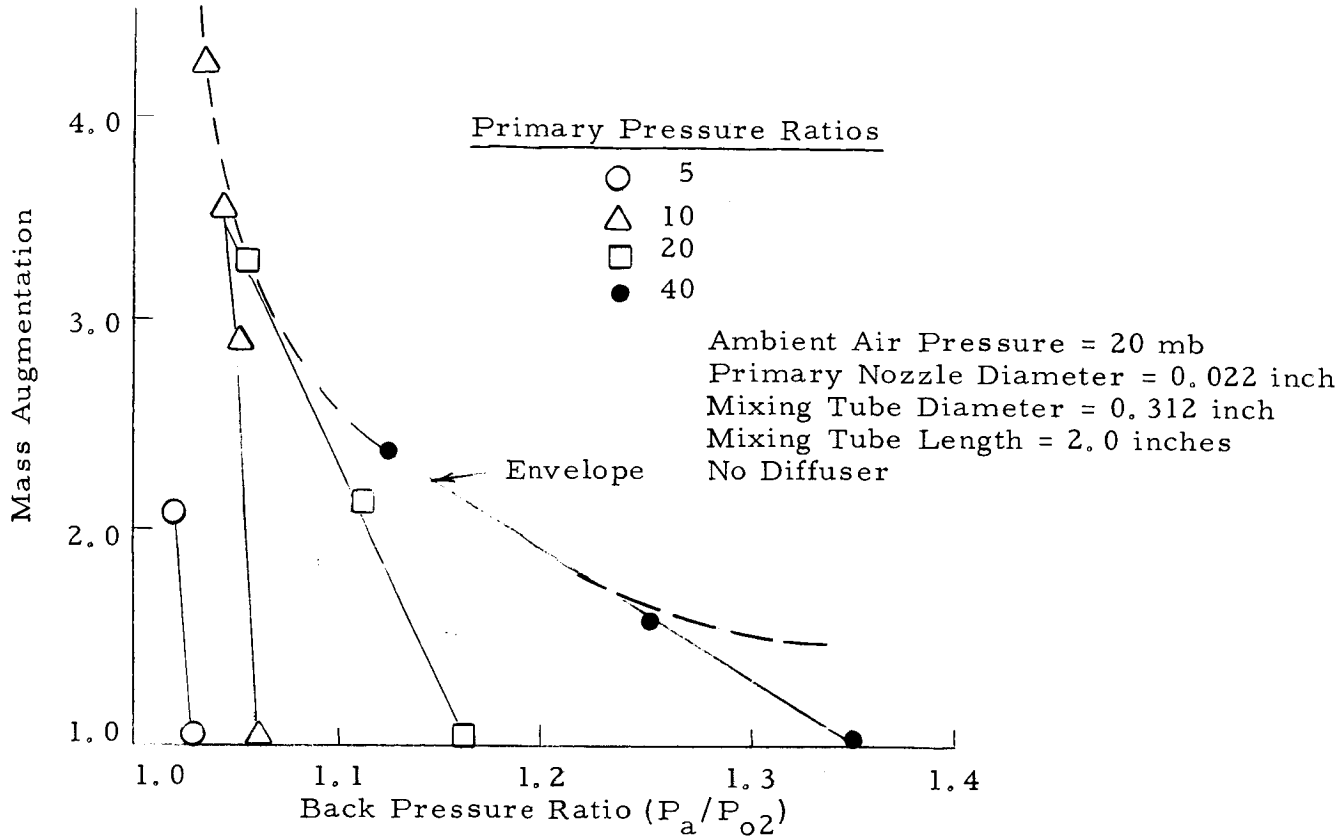


Figure 2-37. Mass Augmentation as a Function of Back Pressure Ratio

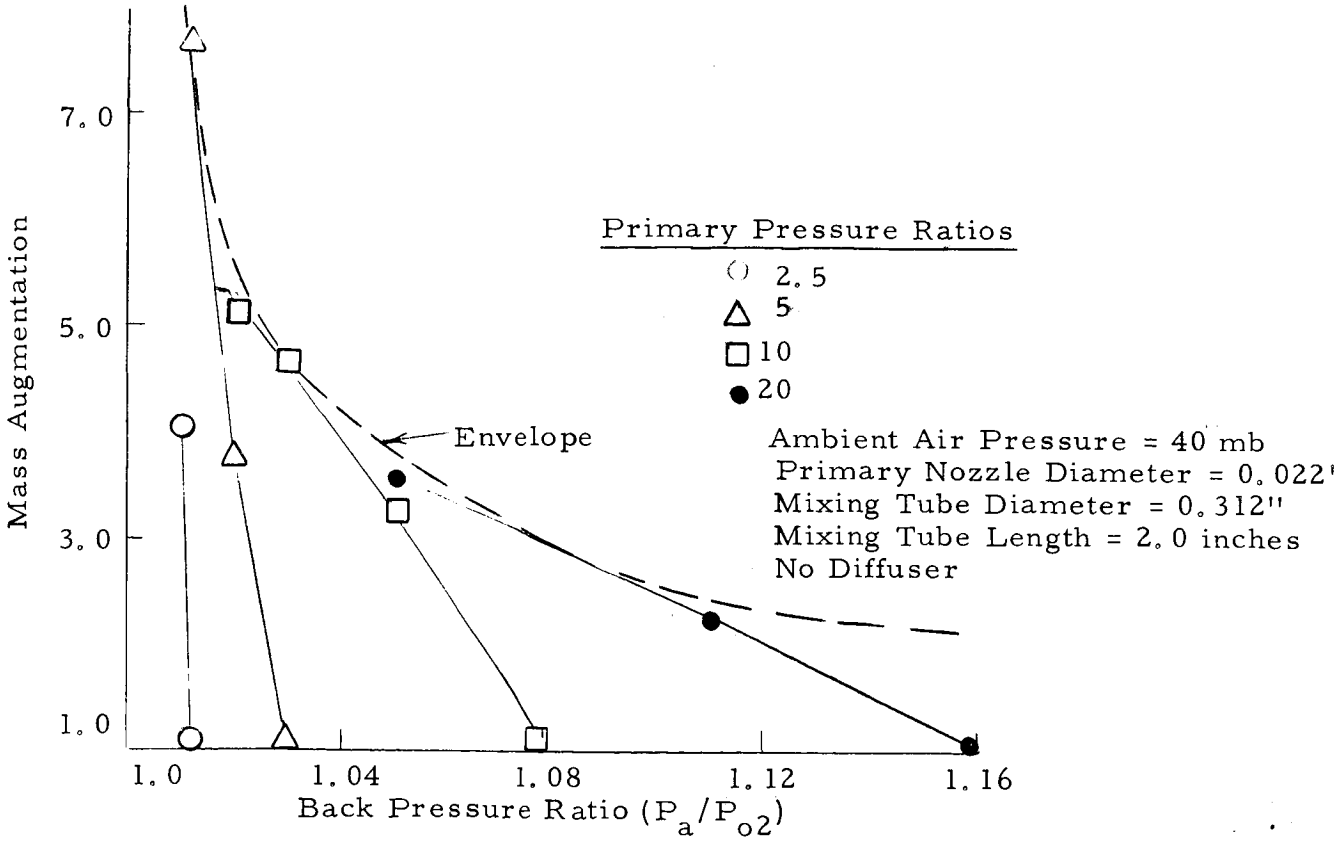


Figure 2-38. Mass Augmentation as a Function of Back Pressure Ratio

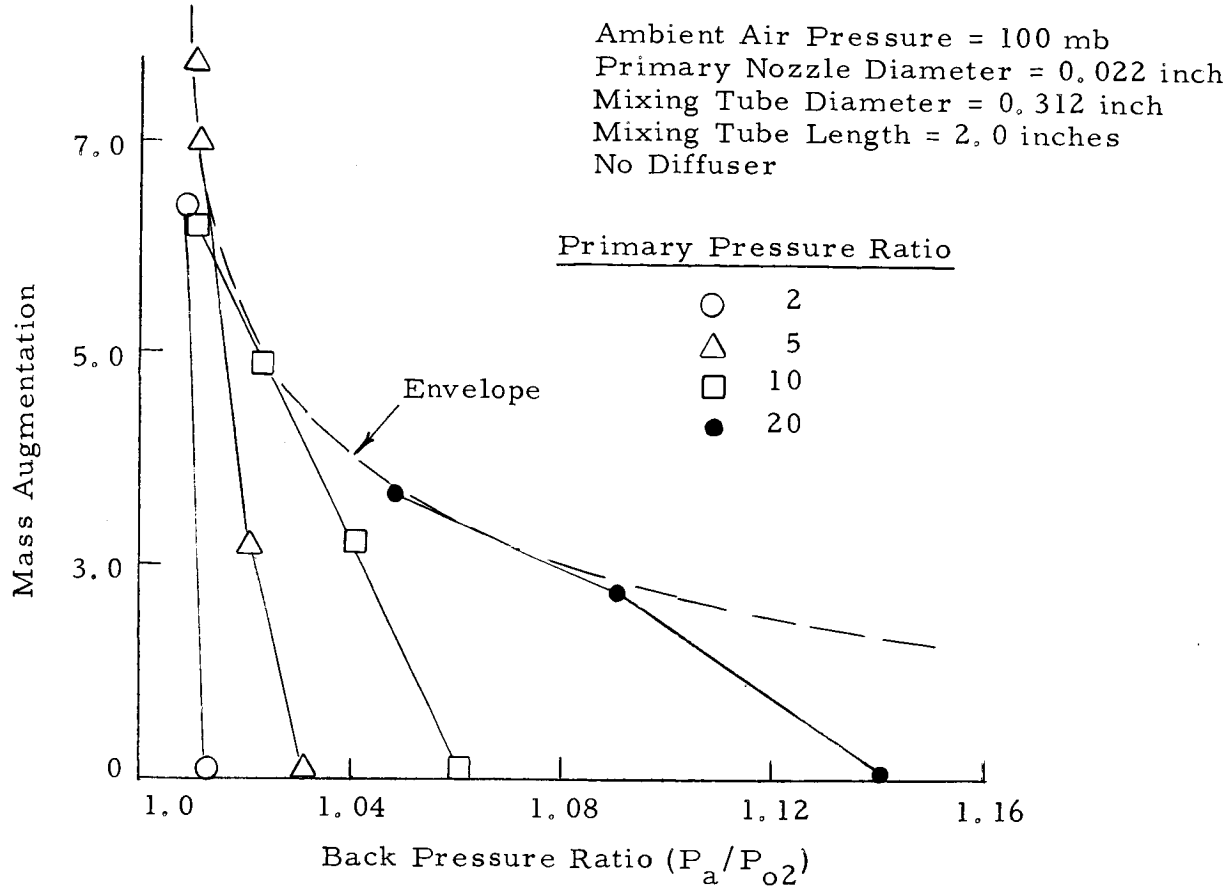


Figure 2-39. Mass Augmentation as a Function of Back Pressure Ratio

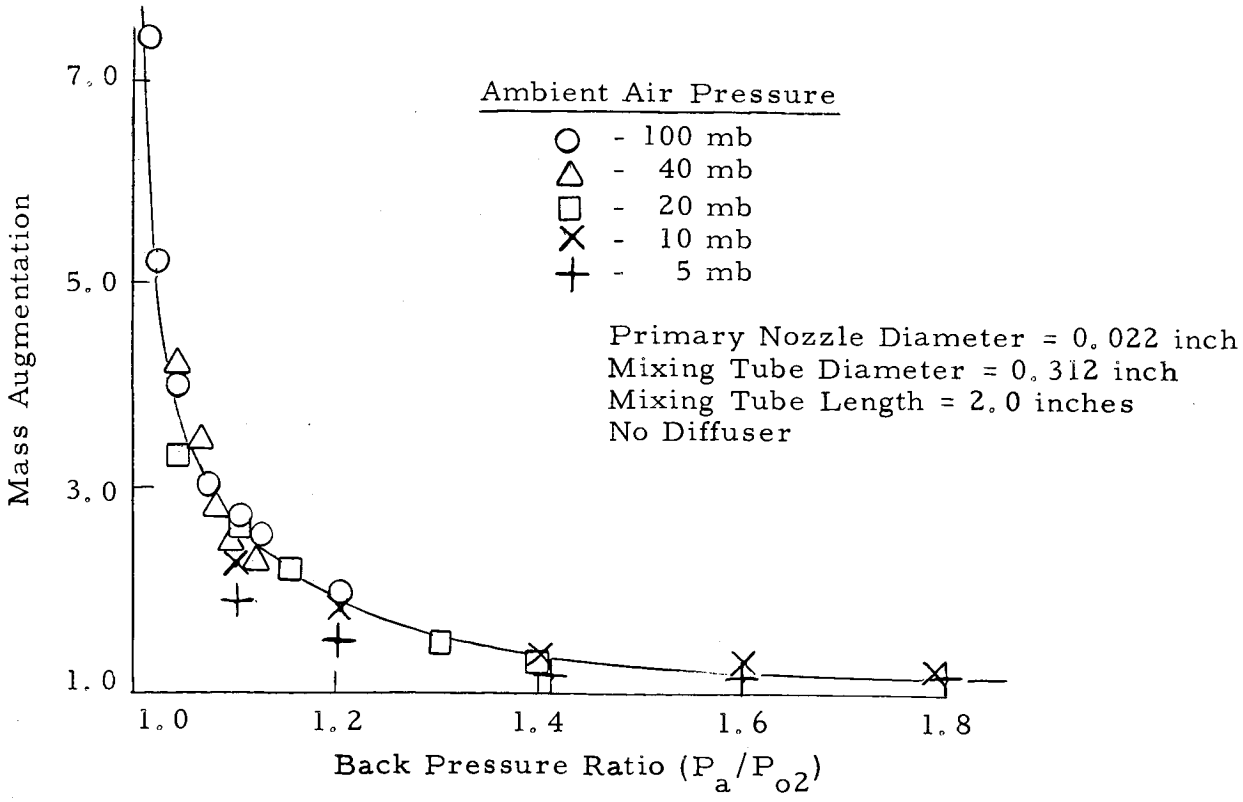


Figure 2-40. Mass Augmentation as a Function of Back Pressure Ratio Obtained with Optimum Primary Pressure Ratios

For these test conditions the mass augmentation is relatively independent of the ambient air pressure.

The above data are also presented with the secondary flow rate as a function of back pressure ratio for constant primary pressure ratios. The wide range of secondary flow rates obtainable with changes in the primary pressure ratio is shown in Figures 2-41 through 2-45.

Thus, it can be seen that increasing primary pressure ratio produces an increasing back pressure ratio, for a given secondary flow rate. However, the efficiency is reduced under these conditions. A minimum primary pressure ratio is, therefore, selected to produce adequate performance over the range of conditions imposed upon the air ejector.

The effect of changing the primary pressure ratio can be offset by changing the primary nozzle area ratio to keep the primary mass flow rate constant. Figure 2-46 presents data with approximately the same mass flow rate for different combinations of primary nozzle area ratio and primary pressure ratio.

7. Effect of a Diffuser on Air Ejector Performance

The efficiency of an air ejector pump can be increased by using a diffuser at the outlet of the mixing tube. Two diffusers were made and tested to determine the effect on the performance of the air ejector when using various mixing tube lengths.

A diffuser with a 7-degree included angle and an outlet diameter twice the inlet diameter was used with the 1-1/2-inch, 2-inch, and 3-inch long mixing tubes. With the 2-inch long by 5/16-inch diameter mixing tube, the diffuser increased the mass augmentation approximately 20 percent at 20 mb ambient air pressure with a 1.15 back pressure ratio. This test was run with a primary nozzle area ratio of 0.008 and a primary pressure ratio of 20. At these same conditions using a 1-1/2-inch long mixing tube, the mass augmentation was increased by about 30 percent.

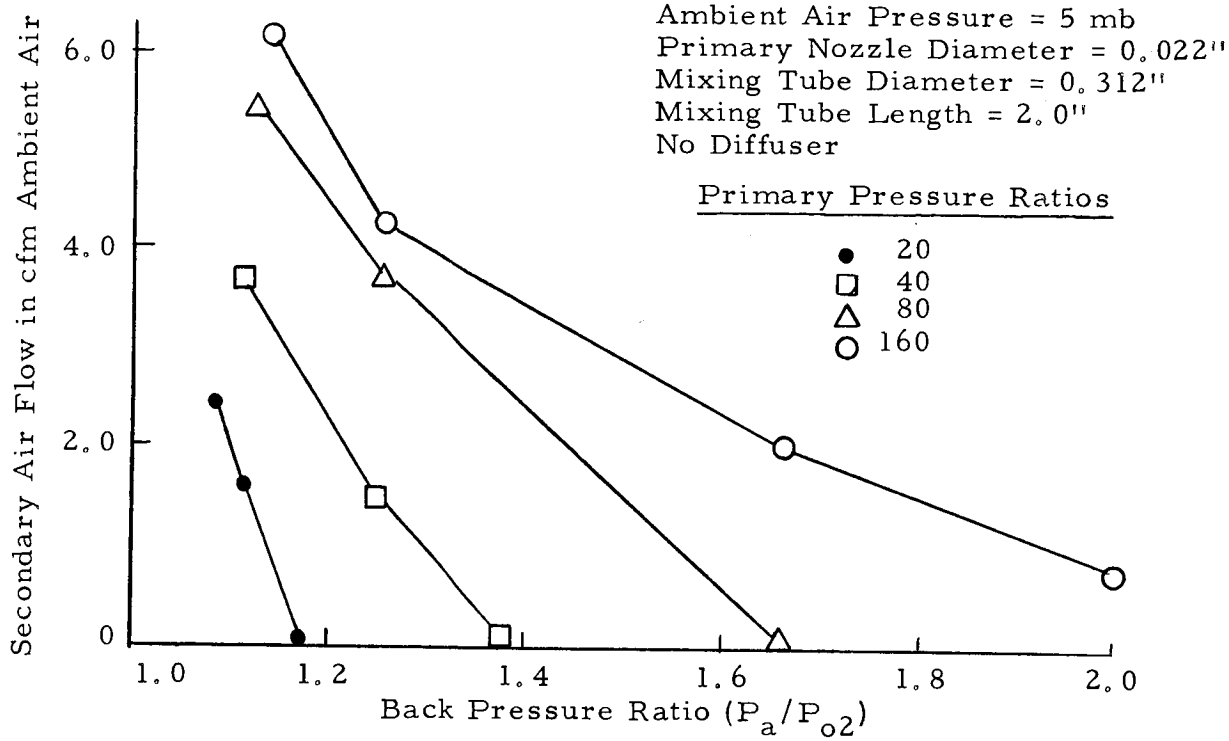


Figure 2-41. Secondary Air Flow Rate as a Function of Back Pressure Ratio

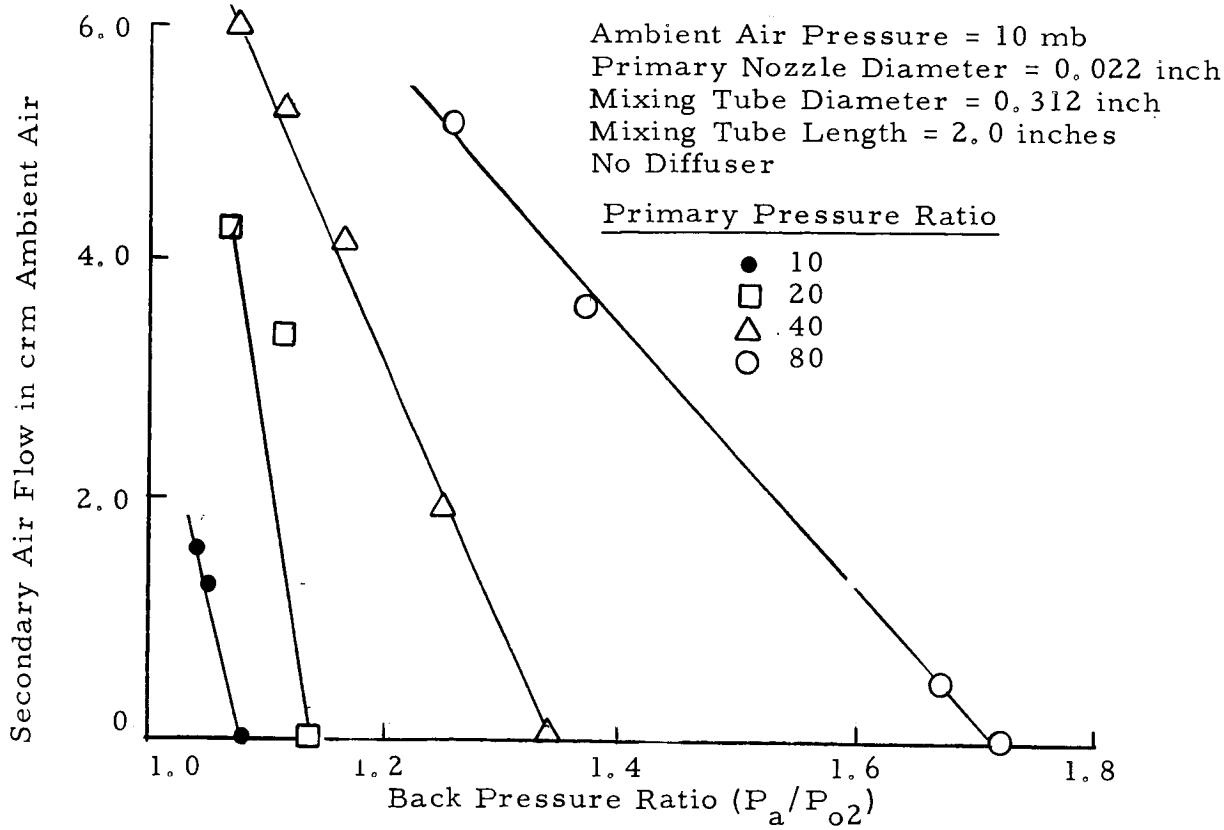


Figure 2-42. Secondary Air Flow Rate as a Function of Back Pressure Ratio

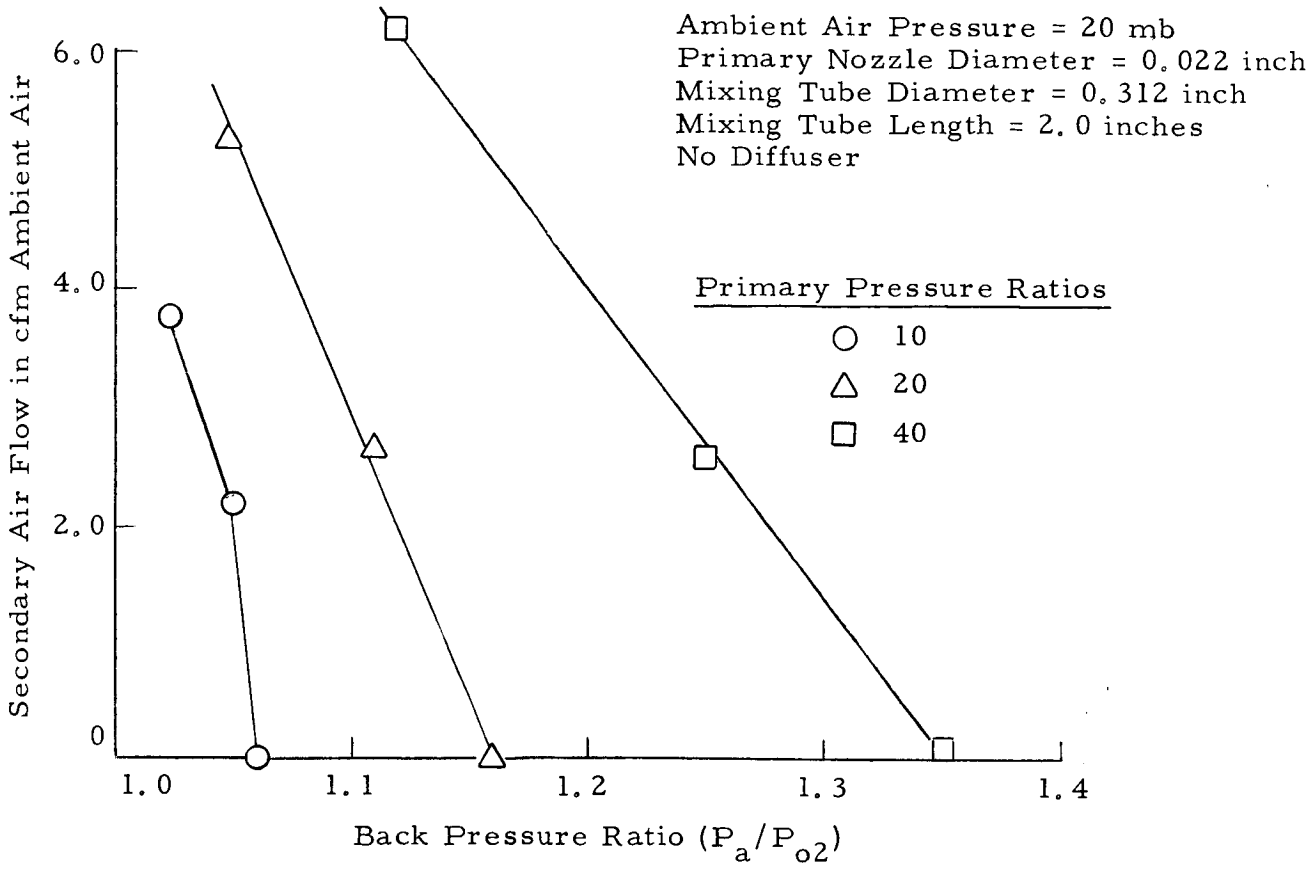


Figure 2-43. Secondary Air Flow Rate as a Function of Back Pressure Ratio

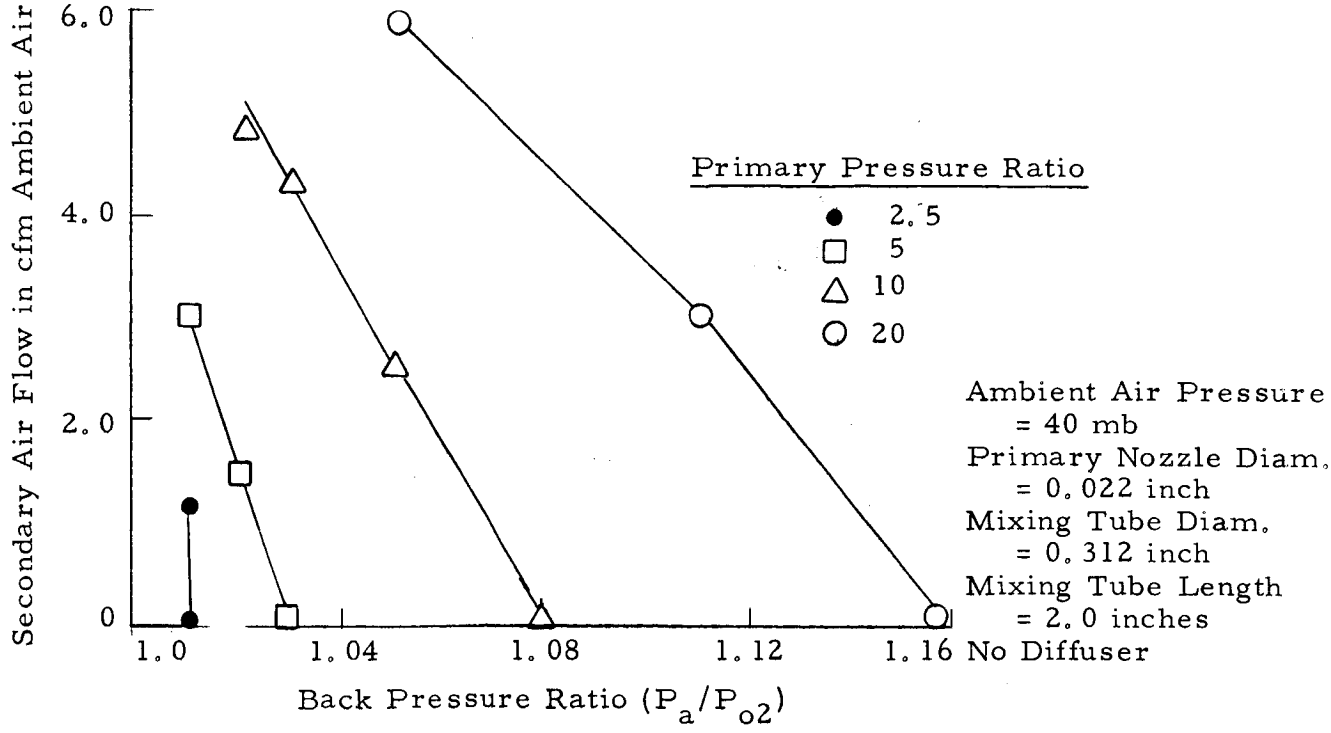


Figure 2-44. Secondary Air Flow Rate as a Function of Back Pressure Ratio

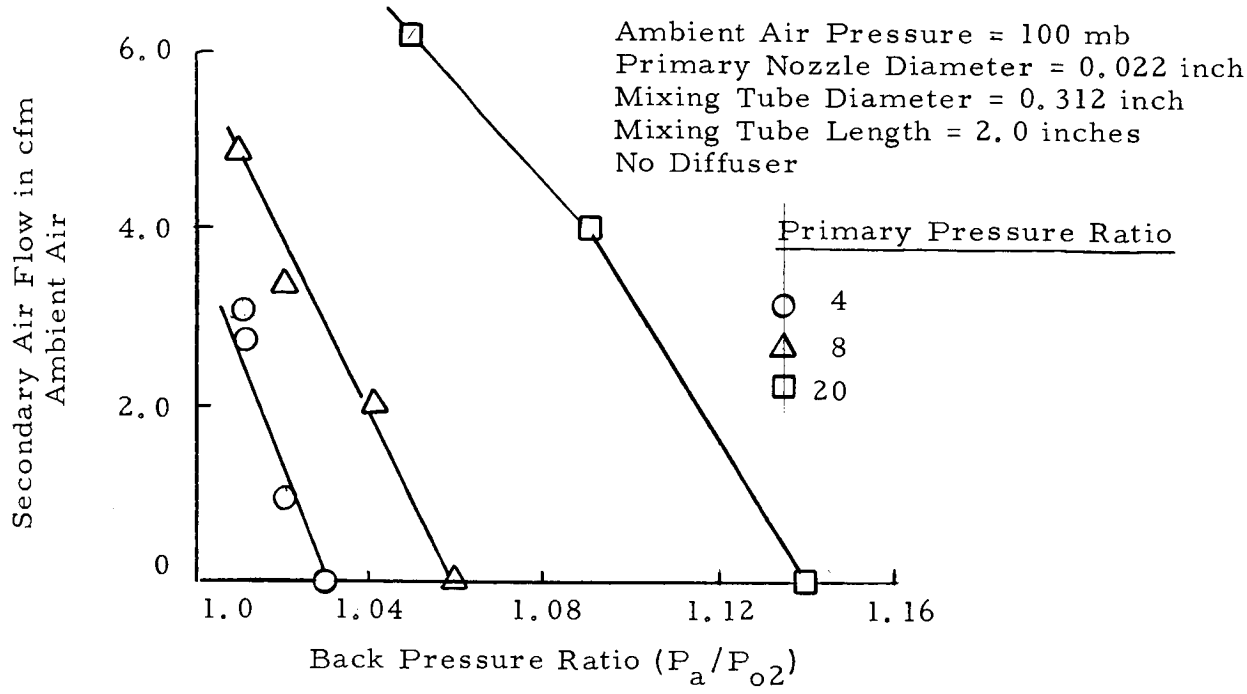


Figure 2-45. Secondary Air Flow Rate as a Function of Back Pressure Ratio

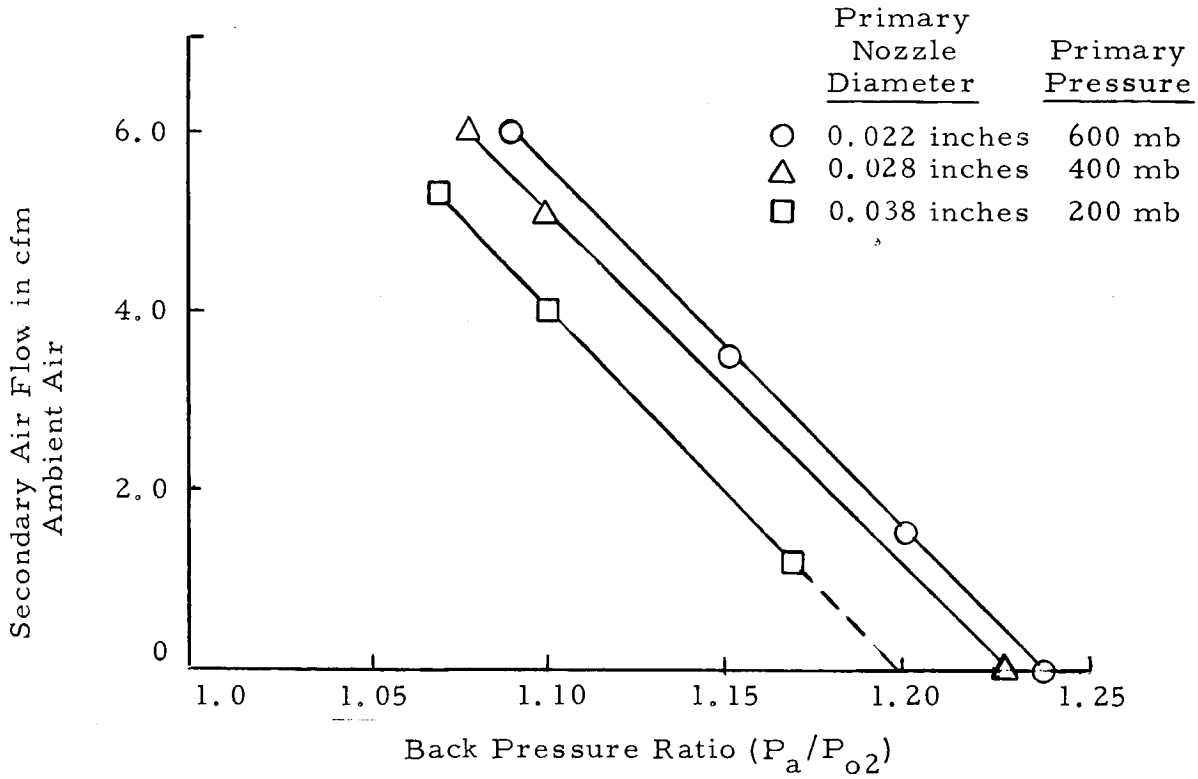


Figure 2-46. Secondary Airflow Rate as a Function of Back Pressure Ratio for Constant Primary Mass Flow Rate

A diffuser with a 14-degree included angle and an outlet diameter three times the inlet diameter was tested under the same conditions. Figures 2-47, 2-48 and 2-49 show that the 14-degree diffuser gives a lesser increase in performance than the 7-degree diffuser.

J. Optimal Sampler Design

It is necessary to know the airflow pressure drop requirements of the pneumatic particle transport system as a function of transport tube size, atmospheric pressure, particle size, shape, and density. These data determine the air ejector performance required for a particular system capable of sampling a given soil. Tests were conducted to determine the pressure drop through the cyclone collector and transport tube at airflow rates sufficient to transport sand particles up to 100 microns in diameter.

The cyclone collector shown in Figure 2-30 was tested to determine the pressure drop over the airflow range of 0.5 to 6 cfm at various ambient air pressures. The pressure drop data is presented in Figure 2-50 and shows that for a given flow rate the pressure drop expressed as a percentage of the ambient air pressure remains reasonably constant. An example is shown in Table 2-6 for a 4-cfm flow rate.

Table 2-6. Pressure Drop through a Cyclone Separator at 4 cfm

Ambient Air Pressure (mb)	Pressure Drop (percent of ambient air pressure)
5	3.4
10	2.8
20	2.1
100	2.2
980	2.7

Mixing Tube Length = 1-1/2 inches

Primary nozzle diameter = .028"
Mixing tube diameter = .312"
Primary pressure = 400 mb
Ambient air pressure = 20 mb

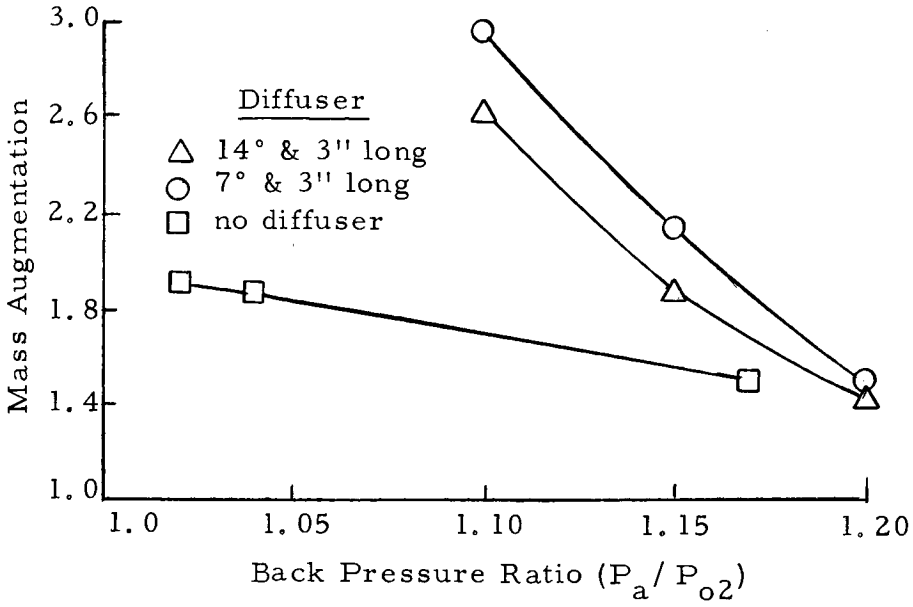


Figure 2-47. Mass Augmentation as a Function of Back Pressure Ratio

Mixing Tube Length = 2 inches

Primary nozzle diameter = .028"
Mixing tube diameter = .312"
Primary pressure = 400 mb
Ambient air pressure = 20 mb

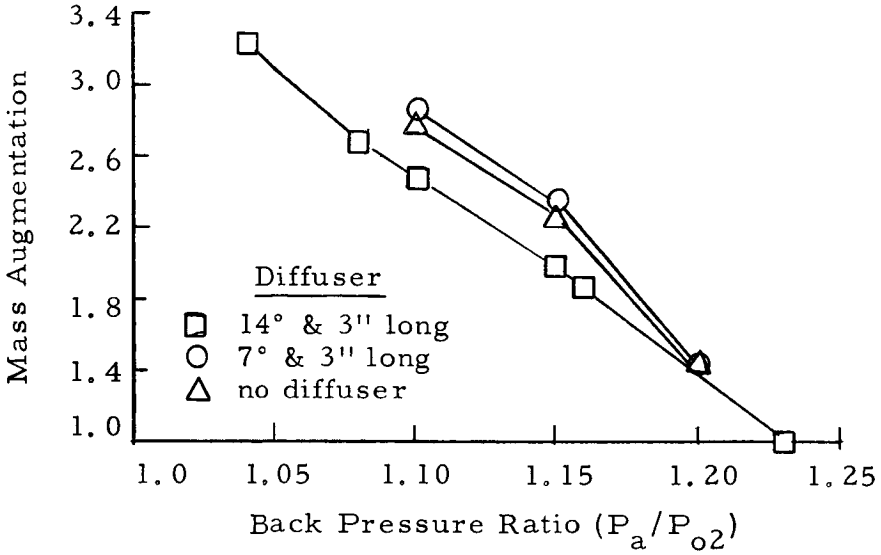


Figure 2-48. Mass Augmentation as a Function of Back Pressure Ratio

Mixing Tube Length = 3 inches

Primary nozzle diameter = .028"
Mixing tube diameter = .312"
Primary pressure = 400 mb
Ambient air pressure = 20 mb

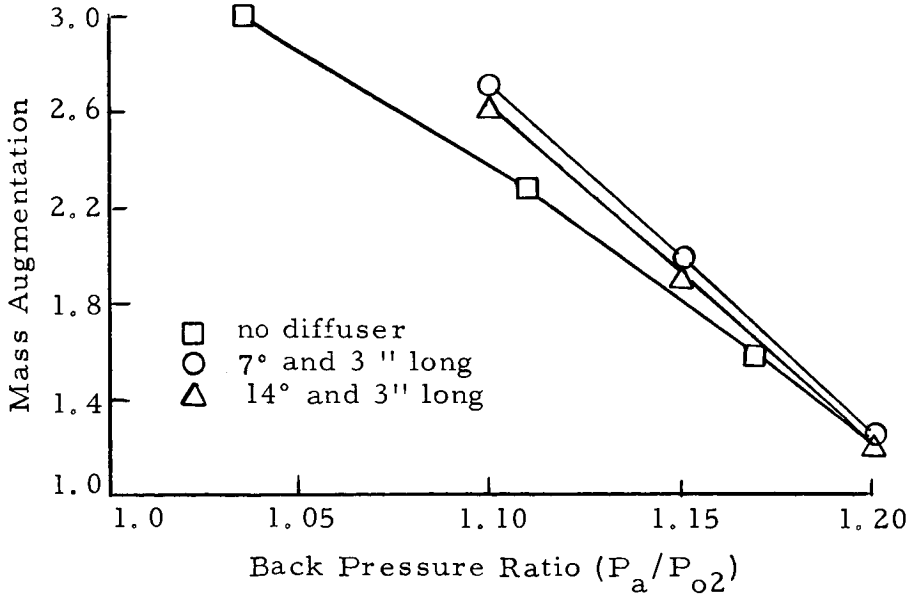


Figure 2-49. Mass Augmentation as a Function of Back Pressure Ratio

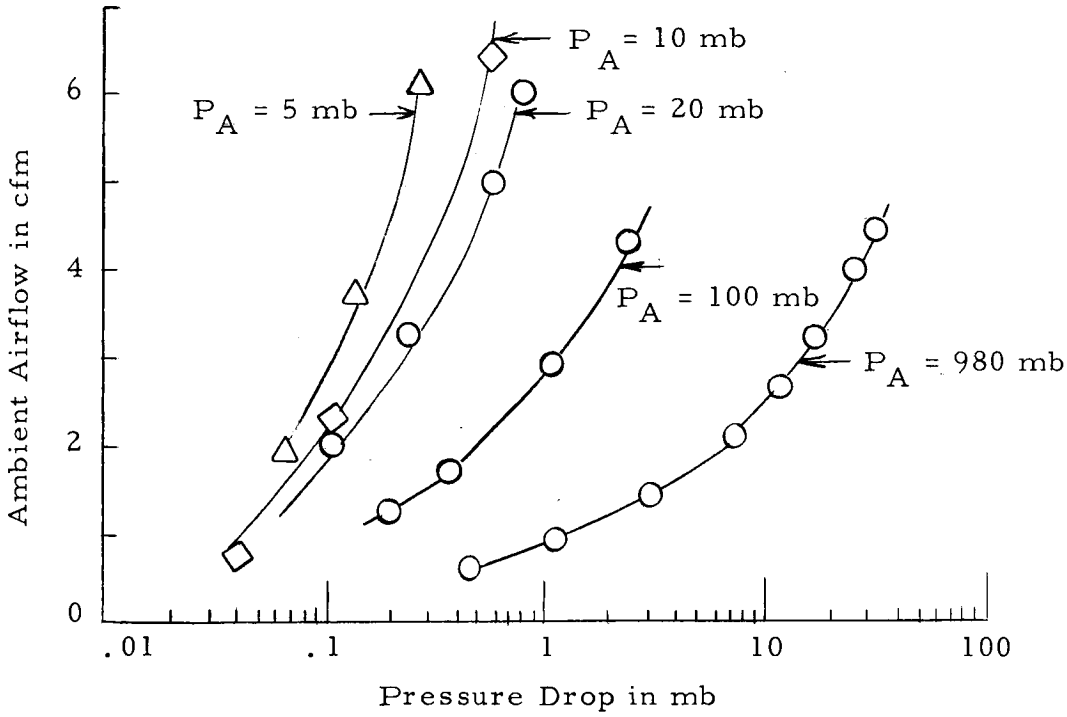


Figure 2-50. Pressure Drop through a Small Cyclone Separator

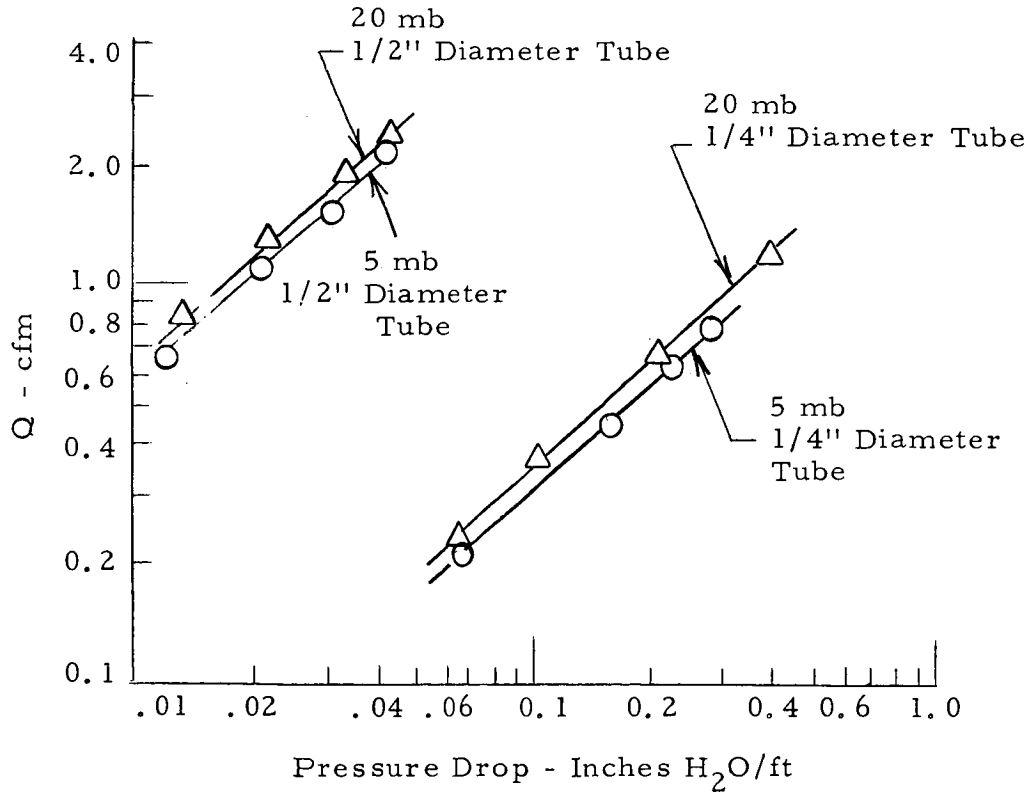


Figure 2-51. Pressure Drop - Flow Rate Relationship for Smooth-Walled Tube

Pressure drops through small diameter smooth-walled tubes were also determined. Two tube diameters were tested: 1/4-inch I.D. and 1/2-inch I.D. vinyl tubes. Tests were conducted with the range of flow rates up to those for complete transport of 100 micron particles. Figure 2-51 presents these data.

We used these data on airflow rate-pressure drop requirements to determine how the tested air ejector would perform from the standpoint of sample collection. The most severe Martian condition would probably be 5 millibars ambient air pressure. From Figure 2-50 and 2-51 it was determined that 10 feet of 1/2-inch I.D. Tube could be used with the cyclone separator for a sampling system. If the air ejector is fixed in geometry and primary pressure, and the secondary air inlet is connected to the cyclone separator, the airflow versus atmospheric pressure relationship of Figure 2-52 would be obtained. Several combinations of primary nozzle diameter and primary air pressure which give approximately the same primary mass flow rate are shown indicating that the primary mass flow rate in a given air ejector has an appreciable effect on the secondary flow rate.

With this system it was possible to transport soil particles even down to 5 millibars ambient air pressure, since approximately 2.5 cfm airflow is required for adequate pneumatic transport. Over the range of 5 millibars to 100 millibars (the extremes of estimated Martian air pressure) the system is completely suitable for pneumatic sampling.

This system was also used to determine the effect of mixing tube diameter on the performance. Figure 2-53 presents the data for mixing tubes of three diameters: 1/4-inch, 5/16-inch, and 3/8-inch. The performance of the 1/4-inch diameter tube is less than the other two at ambient pressures less than 20 millibars, while the 3/8-inch diameter tube performance is less than the 5/16-inch diameter tube in all cases.

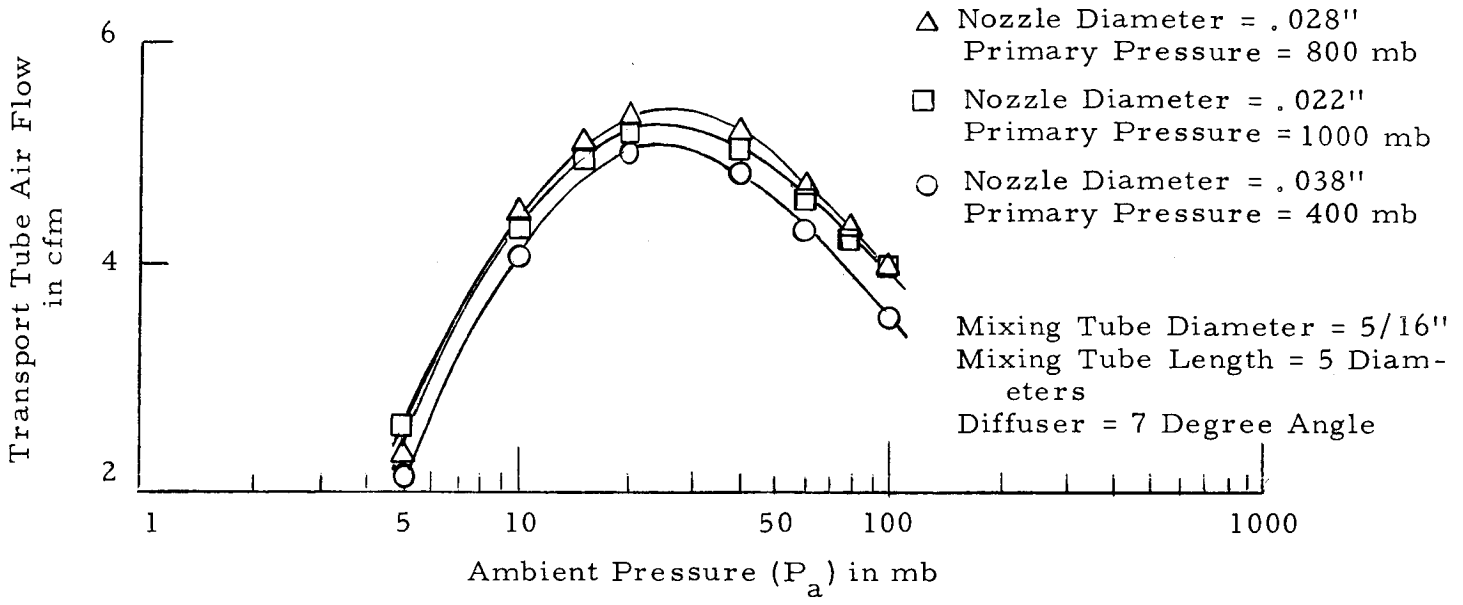


Figure 2-52. Air Flow Through 10 Feet of 1/2-Inch Diameter Tubing versus Ambient Air Pressure

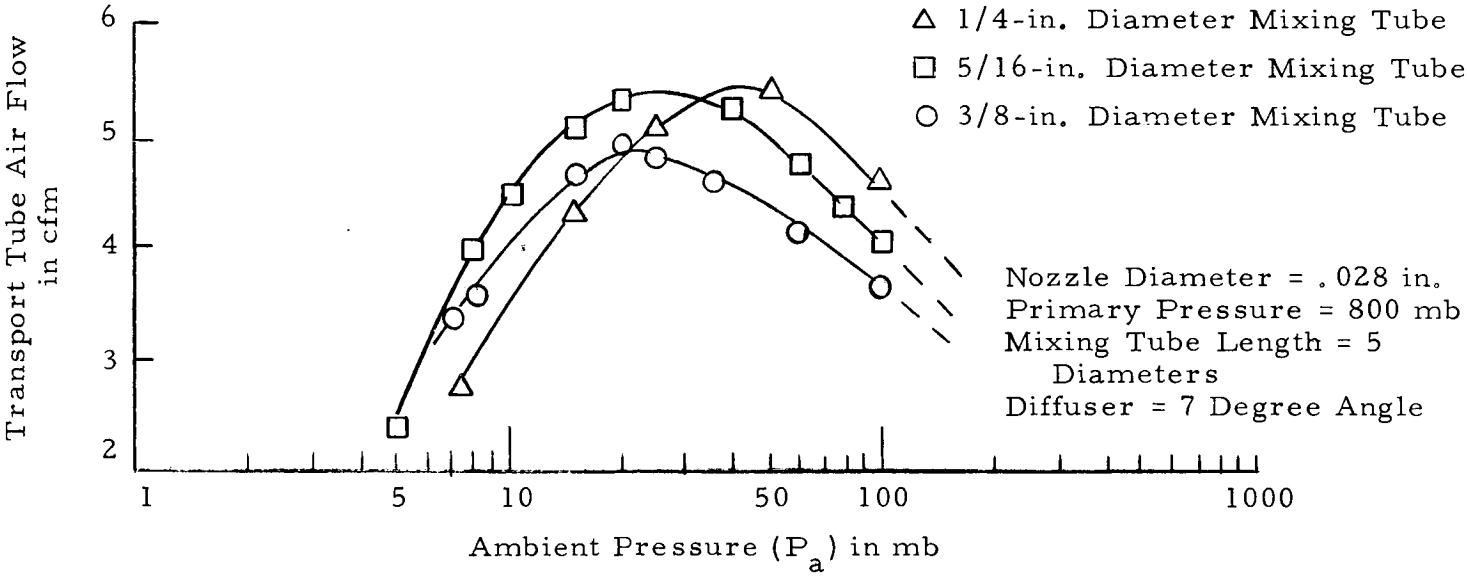


Figure 2-53. Air Flow through 10 Feet of 1/2-Inch Diameter Tubing Versus Ambient Air Pressure

From this data the air ejector selected for the pneumatic transport system would be the following:

Primary Nozzle Diameter	= 0.028 inches
Primary Nozzle Air Pressure	= 800 mb
Mixing Tube Diameter	= 0.312 inches
Mixing Tube Length	= 1.5 inches
Diffuser	= 7° angle, 3.0 inches long

III. BIOLOGICAL SUPPORT STUDIES

A. Introduction

The aims of the biological phase included the following:

1) To develop and apply realistic biological and biochemical laboratory techniques that would be useful in evaluating sampling concepts and devices and processing techniques for exobiological experiments.

2) To carry out the chemical and microbiological analyses on dust and soil samples collected by the pneumatic and mechanical sampling devices being developed in the engineering phase of the program.

3) To develop and improve soil and dust processing techniques that would facilitate life detection in a variety of exobiological experiments.

4) To accumulate a backlog of information on the biological aspects of surface dust sampling and processing and to provide the necessary input to engineers responsible for making decisions about sampling and processing equipment for a Martian lander.

During the course of the program, the engineering phase and the biological support phase were closely coordinated and experiments were always planned cooperatively.

B. Description of Soil Samples Studied

Soil Sample No. 1. Light-brown fine sand; viable count 3×10^5/gm; moisture content 0.3 percent; specific gravity 2.62; organic carbon content 0.07 percent (Minnesota).

Soil Sample No. 5. Light-brown clay loam; viable count 1×10^6/gm; moisture content 1.0 percent; specific gravity 2.57; organic content 1.26 percent (Minnesota).

Soil Sample No. 6. Dark-gray silty clay loam; viable count 4×10^6/gm; moisture content 1.4 percent; specific gravity 2.24; organic carbon content 3.8 percent (Minnesota).

Soil Sample No. 7. Grayish-brown coarse sand and gravel; viable count 3×10^5/gm; moisture content 0.2 percent; specific gravity 2.64; organic carbon content 0.4 percent (Minnesota).

Soil Sample No. 100. Light-brown fine to medium sand; viable count 2×10^4/gm; moisture content 0.3 percent; specific gravity 2.64 (Arizona).

Soil Sample No. 74-2. Light-grayish-brown fine sand; viable count 2×10^6/gm; moisture content 0.2 percent; specific gravity 2.61; organic carbon content 0.35 percent (California).

Soil Sample No. 75-2. Light-grayish-brown well-graded sand; viable count 3×10^6/gm; moisture content 0.4 percent; specific gravity 2.56; organic carbon content 2.0 percent (California).

Limonite Sample. Hydrous iron oxide, $Fe_2O_3 \cdot H_2O$; viable count of surface layer 1×10^5/gm; specific gravity 3.31 (Minnesota).

Lake Mead sand. White, fine to medium sand; viable count < 1000/gm; specific gravity 2.62 (California).

Naranjo farm soil sample. Reddish-brown clay; viable count 7×10^6/gm; specific gravity 2.56; organic carbon content 1/2 percent (Honduras).

Trojas farm soil sample. Grayish-brown clay; viable count 6.5×10^6/gm; specific gravity 2.56; organic carbon content 2 percent (Honduras).

Sample No. 20. Rock dust and fragments, and organic debris collected from the surface of the Plotteville limestone formation; viable count 5×10^6/gm (Minnesota).

Soil Sample No. 21. Light-grayish-brown sand loam; viable count 3.5×10^6/gm; specific gravity 2.45 (Minnesota).

Soil Sample No. 22. Light-brown loamy sand; viable count 3×10^6/gm (Minnesota).

Volcanic ash sample. Gray volcanic ash with cinders and subhedral crystals ranging from clay and silt size to coarse sand size. Mt. Irazu, Costa Rica.

In addition, twenty small samples of 1 gm (more or less) were collected with the pneumatic sampler in the field. No chemical analyses or viable counts were made. Most of these samples were studied by the phosphatase test.

C. Description of Analytical Techniques

During the course of this program a number of chemical and biological procedures were employed to evaluate sampling devices, sampling concepts, and processing techniques. Some of these procedures were "classical", i.e., they are normally employed in agricultural and public health laboratories for soil analyses. Other procedures were "adapted", i.e., they are normally employed in food and water microbiology but were used in this program for soil and dust analyses. Still other procedures were especially designed for this program and are based essentially on the more prominent "life detection" experiments being developed for NASA's exobiology program.

It should be emphasized that no attempt was made to develop life detection experiments per se nor to evaluate any life detection instruments or concepts. The prime purpose of this study was to evaluate sampling and processing concepts. To this end, a variety of techniques were chosen which yielded suitable and reproducible data and which provided critical criteria for improving sampling and processing. In some cases classical techniques and exobiology "life detection"

techniques were inadequate for the purposes of this program and were discarded. In other cases they were altered to suit the needs of this program. Any inferences about the relative merit of one "life detection" technique over another are valid only insofar as they refer to the objectives of this program, and are not intended as criticisms of these techniques as exobiological experiments.

For the purpose of uniformity, a brief description of each analytical procedure is presented below:

1. Moisture Analysis

A tared sample (≈100 g) was dried in an oven at 100°C to constant weight (overnight) and weighed again. The weight loss was calculated as percent moisture.

2. Specific Gravity

The picnometer method of measuring the amount of water (23°C) displaced by a known weight of dry soil was employed:

$$\text{Sp gr} = \frac{Wo}{Wo + (Wa - Wb)}$$

where Wo = weight of soil
Wa = weight of picnometer and water
Wb = weight of picnometer and water and soil.

This technique described the specific gravity of the individual soil particle.

3. Bulk Density

Tared metal cylinders of known volume were filled with oven dried soils and weighed. The bulk density was the ratio of total weight to total volume and is a measure of the compactness of the sample (i.e., the ratio of solid particles to interstitial spaces).

4. Organic Matter

The Walkley-Black wet oxidation method was used. A weighed soil sample was diluted in concentrated H_2SO_4 and was oxidized by a known volume and concentration of chromic acid. The solution was then back-titrated with a reducing substance, and the exact amount of chromic acid employed in the oxidation was calculated. Internal controls, using natural and sterilized soil to which known concentrations of organic matter (glucose) were added, checked on the reliability of this method.

5. Particle Size Analysis

A simple National Bureau of Standards sieving test (shaking a 100 gm sample on a Tyler shaker for 15 min) was employed to provide a rough classification of particle size. The sieve sizes employed were

#20 = 841 microns
#70 = 210 microns
#120 = 125 microns
#170 = 88 microns
#230 = 62 microns
#325 = 44 microns.

For clays, whose particles were too small to be analyzed by sieving, a hydrometer analysis of a hexametaphosphate suspension was employed. This involved making hydrometer readings at elapsed time of 2 min, 5 min, 15 min, 30 min, 60 min, and 250 min, and calculating

the percentage (P) of soil remaining in suspension at a given depth according to the following formula:

$$P = \left[\frac{100,000}{W} \times \frac{G}{G - G_1}\right] (R - G_1)$$

where G = specific gravity of the soil (by picnometer)

G_1 = specific gravity of metaphosphate solution

R = temperature corrected hydrometer reading

W = weight of soil.

The size of the particles which were contributing to the hydrometer reading during each observation were calculated as follows:

$$D = K\sqrt{\frac{L}{T}}$$

where D = diameter of particle

K = specific gravity (corrected for temperature)

L = depth at which hydrometer is measuring

T = time in minutes.

These calculations permitted the determination of percentage of particles of a given size in a collodial clay sample.

6. Microscopy

Several milligrams of dry soil were sprinkled as a thin film on a microscopic slide and were observed at 50x and 100x magnification. Photomicrographs were made with a Unitron camera with polaroid attachments.

7. Plate Counts and Growth Curves

Routine plating of aqueous soil suspensions were made on tryptone-glucose-yeast extract agar (TGE), mycophil agar, and soil extract agar[1]. All plates were incubated at 35°C for 24 hours, followed by 6 days at room temperature. Representative colonies were picked and gram stained for microscopic characterization. Growth curves were prepared by taking aliquots from nutrient broth soil suspensions at hourly intervals and plating the appropriate dilutions on TGE agar.

8. Viability Detection by pH Change

One gram of soil was inoculated into 99 ml of a 2 percent aqueous glucose solution and incubated at 35°C. Readings were made hourly for 6 hours and, after a 24-hour period, with a Beckman Model 72 pH meter.

9. Viability Detection by Dye Reduction

Quantities of soil ranging from 0.25 gm to 1.0 gm were inoculated into 9 ml of cysteine-glucose-phosphate saline containing 1 ml of tetrazalium chloride (0.1 percent) and incubated at 35°C. At 30-min intervals the tubes were visually observed for formazan production. Some trials employed Thunberg tubes in which the reduction of methylene blue in glucose broth was measured.

[1] Soil extract agar was made by steeping equal volumes of soil with boiling distilled water, filtering through cheesecloth and coarse filter paper and adding 1.5 percent agar before autoclaving. The respective soils used for the extracts were the ones which were to be plated on that medium.

10. Viability Detection by ATP Assay

One ml of the supernatant from a 10-percent soil suspension was assayed for ATP by the bioluminescence method using firefly lantern extract (Chase 1960, Methods of biochemical analysis, vol. 8, Interscience, N. Y., p. 61-117). Samples were assayed untreated, after ultrasonication and after perchloric acid treatment. Intensity of bioluminescence was measured in a Beckman DK-2 recording spectrophotometer.

11. Viability Detection by Microrespiration

Oxygen uptake and CO_2 evolution were measured by standard Warburg manometric techniques. One ml of a 10-percent soil suspension was added to 2 ml of a complex soil extract medium (Science 138: 114-121, 1962) containing 0.01 M glucose and 0.001 M sodium formate. Observations were made at hourly intervals for 8 hours.

12. Viability Detection by Phosphatase Assay

Fifty ml of substrate (0.50 mg/ml) disodium phenyl phosphate in 0.0745 M borate buffer, pH 9.6) were inoculated with 1 gram of soil and incubated at 35°. At hourly intervals, 5 ml aliquots were removed and treated with 5 drops of BQC reagent (40 mg of 2.6 dibromoquinone-chloroimide in 10 ml ethanol). After 5 min of color development, the sample was extracted with 4 ml butanol. The butanol layer was decanted and color intensity was measured in a colorimeter at 650 millimicrons. A standard curve was prepared relating light transmission at 650 millimicrons to known concentrations of phenol and BQC. Phosphatase activity in the soil was expressed as micrograms phenol liberated per unit weight of soil in the time period specified.

13. Viability Detection by Turbidimetry Changes

This technique was studied in detail, and the various stages in the development of a suitable standard procedure are described below. Essentially the final technique adopted involved inoculating 1 gm of soil into 50 ml of nutrient broth (DIFCO), shaking 25 times to disperse the soil, centrifuging at 10^3 g for 10 min, decanting the supernate into a sterile 100 ml bottle, and shaking on a reciprocating platform shaker at 35°C. At hourly intervals 5 ml aliquots were aspetically removed and their turbidity measured in a B&L Spectronic 20, standardized to 100 percent transmittance with sterile nutrient broth.

D. Evaluation of Microbial Assay Techniques and Detection Criteria Employed

In the future, further work might be performed to evaluate sampling and processing concepts for exobiological research. It is, therefore, appropriate to provide a subjective evaluation in this report of the analytical techniques we used. Some of the techniques were time-consuming and did not yield sufficient critical information to serve as valid criteria for sampling and processing evaluation. On the other hand, certain analytical techniques were extremely useful in this study and could be employed as a battery of tests for further work in this area.

The physical and chemical characterizations (i.e., moisture, specific gravity, bulk density, organic analysis, and particle size distribution) should be performed on each original soil to be studied. At the very least these characterizations may describe some quantitative parameters that can be correlated with ease of sample acquisition. Moreover, the organic content of the soil is a fundamental parameter in several life detection instruments, and any sampling or processing technique which influences organic composition might be acceptable or rejectable on that basis alone. During the course of this study program

it was found that the moisture content and specific gravity of soils sampled by classical and pneumatic means respectively did not vary, whereas organic content and particle size distribution were often affected. Bulk density was not shown to be a meaningful property in comparing sampling techniques.

Microscopy and standard plating procedures were extremely useful criteria in evaluating sampling and processing. In fact, the standard plate count on TGE agar was chosen as the ultimate criterion for comparing the different procedures studied. It should be recognized, however, that this process is probably the most time-consuming and expensive of all the analytical techniques. Valid results from plating are not available for nearly a week; consequently, plating serves mainly as a backup to more rapid but less quantitative tests. Table 3-1 shows the precision obtainable with the plate count procedure when known mixtures of sterilized and natural soils were used to provide samples with known quantitative differences and qualitative similarities.

Table 3-1. Plate Count of Mixtures of Sterilized and Natural Soil

(Soil No. 1)

Sample	Theoretical (viables/gm)	Actual (viables/gm)
Natural		1×10^6
20% Sterilized - 80% Natural	8×10^5	5.5×10^5
40% Sterilized - 60% Natural	6×10^5	8×10^5
60% Sterilized - 40% Natural	4×10^5	2.6×10^5
80% Sterilized - 20% Natural	2×10^5	1.6×10^5

The use of pH change, Eh change (dye reduction) and ATP assay for rapid detection of microorganisms in soils sampled and/or processed by different techniques was only partially successful. These techniques were not sufficiently sensitive, and in many cases biological reactions were confounded by the presence of nonviable soil components. Although these three criteria might have application for this type of study, they were not as useful as the phosphatase tests, turbidimetry assays, and microrespiration studies on the same soils.

The phosphatase test was quite successful for the rapid semi-quantitative evaluation of microbial content of samples (see Table 3-2, page 3-16). It is significant to note that whereas standard plate counts were obtained at the earliest only after 48 hours, the phosphatase results, which correlated well with viable counts, were available within 8 hours. It was evident that variations in microbial types significantly influenced the phosphatase assay. Nevertheless, the speed with which viable organisms could be detected by the phosphatase test permitted rapid and fairly accurate comparison of samples—especially if we were interested in comparing different types of samplers for use on the same soil (i.e., the same qualitative population).

Microrespiration studies were also quite useful and provided rapid results which correlated fairly well with total initial population. Figure 3-1 illustrates the clear-cut results obtained using this technique within 5-6 hours after sample acquisition. The major limitation of this technique is the amount of equipment necessary for Warburg analyses, and the consequent reduction in the number of samples which can be analyzed simultaneously.

When we initially tried to use turbidimetric techniques to evaluate the microbial content of soils, the results were very unsatisfactory (see Figure 3-2). It was difficult to ascertain the time when growth was initiated; the results were not clear cut; and there was no satisfactory correlation between turbidimetric results and actual viable count.

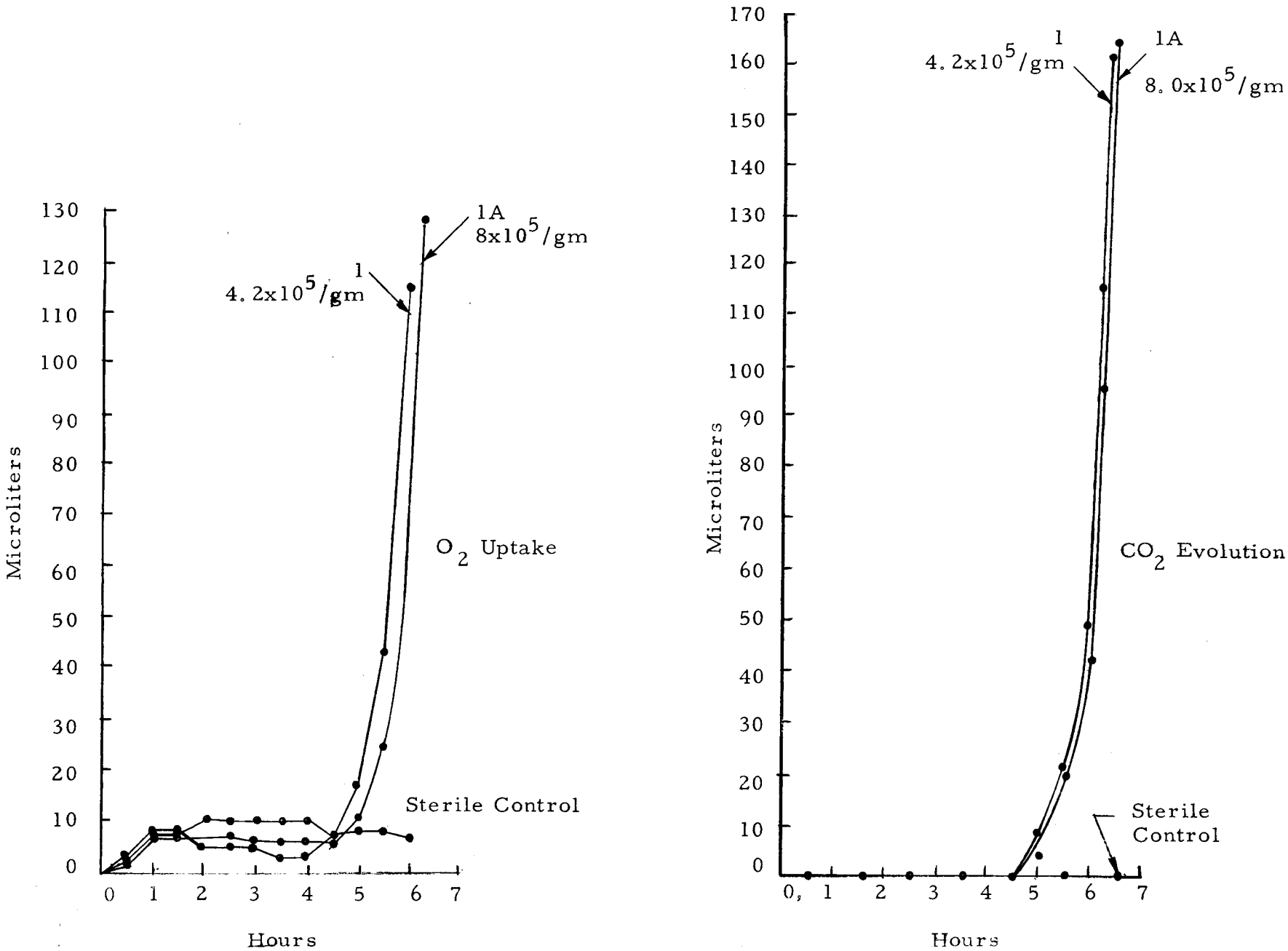


Figure 3-1. Detection of Viable Organisms by Microrespiration Techniques with Soil Sample 1

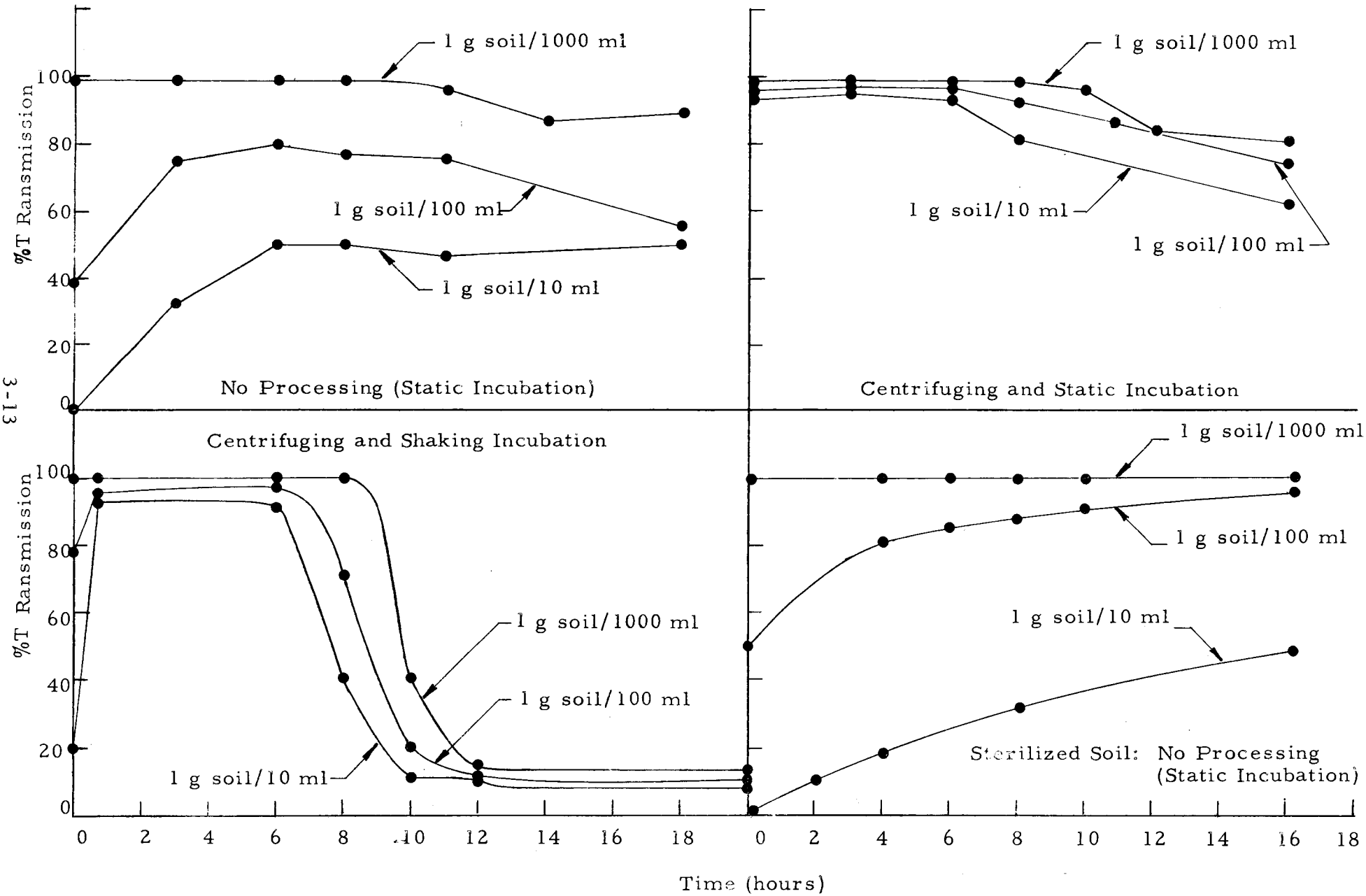


Figure 3-2. Effect of Various Pre-Incubation Processes on Turbidimetric Detection of Viable Microorganisms in Soil (Soil 1).

Essentially we were encountering the same frustrations as everyone who had studied this technique before:

1) Interference from the natural turbidity of the soil.

2) Continual changes in the assay tubes wherein the contribution from sedimenting soil particles was diminishing simultaneously with an increasing contribution from microbial multiplication.

3) Pellicle formation by aerobic strains which indicated presence of viable entities, but which did little to augment the turbidity of the sample.

4) Mutual antagonism by soil organisms which permitted growth initiation but no continued development.

To counteract these drawbacks, the detection technique was modified by introducing two changes:

1) The soil samples were mixed with the broth, shaken by hand, and then centrifuged (1000g x 10 min) to remove most of the suspended particles; and

2) The supernatant broth was removed to another sterile tube and incubated under continuous agitation.

Figure 3-2 illustrates the advantages gained by these processes, and Figure 3-3 summarizes the growth detection results using our modified turbidimetric technique on six different soils.

To evaluate our analytical techniques objectively, a series of comparative experiments was performed on soil samples which were qualitatively identical and which contained graded concentrations of viable microorganisms. To prepare these soils, a quantity of sand (Soil No. 1) was autoclaved, and the sterile sand was mixed in various proportions with the 'nutrient' original soil. Each mixture was analyzed by plate count, the phosphatase test, pH change, dye reduction, specific gravity test, and the modified turbidimetry test described above. The results of these trials are shown in Table 3-2 and Figure 3-4.

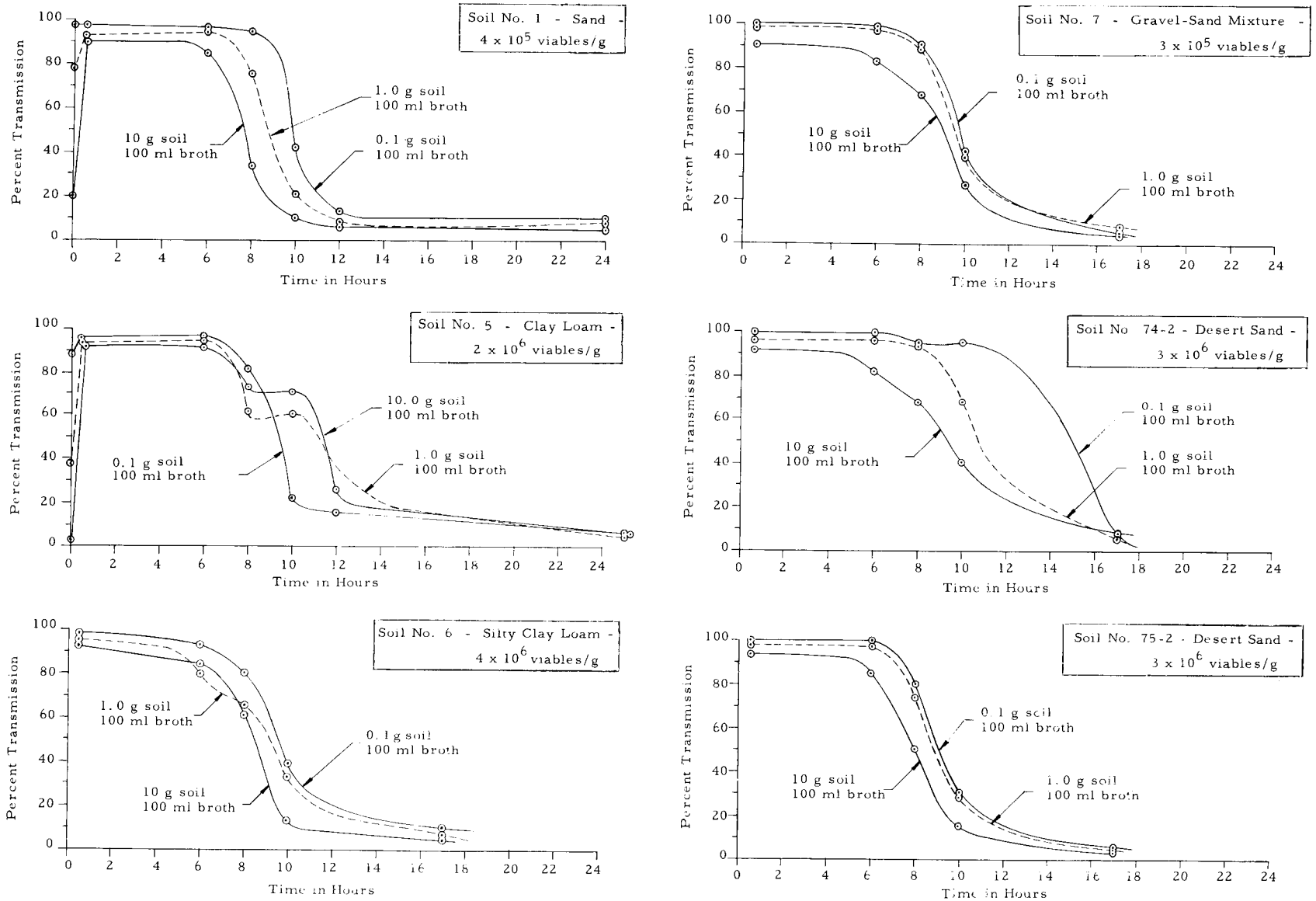
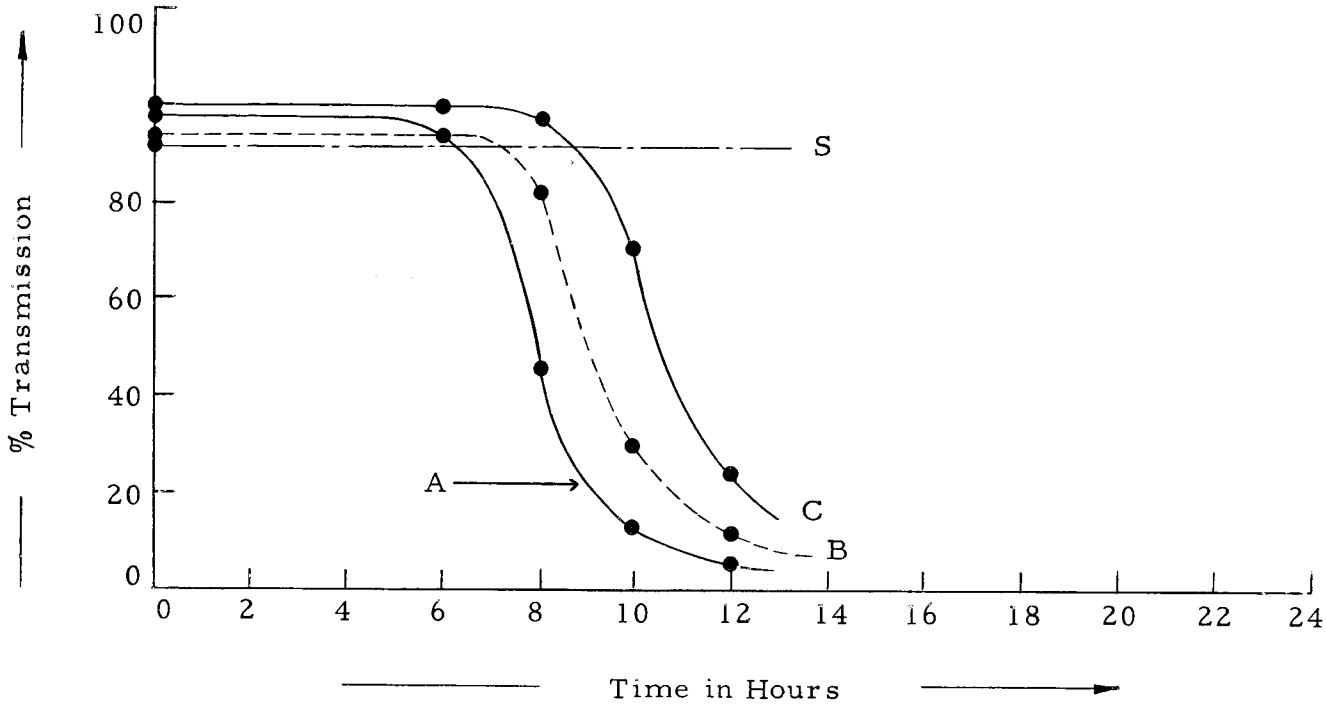


Figure 3-3. Growth Detection in 6 Soils by Turbidimetric Technique

Table 3-2. Relationship of Plate Counts and Biochemical and Physical Properties of Soils with Known Quantitative Differences and Qualitative Similarities

(Soil Sample No. 1: Light Brown Fine Sand)

Test	Natural	1 Part Natural 9 Parts Sterilized	1 Part Natural 99 Parts Sterilized	Sterilized
Plate Count Viables/gm (T.G.E.)	7×10^5	2.7×10^4	4.4×10^3	<10
Phosphatase (Micrograms phenol produced)	2.6 in 8 hr 5.4 in 24 hr	0.7 in 8 hr 1.2 in 24 hr	0.7 in 8 hr 0.7 in 24 hr	0 in 8 hr 0 in 24 hr
pH Change in 22 hr	Initial = 6.6 Final = 5.6	Initial = 6.5 Final = 5.0	Initial = 6.4 Final = 5.8	Initial = 6.4 Final = 6.4
Dye Reduction	No change 5 hr	No change 5 hr	No change 5 hr	No change 5 hr
Specific Gravity	2.62	2.62	2.60	2.62
ATP	No response in 8 hr; equivocal response after ultrasonicating and shaking for 8 hr	---	---	---



Soil No. 1 - 7 x 10^5 viables per gram

Samples shown above were centrifuged in the nutrient broth at 10^3 g.

All test samples were 1.0 gm of soil in 100 ml of broth.

Sample A - Natural soil

Sample B - 1 to 9 mixture of natural soil to sterilized soil

Sample C - 1 to 99 mixture of natural soil to sterilized soil

Sample S - Sterilized Soil

Figure 3-4. Growth Detection by the Turbidimetric Method (Constant Shaking) on Soils with Known Quantitative Differences and Qualitative Similarities

E. Evaluation of Sampling Techniques and Concepts

A major function of the biological laboratory during the tenure of this contract was microbial and chemical evaluation of pneumatic sampling and transportation concepts. To this end, we attempted to assay parallel soil samples obtained from different terrestrial environments by classical techniques and by pneumatic techniques. We were primarily concerned with any bias that might be introduced by sampling methods. If a certain device or process exerted a deleterious effect on the organic matter or microbial population of a soil sample, or if it significantly altered our ability to detect "life" in that sample, it would be necessary to recognize that phenomenon. Subsequently, the biological bias might become an important consideration in the ultimate choice of sampling devices and/or approaches.

Nine soils were employed for these comparative studies. Lots 1, 5, 6, and 7 were soil "blocks" (2 ft x 2 ft x 6 inches) which were cut out of the frozen ground, placed in clean pans, and returned to the laboratory where they were thawed and dried. Pneumatic samples were taken with the collection device described on page 2-5 . Classical samples were taken with a spatula. Soil lots 74-2 and 75-2 were bulk sand which was layered into a shallow pan for pneumatic and classical sampling as described above. Sample 20, a limestone bedding plane with algae and lichen overgrowth, was sampled in the field with the pneumatic sampler described on page 2-32 and by scraping the surface with a sharp knifeblade. Soils 21 and 22 were also sampled in the field using the same pneumatic sampler used on soil 20. The classical samples from these soils were taken by shovel (3-5 lb sample through a 4-6 inch depth); by probe (a 1-inch diameter core removed to a depth of 6-8 inches); by auger (6-8 inch depth); and by scraper (a 6-inch knifeblade scraped over several square feet to a depth of 1/2 inch).

In essence, we found that the classical samples differed considerably from the pneumatic samples insofar as physical parameters and appearance were concerned. However, any biological bias favored the pneumatic approach.

Figure 3-5 illustrates one of the fundamental differences between the two types of sampling techniques. Whereas classical samples contain a heterogeneous distribution of particles of different sizes, pneumatic samples characteristically contained mostly small particles and presented a more uniform distribution. This bias is quantitatively summarized by the sieve analysis data given in Table 3-3.

Table 3-3. Respective Particle Size Distribution of Samples Collected by Classical and Pneumatic Means.

(Results expressed as % by weight associated with respective size fraction)

		>2000μ	841-2000μ	210-841μ	125-210μ	88-125μ	62-88μ	44-62μ	<44μ
1	Classical	0	0.3	41.6	42.6	8.5	4.2	1.6	1.1
	Pneumatic	0	0	2.0	26.0	24.0	22.0	11.0	14.0
7	Classical	48.4	14.0	30.8	7.6	0.8	0.7	0.6	2.0
	Pneumatic	0	0	54.5	17.0	5.1	7.2	4.4	12.5
74-2	Classical	0	2.9	23.7	34.7	17.6	12.1	5.2	3.2
	Pneumatic	0	0	7.3	28.9	24.5	19.6	10.3	8.9
75-2	Classical	0	29.8	44.3	9.7	4.1	3.4	2.8	5.2
	Pneumatic	0	0	6.1	14.0	12.0	15.3	16.2	35.9

(Results expressed as % by weight smaller than a given size)

	31μ	20.6μ	12.6μ	9.1μ	6.5μ	3.0μ	1.4μ
5* Classical	61.0	48.5	36.0	29.8	26.6	20.6	15.7
6* Classical	64.5	52.2	39.9	30.9	27.6	20.0	10.7

*50-60 grams of sample needed for hydrometer analysis; study model of parameter sampler was unable to acquire this amount of sample.

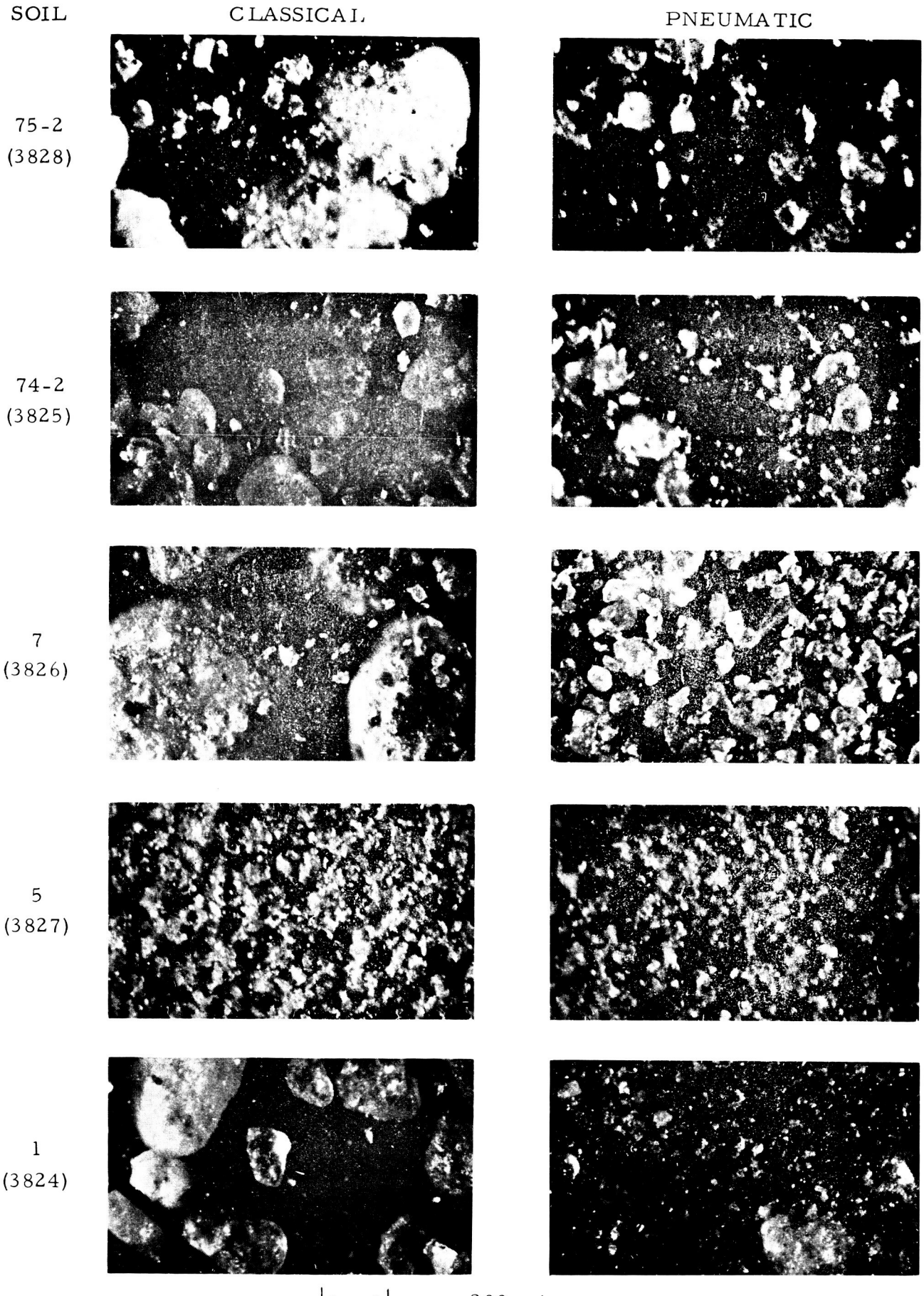


Figure 3-5. Photomicrographs of Soil Samples Obtained by Classical (spatula) and Pneumatic Techniques

Very rarely were any particles >800 microns collected by the pneumatic technique, even from gravels in which half of the particles by weight were greater than 2 mm.

Despite this profound shift in particle size distribution, the pneumatic technique did not discriminate against particles on the basis of their specific gravity. Table 3-4 presents comparative data on the moisture content, specific gravity, and organic carbon content of samples acquired classically and pneumatically.

Table 3-4. Influence of Sampling Technique on Organic Matter Fraction, Moisture Content, and Specific Gravity

Soil		Classical	Pneumatic
1	Organic	0.07%	0.59%
	Moisture	0.30	0.24
	Specific Gravity	2.62	2.60
5	Organic	1.26	2.37
	Moisture	1.0	--
	Specific Gravity	2.57	2.46
6	Organic	3.86	--
	Moisture	1.4	--
	Specific Gravity	2.24	2.28
7	Organic	0.42	0.29
	Moisture	0.20	0.20
	Specific Gravity	2.64	2.60
74-2	Organic	0.34	0.41
	Moisture	0.20	--
	Specific Gravity	2.61	2.62
75-2	Organic	2.09	3.54
	Moisture	0.45	--
	Specific Gravity	2.56	2.29

It is evident that the specific gravities of both types of samples were similar and that any change in the organic content was usually toward the higher side in the pneumatic sample. This suggests that the organic matter in soils is probably associated with the smaller particles in that soil. Furthermore, it indicates that life-detection experiments based on organic analysis would be aided rather than impaired by pneumatic sampling techniques.

This positive bias was also reflected in several other life-detection experiments run on the same soils. Table 3-5 summarizes the viable counts obtained in parallel soil samples. Although most of the differences were negligible and usually within the experimental error inherent in soil plating methodology, it is interesting to note that in almost every case the pneumatic sample had a higher count than its replicate acquired by spatula. On the basis of these data no claims should be made about an "improved sampling technique". However, at the very least, recognition should be given to the fact that the pneumatic sampling concept is as compatible with life detection as are the time-tried classical methods. Furthermore, there is no significant qualitative bias associated with pneumatic sampling. The predominant soil organisms (Table 3-6) determined in samples scooped from the soil by augers, shovels, probes and scrapers were recovered in the same relative distribution by the pneumatic sampler.

A rapid life detection technique (i.e., phosphatase readout in 1 hour) also revealed the applicability of pneumatic sampling. From Table 3-7, it can be seen that in all cases but one, the pneumatic sample provided a more significant readout than parallel classical samples from the same soil. In the one case where the readout was not as intense, the difference was slight.

Table 3-5. Summary of Viable Counts in Soils Sampled by Classical and Pneumatic Techniques

Soil No.	Soil Type	Classical Sample			Pneumatic Sample		
		TGE	Soil Extract	Mycophil	TGE	Soil Extract	Mycophil
1	Fine Sand	$4x10^5$	$2x10^5$	$3x10^4$	$8x10^5$	$1x10^6$	$3x10^3$
5	Clay Loam	$2x10^6$	$1x10^6$	$2x10^3$	$8x10^6$	$1x10^6$	$8x10^4$
6	Silty Clay Loam	$4x10^6$	$8x10^6$	$1x10^4$	$5x10^7$	$2x10^7$	$1x10^5$
7	Coarse Sand and Gravel	$3x10^5$	$2x10^5$	100	$2x10^6$	$2x10^6$	$2x10^3$
74-2	California Desert Sand	$3x10^6$	$2x10^6$	$9x10^3$	$2x10^6$	$3x10^6$	$1x10^4$
75-2	California Desert Sand	$3x10^6$	$3x10^6$	$7x10^3$	$6x10^6$	$8x10^6$	$2x10^4$
21	Sandy Loam	$4x10^{6*}$			$7x10^6$		
22	Loamy Sand	$4x10^{6*}$			$6x10^6$		
20	Limestone Rock	$3x10^6$			$2x10^6$		

*Average of auger, probe, shovel and scraper; for details see Table 3-9.

Table 3-6. Qualitative Distribution of Organisms in Relation to Sampling Technique

(Soil No. 20, 21, and 22)

Classical	Pneumatic
(Auger, Shovel, Probe, and Scraper)	
In order of predominance	In order of predominance
1. Actinomycetes	1. Actinomycetes
2. Gram-positive rods	2. Gram-positive rods
3. Gram-negative rods	3. Gram-negative rods
4. Molds and yeasts	4. Molds and yeasts

TECHNIQUE

Soil samples plated onTryptone-Glucose-Extract agar. Colonies were picked from plates showing 5 to 25 organisms between 24 to 48 hours old. Surface colonies only were picked; Gram method of staining. Colonies that could be identified without staining were done so, i.e., actinomycetes. Organisms grouped into four major classifications: actinomycetes, molds and yeasts, Gram-positive, and Gram-negative.

Table 3-7. Summary of Phosphatase Readouts on Soils Sampled by Classical and Pneumatic Techniques

Soil Number	μgms Phenol/hr/gm sample	
	Classical	Pneumatic
1	< 1.0	1.5
5	< 1.0	6.4
6	6.8	> 20.0
7	< 1.0	2.0
74-2	6.0	9.6
75-2	20.0	> 20.0
20	11.6*	10.0
21	1.6*	1.9
22	5.7	15.0

*Average of scraper, auger, probe and shovel; for details see Table 3-10.

Biodetection by pH change was not enhanced by pneumatic sampling. Even though the results obtained by measuring ΔpH were equivocal at best, it was evident that the pneumatic sampler might be incompatible with life detection by this technique. In most cases (see Table 3-8) the initial pH observed after mixing the soil with broth was considerably higher in the pneumatic sample. This indicates that the alkaline components of soil are probably associated with the smaller soil particles (cf., organic carbon, Table 3-4) and that the large particles which make up the bulk of classical samples are more or less inert. However, before any decision about compatibility of sampling technique-detection technique can be made, a larger variety of soils should be evaluated.

A series of experiments were performed in which several classical techniques were compared to each other, and to a pneumatic sample taken from the same soil. The classical samples were subdivided by sieving into three classified subsamples based on particle size, and each subsample was analyzed for total viable count, phosphatase readout, and speed of response in a turbidimetric experiment. The results of these trials are presented in Tables 3-9 and 3-10 and Figure 3-6.

Table 3-8. Influence of Sampling Method on Initial pH and Δ pH of Soil Samples

	Classical		Pneumatic	
	Initial	Δ pH (22 hr)	Initial	Δ pH (22 hr)
1	6.4	5.6	6.5	6.5
5	6.8	6.0	8.4	6.7
74-2	7.3	7.4	8.0	7.6
75-2	8.4	7.6	8.9	7.2
6	7.1	6.1		
7	8.0		8.1	

Table 3-9. Relationship of Sampling Technique to Quantitative Viable Count

(Soil No. 21)

Particle Size	Viable Count Microorganisms/gram				
	Shovel	Probe	Auger	Scraper	Pneumatic
Unfractionated	2.5×10^6	4.5×10^6	3.0×10^6	5.5×10^6	7.3×10^6
< 44 microns	1.0×10^7	1.0×10^7	1.2×10^7	1.3×10^7	
44 to 210 microns	3.0×10^6	2.5×10^6	1.5×10^6	2.6×10^6	
> 210 microns	2.6×10^5	3.7×10^5	3.6×10^5	3.2×10^5	

(Soil No. 22)

Unfractionated	3.2×10^6	3.0×10^6	8.0×10^6	3.0×10^6	5.7×10^6
< 44 microns	1.1×10^7	5.0×10^6	5.0×10^6	6.0×10^6	
44 to 210 microns	---	4.0×10^6	4.0×10^6	5.0×10^6	
>210 microns	3.0×10^5	1.5×10^5	6.0×10^5	7.0×10^5	

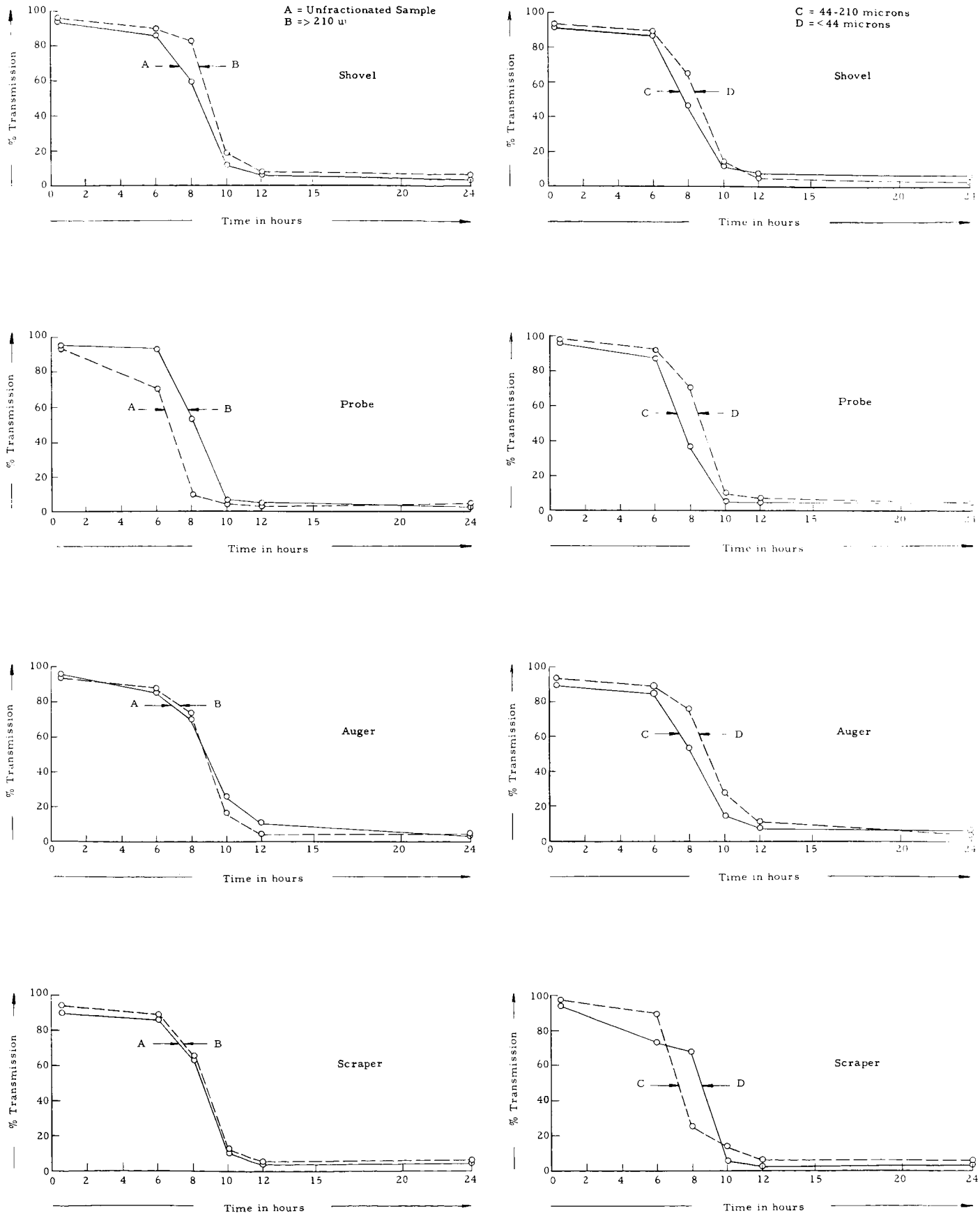


Figure 3-6. Relationship of Sampling Technique to Growth Detection by Turbidimetric Methods (Soil #21 = Loamy Sand)

Table 3-9 suggests the reason for the positive biological bias demonstrated by pneumatic sampling. There appears to be a definite correlation between particle size and viable count/gm. (This relationship is examined in greater detail in Section G of this report.) The viable organisms are associated with all particles, but there is a one or two order of magnitude difference between the count per gram in the $>$210 micron fraction and the $<$44 micron fraction. Since it had been shown earlier that the pneumatic sampler discriminates against the large particles and acquires mainly small particles, the count in the pneumatic sampler should be higher. It is also evident that there is little difference in count among the samples obtained respectively by the four classical techniques, suggesting that for these soils, at least, depth of sampling was not a significant factor in influencing viable count.

The phosphatase readouts in Table 3-10 refer to the identical samples described in Table 3-9.

Nonetheless, the inferences gained from these data differ somewhat from those of the total count data. It is apparent that particle size alone was not the overwhelming factor controlling the phosphatase content. Similarly, there appears to have been a considerable difference among the methods of classical sampling insofar as they were reflected in phosphatase readout. Thus, the scraper sample provided the lowest readout for soil 21 but provided the highest readout for soil 22. Furthermore, the auger and the probe provided similar samples from soil 22 but dissimilar samples from soil 21. It appears that we were dealing with phosphatase sources other than microbial. Visual inspection of the samples showed a marked variation in plant debris. This debris contained phosphatase which would not show up in the plate counts but which contributed to the readouts in Table 3-10. It is possible that in soil 21 the phosphatase-rich strata were deep in the soil and not at the surface; thus, the scraper sample had less phosphatase than the others. On the other hand, in soil 22 there was a

Table 3-10. Relationship of Sampling Technique to Soil Phosphatase Readout

Particle Size	Incubation Time (hours)	Micrograms Phenol/gram Shovel	Probe	Auger	Scraper	Pneumatic
		(Soil 21)				
Unfractionated	1	1.5	1.9	1.6	1.4	1.9
	2	2.2	5.4	3.2	2.4	3.4
	4	3.7	12.0	6.8	3.4	15.0
	6	8.0	14.2	11.6	6.4	>18.0
	24	14.2	>18.0	>18.0	16.7	>18.0
< 44 microns	1	1.5	2.4	1.3	1.4	
	2	2.6	5.2	2.0	2.5	
	4	5.2	10.6	3.4	3.6	
	6	10.0	14.8	7.6	4.6	
	24	17.4	>18.0	14.2	6.4	
44 to 210 microns	1	1.6	2.9	1.6	1.5	
	2	2.9	11.0	2.9	2.2	
	4	5.8	17.4	7.6	3.4	
	6	10.0	>18.0	12.7	8.4	
	24	>18.0	>18.0	>18.0	16.7	
> 210 microns	1	1.2	1.5	0.8	0.5	
	2	1.6	3.1	1.5	1.5	
	4	2.2	8.8	1.7	1.5	
	6	3.2	12.0	2.5	1.8	
	24	11.6	>18.0	6.0	4.0	
		(Soil 22)				
Unfractionated	1	3.2	1.0	0.6	>18.0	15.0
	2	5.4	1.3	1.7	>18.0	>18.0
	4	11.0	1.4	1.5	>18.0	>18.0
	6	16.6	1.6	1.6	>18.0	>18.0
	24	>18.0	4.4	4.6	>18.0	>18.0
<44 microns	1	3.2	1.4	1.1	>18.0	
	2	8.4	1.5	1.7	>18.0	
	4	13.0	1.9	2.2	>18.0	
	6	> 18.0	2.9	3.6	>18.0	
	24	> 18.0	12.0	14.2	>18.0	

Table 3-10. (Continued)

Particle Size	Incubation Time (hours)	Micrograms Phenol/gram Shovel	Probe	Auger	Scraper	Pneumatic
		(Soil 22 Continued)				
44 to 210 microns	1	1.6	1.1	0.5	>18.0	
	2	1.9	1.4	1.1	>18.0	
	4	3.9	1.5	1.4	>18.0	
	6	7.2	1.9	1.4	>18.0	
	24	>18.0	4.6	2.2	>18.0	
>210 microns	1	5.2	0.6	0.7	>18.0	
	2	8.4	1.0	1.3	>18.0	
	4	13.0	1.3	1.4	>18.0	
	6	>18.0	1.5	1.4	>18.0	
	24	>18.0	2.4	1.7	>18.0	

macroscopic concentration of vegetative matter at the surface; thus, both the scraper sample and pneumatic sample yielded more rapid and higher phosphatase readouts than did the others.

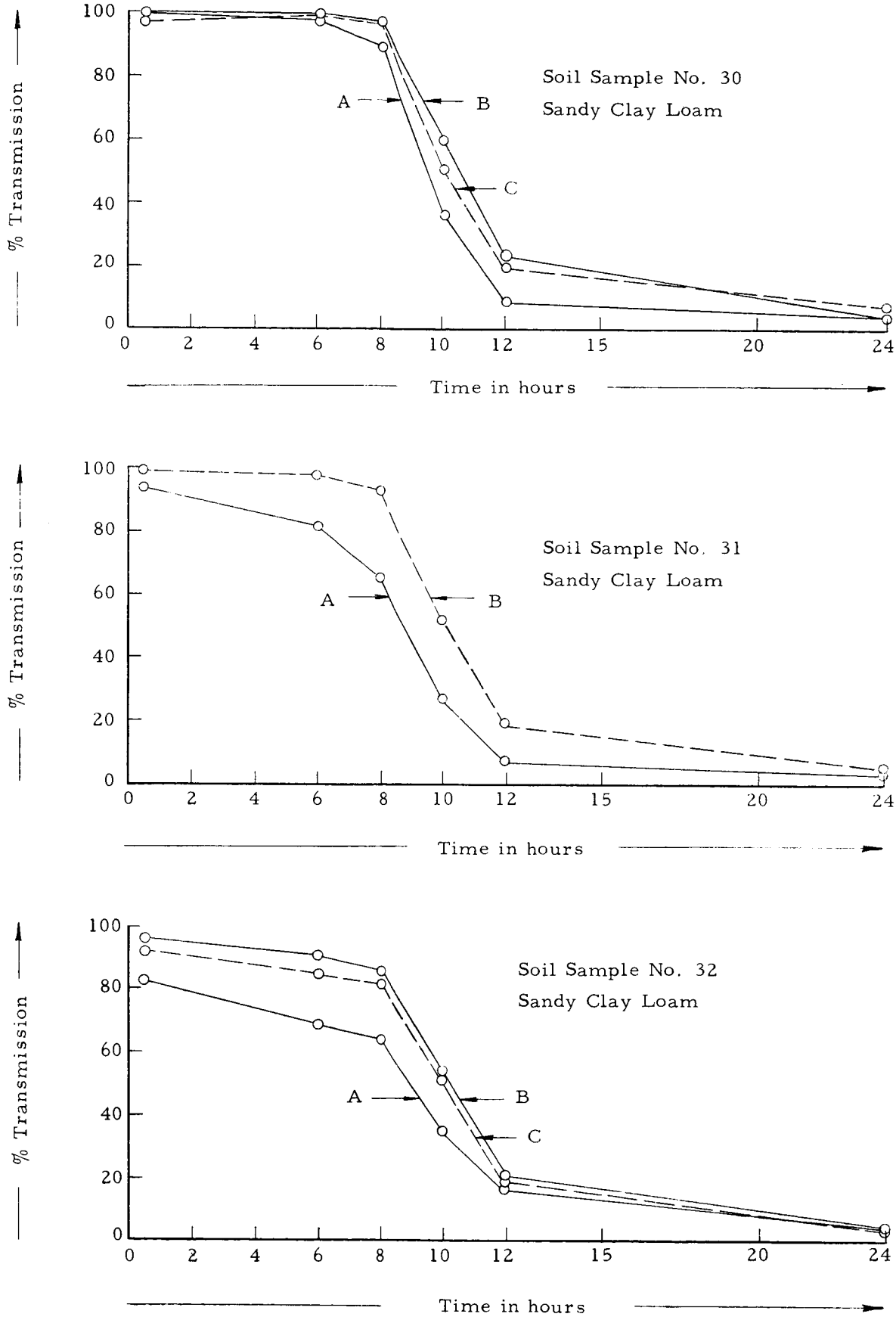
The summarized turbidimetric tests on the four classical sampling techniques (Figure 3-6) show that growth was detected at approximately the same time (between 6-8 hours) in each case. There was no significant difference between the various sampling methods, nor was there any significant influence of particle size on speed of life detection. Consequently, sampling methods exerted no bias, either positive or negative, on life detection by this technique.

Several experiments were carried out to evaluate the influence of certain aerosolizing devices employed in conjunction with the pneumatic sampler (page 2-32). These experiments, performed in the field, tested the concepts of mechanical (i.e., brush) and pneumatic (i.e., jet) methods

Table 3-11. Bacterial Count of Soil Samples Collected Pneumatically Using Various Aerosolization Procedures

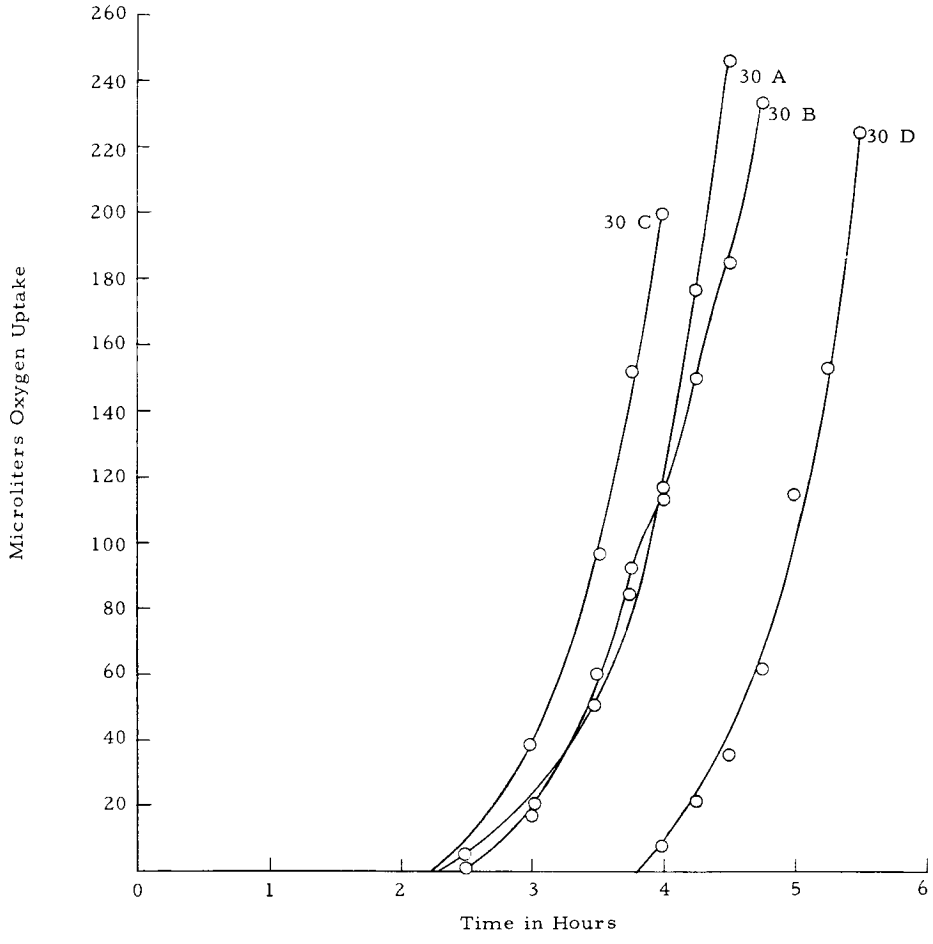
Soil Number		Total Viable Count/gram
30	A brush only	5.5×10^6
	B jet plus brush	5.2×10^6
	C brush only (blown surface)	8.5×10^6
	D jet plus brush (blown surface)	8.0×10^6
31	A brush only	4.8×10^6
	B jet plus brush	2.3×10^6
	C brush only (blown surface)	2.5×10^6
	D jet plus brush (blown surface)	2.4×10^6
32	A brush only	5.5×10^5
	B jet plus brush	2.0×10^6
	C brush only (blown surface)	5.0×10^5
	D jet plus brush (blown surface)	1.0×10^6

for aerosolizing native dusts in situ to facilitate pneumatic sampling. The biological results of several preliminary trials are shown in Table 3-11 and Figures 3-7 and 3-8. It is evident from both the plate counts and the turbidimetric observations that there was no biological bias introduced by these devices. It should be emphasized that the results presented in Table 3-11 are counts per gram, and that the innoculum size for the turbidimetric and microrespiration experiments were kept constant. Thus even though the different aerosolization devices had different efficiencies--and would have had a profound influence on life detection readout from a total sample--the inherent



A = Mechanical Brushing
B = Brush and Jet
C = Brush and Jet on Clean Surface

Figure 3-7. Turbidimetric Analyses of Soil Samples Collected Pneumatically after Various Aerosolization Procedures



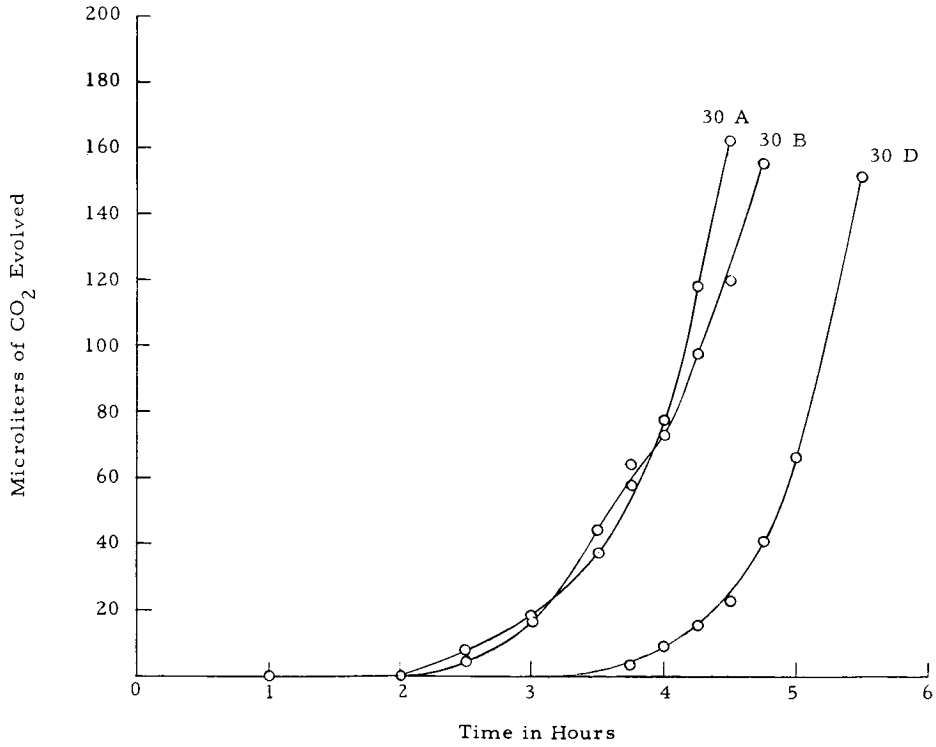


Figure 3-8. Microrespiration Analyses of Soil Samples Collected Pneumatically after Various Aerosolization Procedures.
A = Brush Only
B = Brush and Jet
C = Brush only on Clean Surface
D = Brush and Jet on Clean Surface
Soil # 30

microbiological picture per unit quantity of the sample was unchanged. A significant microrespiration lag observed in sample 30D (brush and jet on a "windblown" surface) might represent a biodetection bias introduced by that aerosolization technique, although not enough data were obtained to either verify or discount this phenomenon.

A rather elaborate field test was carried out to compare pneumatic sampling concepts, using phosphatase readouts per one minute sample as a criterion of biodetection efficacy. Unlike the results discussed above, the data from these trials encompass both biodetection bias and sampling efficiency. The three pneumatic techniques studied included vacuum only, vacuum and aerosolization by jet, and vacuum and aerosolization by jet and brush. Eight different topographical surfaces were studied—both undisturbed and "windswept" (i.e., a jet of dry nitrogen was directed at the surface until no visible dust could be seen). These results are summarized in Table 3-12.

Two observations from this experiment are worth mentioning: 1) Viable organisms (or at least phosphatase sources) were detected in nearly every case after only 1 minute of pneumatic sampling and 2) The devices which enhanced sampling efficiency did not exert any deleterious effect on either the biological fraction within the sample or the technique employed to recognize them but rather facilitated the overall process of rapid biodetection.

Table 3-12. Microbial Detection (Phosphatase Assay) in "One Minute" Pneumatic Samples After Various Aerosolizing Procedures

Legend:
- A Vacuum only
- B Aerosolizing jet only
- C Aerosolizing jet plus brush
- D Surface blown clean, then vacuum only
- E Surface blown clean, then aerosolizing jet only
- F Surface blown clean, then aerosolizing jet plus brush

	Soil	Test A (gm/min)	Test B (gm/min)	Test C (gm/min)	Test D (gm/min)	Test E (gm/min)	Test F (gm/min)
Quantity Collected	#40 Clay-gravel	0.1	1.2	1.6	0.05	0.5	1.4
	#41 Clay	0.3	4.7	4.9	0.2	1.5	4.0
	#42 Black Dirt	0.2	10.1	11.2	0.1	1.6	2.8
	#43 Soft Sandstone	0.2	7.9	12.2	0.1	2.5	6.3
	#44 Hard Sandstone	0.03	4.8	5.2	0.04	0.15	0.4
	#45 Fine Sand	0.2	18.1	19.2	0.2	17.5	19.0
	#46 Granite	Trace	0.01	0.07	0.0	0.0	0.06
	#47 Limestone	Trace	0.02	0.09	0.0	0.0	0.08

	Soil No.	Incubation Time (hours)	Micrograms Phenol/one minute sample					
			A	B	C	D	E	F
Phosphatase Readout	40	1	2.3	>18	>18	1.8	>18	>18
		2	4.0	>18	--	2.0	>18	--
		4	9.6	--	--	2.3	--	--
		22	>18	--	--	16.6	--	--
	41	1	1.6	9.6	>18	1.5	5.2	10
		2	1.5	14.8	>18	1.5	11	>18
		4	3.2	>18	--	1.5	>18	>18
		22	3.2	>18	--	1.8	>18	--
	42	1	2.7	>18	>18	1.8	>18	>18
		2	3.4	--	--	1.9	>18	--
		4	7.6	--	--	2.0	--	--
		22	>18	--	--	14.2	--	--
	43	1	1.3	17.4	>18	1.4	1.8	1.3
		2	1.2	>18	--	1.3	3.2	1.8
		4	1.3	--	--	1.4	4.0	2.3
		22	4.0	--	--	1.4	4.8	12
	44	1	1.4	>18	>18	1.2	1.7	2.7
		2	1.4	--	--	1.1	1.9	3.2
		4	1.6	--	--	1.4	2.4	4.8
		22	13	--	--	1.4	--	>18
	45	1	0.8	6.8	5.8	1.5	4.0	3.0
		2	1.2	16	10	1.5	13.6	10.0
		4	1.4	>18	14.2	1.7	16	14.2
		22	--	--	--	--	--	--
Viable Count per gram	6		$2x10^6$	$5x10^6$	$8.6x10^6$	$8x10^6$	$7.2x10^6$	$7.6x10^6$

F. Processing Studies

1. Introduction

A certain amount of time was spent in assessing the advantages accruing to "life detection" after crude sample processing. Among the processing techniques studied were density flotation in a variety of fluids, in both batch and continuous operations; flocculation and solid-solid absorption; pre-incubation and pre-soaking of dust samples as enrichment processes; and ultrasonic and detergent treatment of samples to dislodge viable particulates from mineral fragments. Some of the "processing" steps, such as pre-centrifugation of a soil-broth suspension followed by shaker-incubation have already been described above (Section D, Figure 3-2). Whereas, these steps were primarily adopted to facilitate a specific assay technique(turbidimetry), the very fact that ultimate life detection readout was accelerated and intensified by their employment permits them to be considered as useful sample processing steps.

The flotation fluids employed in this study and several comments regarding their usefulness are listed below:

a. Water

Although not strictly a "flotation fluid", it was found that enough soil bacteria remain suspended in the aqueous menstrum (after shaking with soil and centrifuging to remove the occluding material) to permit subsequent detection. The count remaining is, of course, a function both of the original soil population and the force of centrifugation. On the basis of the various soils studied and the various G forces employed, it was found that from 0.1 percent to 10 percent of the original viable count remained in the supernatant after it had been clarified to a light transmission of 95 percent.

b. Ludox

This was the most useful flotation fluid encountered. It could be used in microscopic work, plate counts, turbidity tests, microrespiration tests (where turbidity is no problem anyway), organic analyses, and dye reduction. The silica is inert chemically and inocuous biologically. Its only drawbacks were extreme pH sensitivity on the alkaline side—precluding its use in an alkaline phosphatase assay, and its instability after freezing.

c. Fluorochemicals

Although these materials are excellent flotation fluids from a density standpoint, their immiscibility with water limits their usefulness in any life detection work using aqueous systems. They might yet prove useful if techniques are developed to facilitate homogeneous suspension of solid particles throughout their volume.

d. Bromoethanol
e. Cesium chloride
f. Rubidium chloride
g. Ammonium Sulfate

These are excellent flotation fluids for preparation of semi-purified specimens for microscopic examination—but the concentrations necessary for adequate density flotation are toxic to soil microbes.

h. Glucose
i. Glycerin

The concentrations necessary for adequate purification by density flotation yield highly viscous solutions which interfere with easy centrifugation.

2. Purification of Specimens for Microscopic Observation

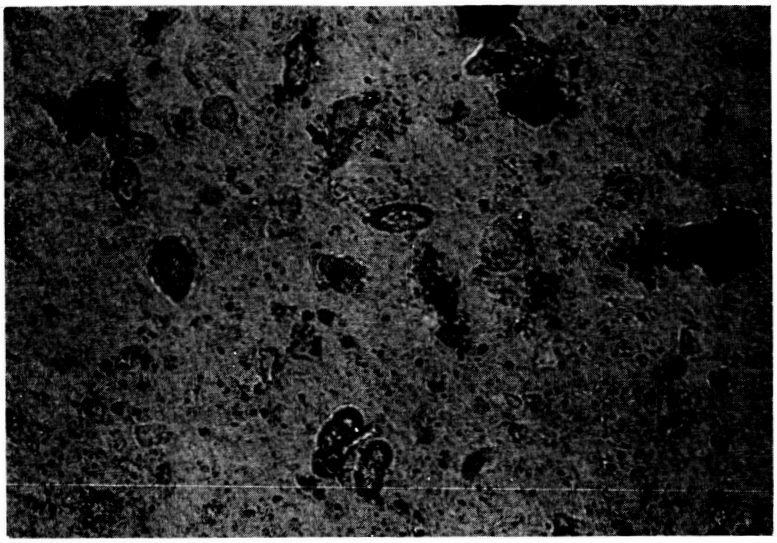
Several experiments were performed using different soils and their classified fractions to which mixtures of rust spores (~35 microns diameter) and Cladosporium spores (~10 microns) had been added. The soils were mixed with the respective flotation fluids in centrifuge tubes, aliquots were removed as "unprocessed" samples, and the tubes were then spun at 1000 G for 20 minutes. Aliquots of the supernates were then taken as "processed" samples. Both the processed and unprocessed samples were filtered through membrane filters (MF-HA-0.45 micron) which were microscopically examined. A summary of these trials is shown in Table 3-13, and photographs illustrating the extent of purification attained in Ludox is shown in Figure 3-9.

Table 3-13. Microscopic Enumeration of Mold Spores and Rust Spores in Processed Soil Samples

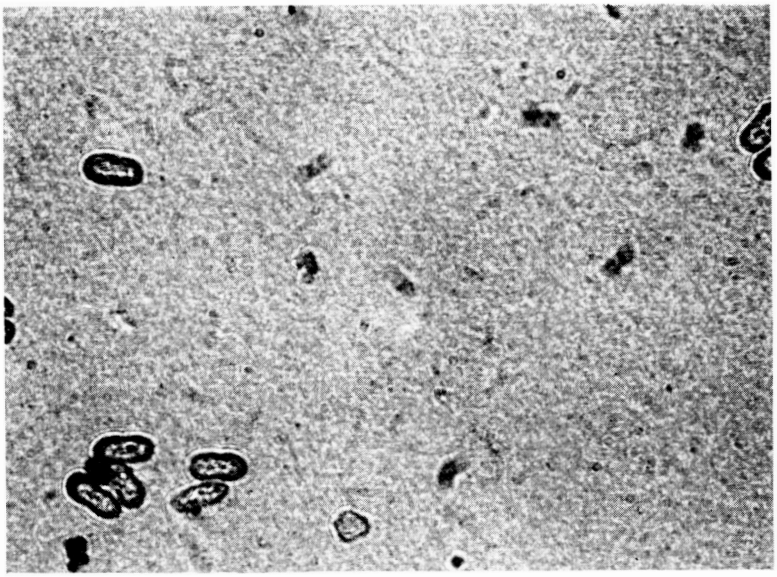
Soil	Microscopic Count of Recognizable Spores/cm^2					
	Water		Ludox		Fluorochemical**	
	*Unprocessed	1000g x20min	*Unprocessed	1000g x20min	*Unprocessed	1000g x20min
#1 Unfractionated	330	0	350	183	30	0
#1 62-88 μ	400	0	600	800	150	60
#1 <62 μ	-	0	-	1000	-	50
#6 Unfractionated	300	0	300	280	-	-
#6 62-88 μ	800	13	300	280	100	100
Lake Mead Sand Unfractionated	600	0	800	450	175	100

*Unprocessed Samples extremely occluded; counts difficult and inaccurate.

**In fluorochemical processing, spores not evenly distributed on viewing surface, but irregularly clustered; counts difficult and inaccurate.



Soil No. 6 Before Centrifuging



Soil No. 6 After Centrifuging

Figure 3-9. Soil No. 6 with Rust Spores Added Showing Effects of Centrifuging in Ludox

Magnification 200X

It is evident that both quantitative and qualitative recovery of these rather large biological entities was facilitated in Ludox by the treatment employed. In each case, the unprocessed sample was heavily occluded, and it appeared that fine soil particles were actually coating the spores, preventing easy recognition. After centrifuging, however, a good yield of readily recognizable spores was present in the Ludox supernate. On the other hand, the spores were sedimented out of the water along with the soil particles. The results with fluorochemicals were inconsistent.

3. Density Flotation and Life Detection

In theory, density flotation can contribute to life detection in two ways. It may serve to concentrate the viable organisms into a small volume and thus facilitate their detection in soils with a low viable count; and it may help to purify the biological entities by separating them from occluding nonbiological particles of higher density.

To study the concentration approach, several experiments were performed in which soil suspensions were mixed with flotation fluids and centrifuged (385 G x 10 min). Viable counts were made of the original shaken suspension, of the supernate after centrifuging, and of the sediment. These data, for soils 1, 6, and 21 are shown in Table 3-14. It is apparent that in the case of water, between 90 and 99 percent of the viable organisms were deposited with the sediment, whereas in the case of Ludox most of the organisms remained in the supernate.

A further series of experiments was performed to measure the distribution of viable organisms in the centrifuge tube. If true concentration were taking place, there would be a significant difference in count along the vertical axis. Soil 1 was suspended in several respective fluids (1 gram to 3 ml), shaken, and centrifuged at 550 G x 10 min.

Table 3-14. Distribution of Organisms Before and After Centrifuging in Water and Ludox

(1 gm soil and 5 mls Fluid; 385G x 10 min)

Soil No.	Fraction	Water	Ludox
1	Initial count/milliliter	8.0×10^4	8.0×10^4
	Supernatant count/milliliter	8.0×10^3	7.0×10^4
	Sediment count/gram	2.6×10^5	2.2×10^4
6	Initial count/milliliter	1.8×10^6	7.0×10^5
	Supernatant count/milliliter	1.7×10^4	7.0×10^5
	Sediment count/gram	4.4×10^6	4.5×10^5
21	Initial count/milliliter	2.0×10^6	1.9×10^6
	Supernatant count/milliliter	1.0×10^5	1.3×10^6
	Sediment count/gram	5.7×10^6	6.0×10^5

Table 3-15. Distribution of Organisms After Centrifuging Soil No. 1 in Various Liquids

(1 gm soil and 3 ml Fluid; 550G x 10 min)

Fraction	Water	Ludox	Glycerin	Cesium Chloride
Top milliliter	1.4×10^3	1×10^4	1.5×10^4	1.5×10^4
Middle milliliter	7.0×10^2	1.3×10^4	1.2×10^4	1.5×10^4
Bottom milliliter	3.5×10^3	1.1×10^4	2.4×10^4	7.5×10^3
Total in supernatant	5.6×10^3	3.4×10^4	5.1×10^4	3.7×10^4
Total in sediment	4.0×10^5	2.7×10^5	2.0×10^5	1.1×10^5
Total accounted for	4.05×10^5	3.0×10^5	2.5×10^5	1.4×10^5
Initial average viable count/gram	4.0×10^5	4.0×10^5	4.0×10^5	4.0×10^5

Viable counts were made on equal aliquots taken from the top, middle, and bottom layers of the supernate and from the sediment. These data are shown in Table 3-15. Although the results with the flotation fluids were encouraging (significant fractions of the total count remained suspended in the supernate) no real claims for concentration can be made. It appears that the high density fluids merely slow down the sedimentation process so that at the end of the centrifuging period some small particles still remain in suspension.

The conditions of centrifugation were studied to ascertain the limits of centrifugal speed which could be applied for clarification purposes without losing too great a fraction of the viable population. A series of soils, mixed with water or Ludox, were subjected to G forces ranging from 170 G x 10 min to 1000 G x 10 min in a batch centrifuge (subsequently extended to 20,000 G in a continuous centrifuge, Table 3-21). The viable counts of the supernates from these tubes are summarized in Table 3-16. Again it was apparent that anywhere from one to three orders of magnitude reduction in viable count occured in centrifuged aqueous suspensions, depending on the soil. On the other hand, in Ludox, forces as high as 1000 G for 10 minutes effected a loss of less than 1 log.

The advantages of density flotation, therefore, would be most applicable to those life detection processes which require a clarified sample (cf Figure 3-9). The most prominent "biological" approach which requires non-occluded substrates is the turbidimetric technique. Consequently, a series of turbidimetry assays were set up to assess the possible advantages of prior density flotation. These tests involved the mixing of various soils with a given flotation fluid, centrifuging to sediment the occluding matter, and transferring aliquots of the supernate to nutrient broth which was then incubated on a platform shaker. At hourly intervals the light transmission was measured and plotted against time. A series of typical "growth curves" are presented in Figure 3-10.

Table 3-16. Sedimentation of Soil Organisms by Centrifugation in Water and Ludox (batch process)

	Viable Count/ml Supernatant				
	No Centrifuging	170G x10 min	385G x10 min	680G x10 min	1000G x10 min
Ludox + Soil 21	7.6×10^6	2.2×10^6			
	8.0×10^6		2.1×10^6		
	8.2×10^6			1.8×10^6	
	1×10^7				2.1×10^6
H_2O + Soil 6	1.8×10^6		1.7×10^4		
	3.0×10^6	2×10^4	3×10^4		8.0×10^3
	6.0×10^6	1.2×10^5			
H_2O + Soil 1	3.5×10^5			3.5×10^4	
	1.8×10^5				2.0×10^4
	8.0×10^4		8.0×10^3		
Ludox + Soil 1	8.0×10^4		7.0×10^4		
	3.5×10^5			9.0×10^4	

The advantages of density flotation insofar as it aids turbidimetric life detection are evident. It had previously been shown that pre-centrifugation was necessary (Figure 3-2) before any reasonable growth detection could take place. It had also been shown (Table 3-16) that >99 percent of the viable matter was sedimented from aqueous mixtures during the centrifugation process. It now appears that this apparent dilemma can be resolved by density flotation. Apparently, enough viable material was retained in the supernates of Ludox and glycerin to initiate detectable growth in 7 hours or less. Cesium chloride, as indicated previously, inhibited growth.

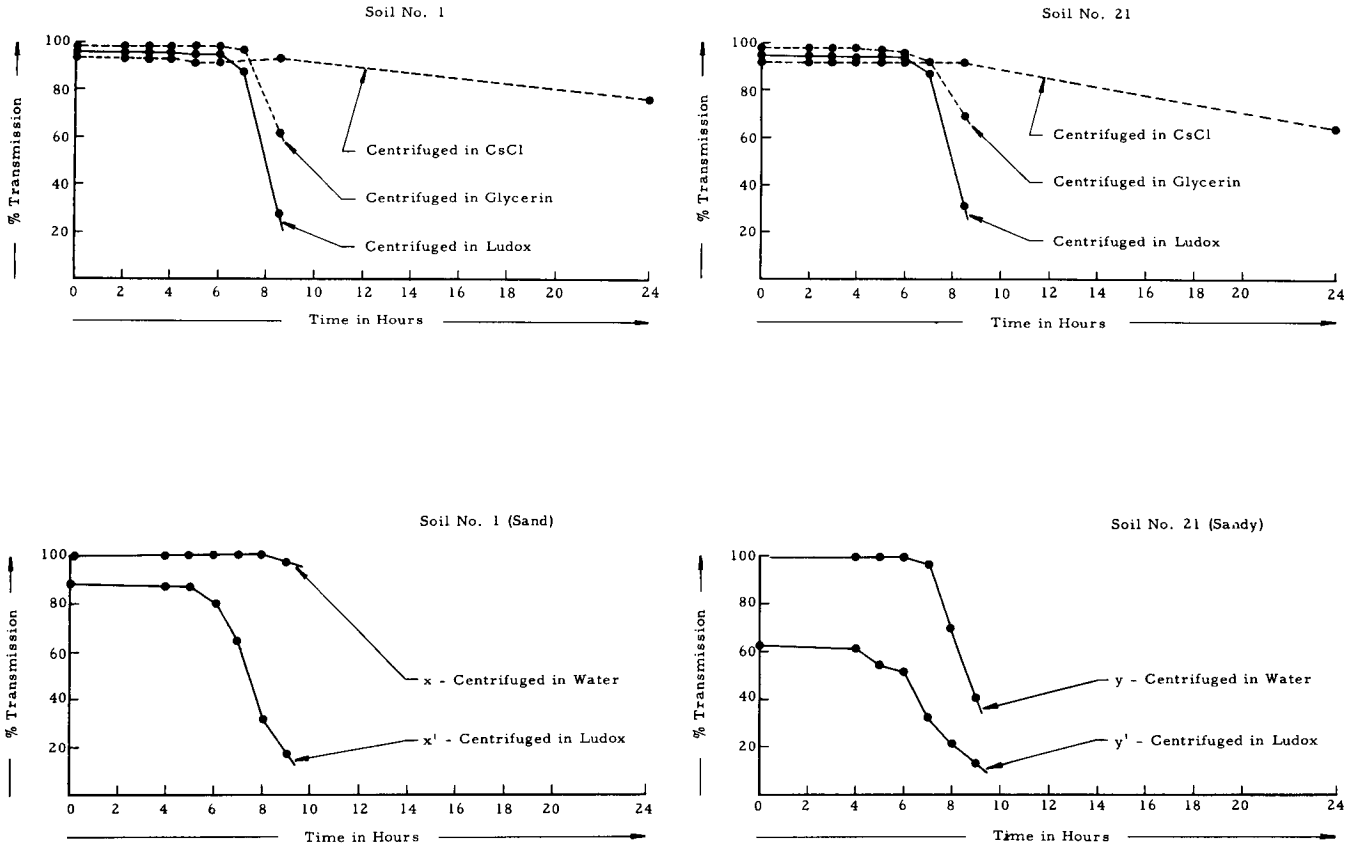


Figure 3-10. Effect of Processing by Density Flotation on Subsequent Growth Detection by Turbidimetry

Table 3-17. Turbidimetric Detection of Growth as Influenced by Various Processing Fluids and Treatments

(Time Unequivocal Turbidity Detected-Hrs)

Technique	Soil	Water	Ludox	Glycerin	$CsCl_2$
1 gm soil & 5 ml fluid spun at 385Gx 10 min	1	>9	6-7		
3 ml supernate transferred to 97 ml broth	21	7-8	6-7		
1 g soil & 3 ml fluid	1	>9	7-8		
spun at 385 Gx10 min	6	>9	6-7		
1 ml supernate transferred to 100 ml broth	21	8-9	6-7		
1 g soil & 3 ml fluid	1		6-7	7-8	
spun at 385 Gx10 min	6		5-6	8-9	
1 ml supernate transferred to 50 ml broth	21		5-6	7-8	
1 g soil & 1 ml fluid	1		6-7	7-8	>9<24
spun at 550 Gx10 min	6		6-7		
decant supernate into 50 ml broth	21		6-7	7-8	>9<24
Volcanic Ash			> 9		
"Low Count Soil" (300/gm)		>10	8-9		

The experiments described in Figure 3-10 were expanded, using a larger variety of soils and experimental techniques. The data from these growth curves are summarized in Table 3-17.

Instead of plotting twenty-six separate diagrams, the table shows the time that turbidimetric readings in the Spectronic 20 showed the first definite and continuous shift away from the original base line. In every case studied, the supernatant from Ludox permitted faster growth detection than parallel supernatants from water or the other flotation fluids.

A simple experiment was performed in this series to verify the compatibility of density flotation with pneumatic sampling. Since density flotation restrained the sedimentation of small particles, and since pneumatic samples contained predominantly small particles (table 3-3), it was feared that both procedures might interreact to lose the advantages gained by each. Three classical samples and three pneumatic samples from the same soils were mixed with Ludox and centrifuged at 1000 G. The supernates from each were inoculated into broth for shaker incubation. From Table 3-18 it can be seen that there was no real incompatibility. The positive biological bias of pneumatic sampling is very much in evidence. In each case, the pneumatic

Table 3-18. Turbidimetric Growth Detection in Classical and Pneumatic Samples after Density Flotation

	% Transmission					
	No. 1		No. 6		No. 21	
Time of Incubation	Classical	Pneumatic	Classical	Pneumatic	Classical	Pneumatic
0	94	96	95	95	95	92
5	94	96	93	90	95	86
6	94	90	89	78	91	70
7	91	84	77	77	70	52
8	87	51	50	54	40	30
9	56	17	25	29	12	20

sample permitted growth detection faster than the classical sample. Furthermore, the Ludox processing of the pneumatic sample enabled viable organisms in one-gram soil samples to be detected faster than in any previous attempt.

Despite the advantages gained by density flotation, the precedure of transferring supernate aliquots from flotation tubes to growth media for turbidimetric assay was inconvenient. Consequently, some thought was given to combining several steps into one integrated operation. To this end a nutrient broth was prepared using Ludox instead of water. This high density (HD) broth (8 grams DIFCO nutrient broth in 1 liter Ludox AM) had a Sp Gr of 1.22, could be neutralized to pH 7.5, and supported the growth of soil organisms as well as did its low density counterpart. Although the Ludox itself imparted some turbidity (75 percent transmission on a colorimeter standardized to 100 percent with aqueous nutrient broth) to the medium, turbidimetric growth analyses could be made easily by standardizing the colorimeter to 100 percent transmission with a sterile HD broth. In practice, the soil to be tested was mixed with the HD broth, the suspension was centrifuged at 2500 G, the supernate was decanted into a sterile cuvette, and incubated on the platform shaker. The decanting step was necessary to prevent re-suspension of the sediment during incubation. In an actual processing device, either continuous centrifugation or filterfuge tubes could be used to separate effectively the clarified supernate from the sediment.

Figure 3-11 shows the comparative turbidimetric growth curves obtained from the same soil in ordinary nutrient broth and HD broth respectively. Growth was detected in the HD broth faster (between 4-6 hours) than in the ordinary broth. Although after 8 hours of incubation either medium was adequate, if time for detection is critical, the advantage of clarification in a high density fluid is evident. Figure 3-12 shows the results of processing a variety of soils in HD broth. Again, the speed of life detection was encouraging, especially when it is recognized that this type of processing can be done with a minimum amount of equipment and relatively few steps.

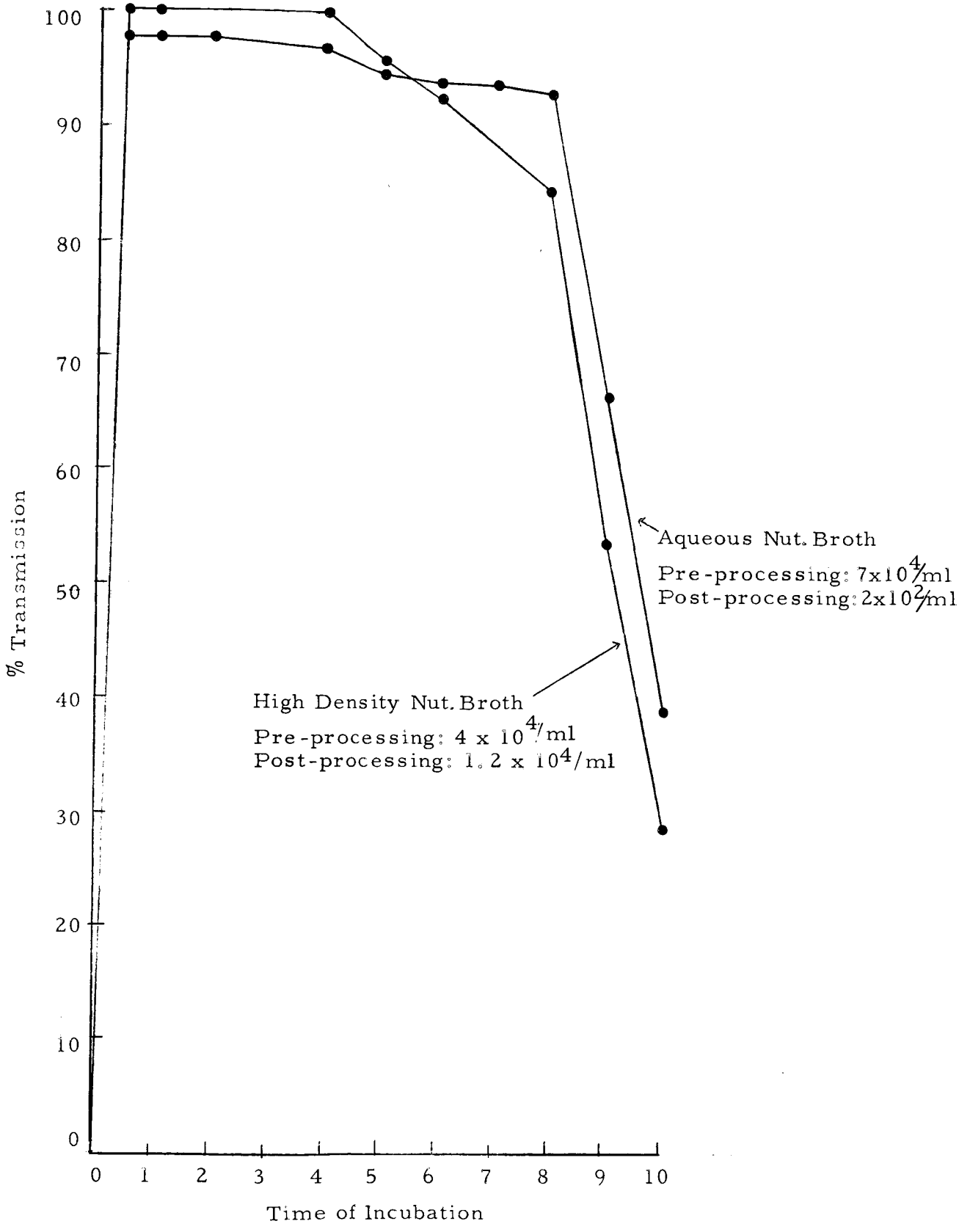


Figure 3-11. Comparative Growth Detection by Turbidimetric Means in Normal and High Density Nutrient Broth

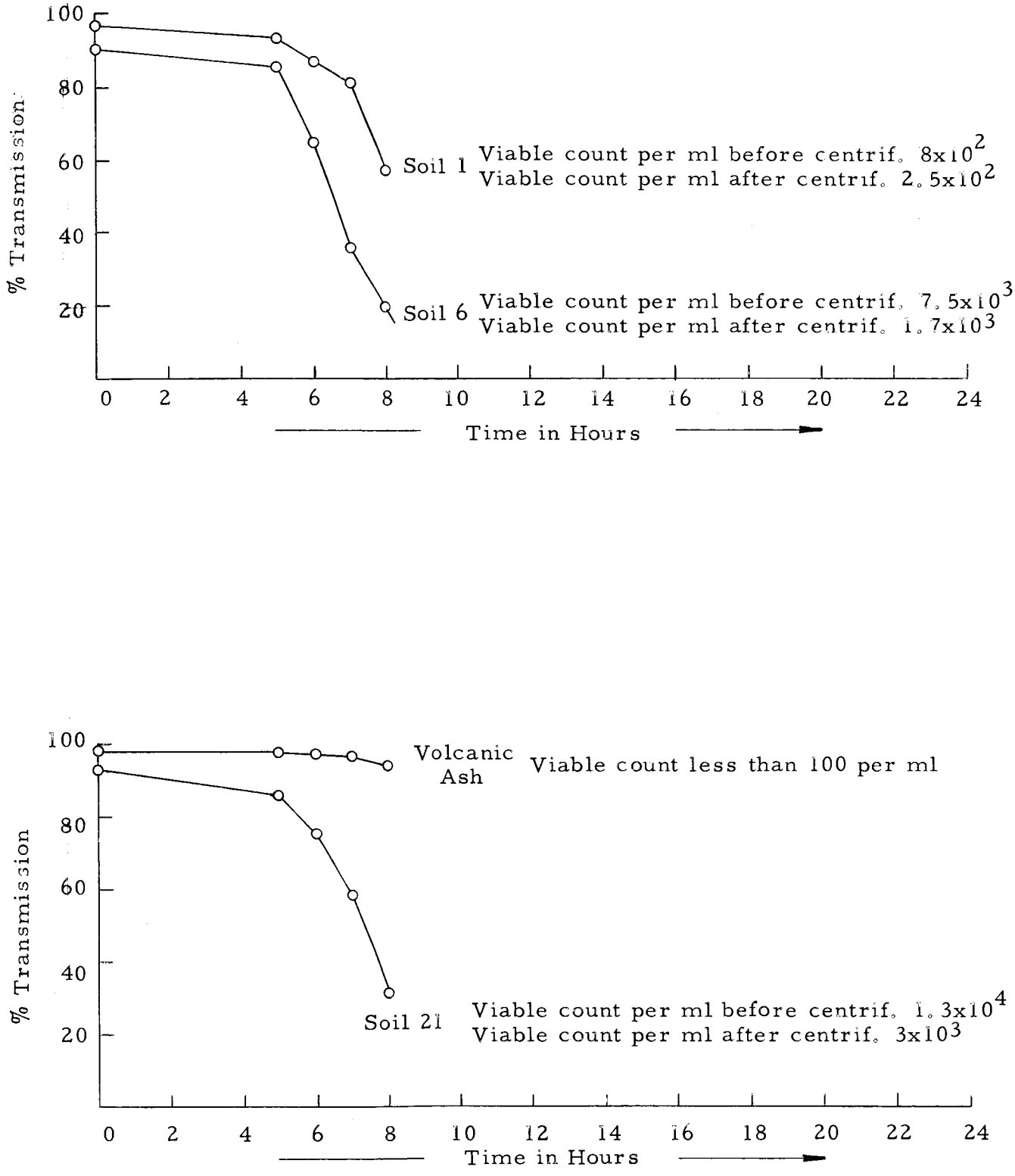


Figure 3-12. Detection of Growth Turbidimetrically in a High Density Nutrient Broth

4. Processing by Continuous Centrifugation

Continuous flow centrifugation has some obvious advantages over batch centrifugation. On the one hand the sediment and supernatant are kept separate from each other, precluding the resuspension of occluding material and concurrently obviating the need for transferring or decanting the supernate. Furthermore, higher G forces can be obtained to improve clarification. Several experiments were performed, using a Sharples laboratory model centrifuge at 6000 G (20 ml/min) and Ludox suspensions of Soil 5 (clay loam). The wet sediment contained 9×10^4 viable organisms/gram and the liquid effluent 3×10^4 viable organisms/ml, indicating good retention of biologicals even at these forces. Unfortunately, the performance of experiments with the Sharples instrument proved quite time-consuming and were discontinued.

During the course of this contract, another type of continuous flow centrifuge was also used. This device (pictured in Figures 3-13 and 3-14) can process glycerin or Ludox at 10 ml/min at any gravitational force setting from 50 G to 20,000 G. Preliminary attempts to process Soils 1 and 6 in Ludox are summarized in Table 3-19. The count/ml of both the original suspension and the clarified effluent was a direct function of the amount of soil originally employed. Significantly, reduction in count at 2500G was less than one log; whereas, the light transmission through the effluent was increased from 0 percent to 54-87 percent.

Table 3-20 contains the results from a series of trials with this continuous flow centrifuge. The variables studied were centrifugal force, density of flotation fluid and source of viable organisms.

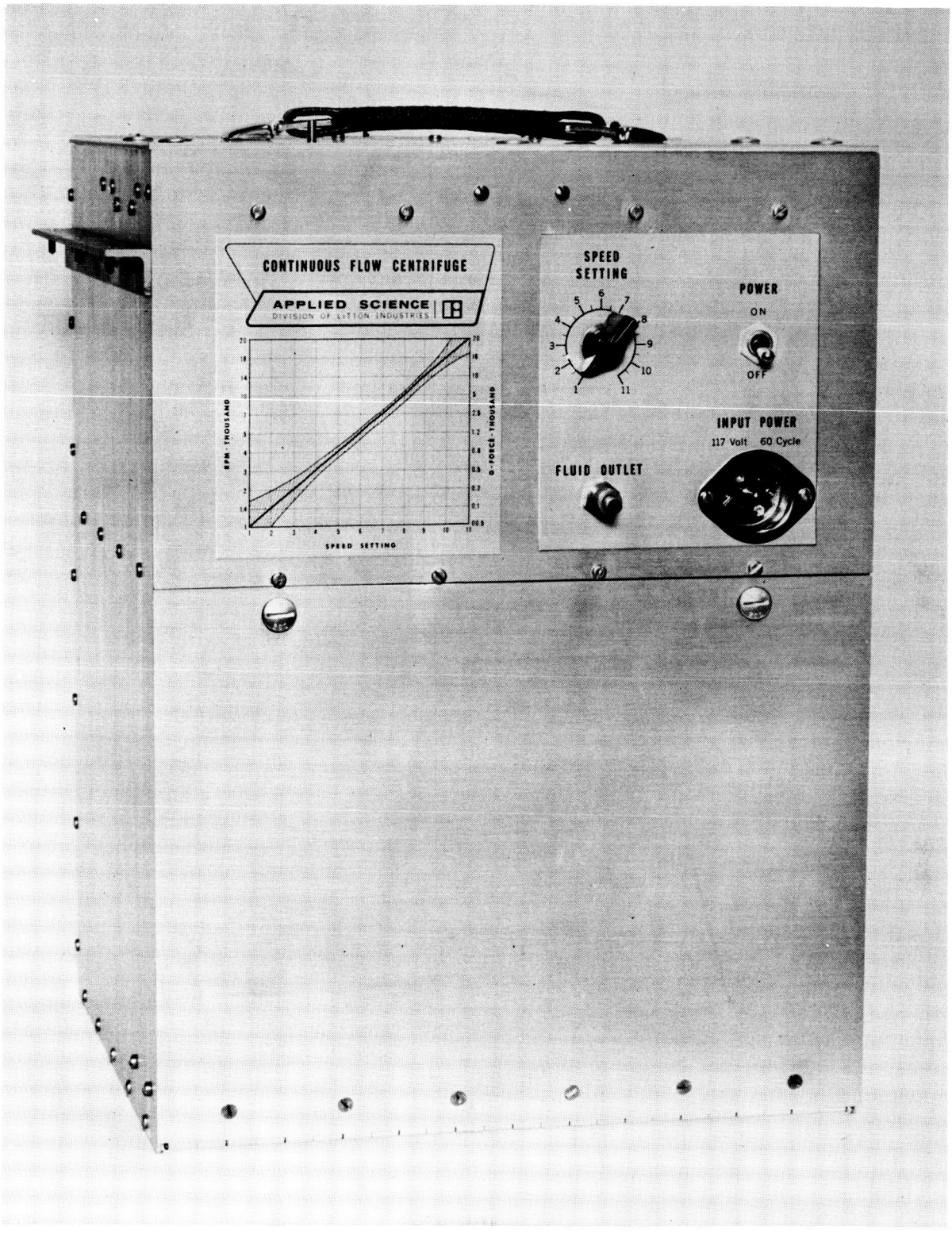


Figure 3-13. Front View of Continuous Flow Centrifuge Showing Speed Control, Speed Setting Graph, Fluid Outlet, and Power Switch

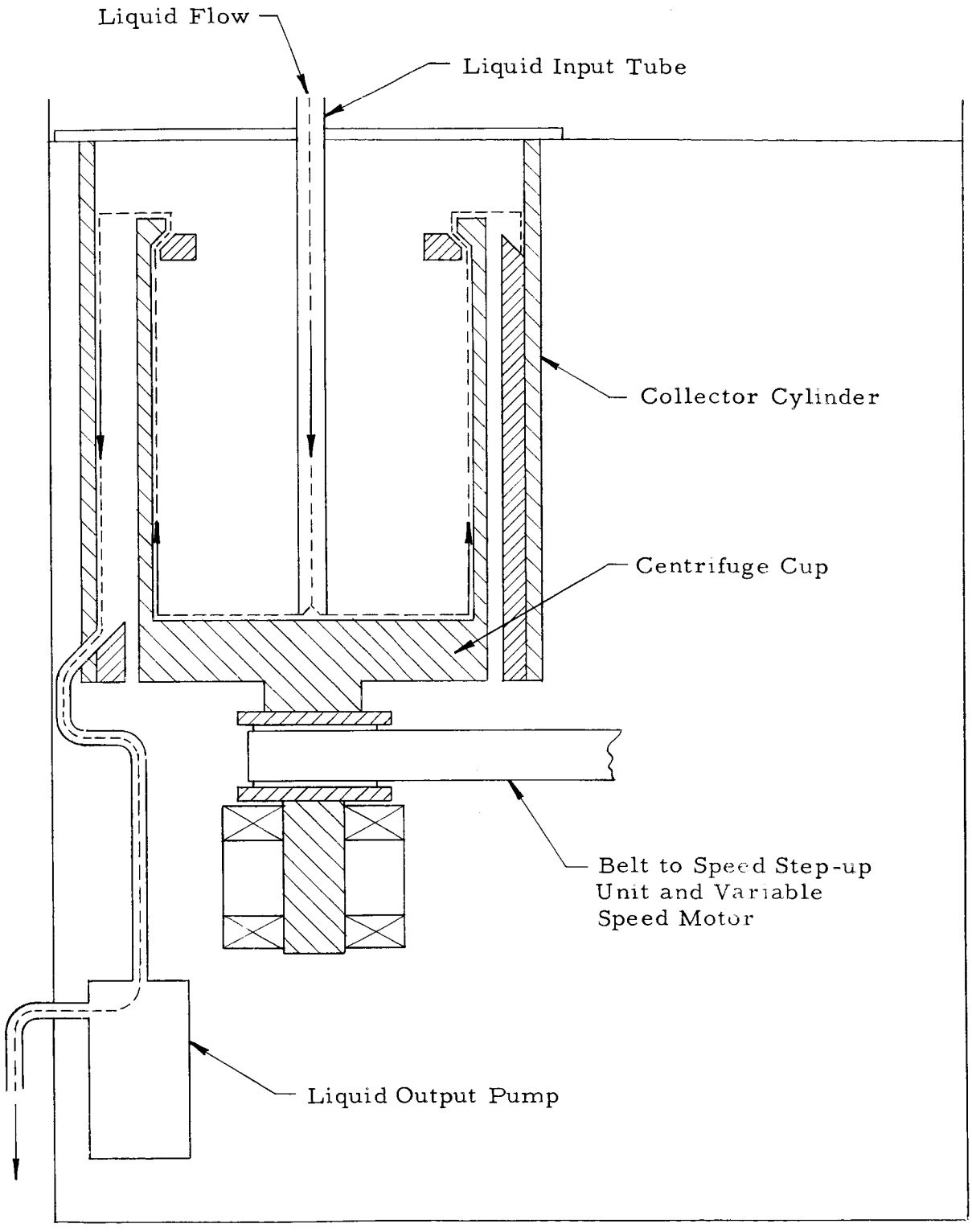


Figure 3-14. Schematic of Centrifuge Liquid Flow System

Table 3-19. Purification of Soil Organisms by Continuous Flow Centrifugation

		Viable Count/ml in Effluent	% Transmission in Effluent
Soil #1	unprocessed	9×10^4	0
20g/100 ml Ludox	processed*	2.3×10^4	71%
Soil #1	unprocessed	1.6×10^5	0
50g/100 ml Ludox	processed*	6×10^4	54%
Soil #6	unprocessed	5.4×10^4	0
20 g/100 ml Ludox	processed*	1.4×10^4	87%
Soil #6	unprocessed	1×10^5	0
20g/50 ml Ludox	processed*	4.2×10^4	66%

*2500 G, 10 ml/minute

The data presented agree for the most part with theoretical expectations. The greater the G force, the lower the viable count in the effluent; the greater the density of the fluid, the greater the retention of viable particles. One observation worth mentioning is the significant difference between retention of Bg spores and native soil organisms. Even at 20,000 G, the Ludox suspensions retained 40-75 percent of the spores, whereas more than 90 percent of native soil organisms were sedimented. This inferred quite strongly that most soil organisms were associated with heavy but small mineral particles. At slow speeds, many of these particles and their associated bacteria would remain suspended and would behave like the less dense Bg. On the other hand, at high centrifugal speeds they were sedimented, and the attached bacteria were sedimented with them.

Table 3-20. Sedimentation of Soil Organisms and Artifically Added Spore Suspensions after Centrifuging in Water and Ludox (continuous flow centrifuge)

Soil	Treatment	Viable Count/ml Effluent Water D = 1.00	Ludox D = 1.11	Ludox D = 1.16	Ludox D = 1.22
Bg	Pre-centrifuge	4×10^4	5.4×10^4		4.6×10^4
	2500 G	3×10^2	2.8×10^3		3×10^4
	Pre-centrifuge	6×10^4	4.3×10^4		5×10^4
	5000 G	$< 1 \times 10^2$	2.3×10^3		3×10^4
	Pre-centrifuge	9×10^4	4.4×10^4	4.5×10^4	4.7×10^4
	20,000 G	$< 1 \times 10^2$	3×10^3	1.8×10^4	3.5×10^4
#1	Pre-centrifuge	7×10^4			4×10^4
	2500 G	2×10^2			1.2×10^4
	Pre-centrifuge	5.7×10^4			5×10^4
	5000 G	1.5×10^2			1.5×10^4
	Pre-centrifuge	4×10^3	4.7×10^3		4.6×10^3
	20,000 G	1×10^2	1.8×10^2		4×10^2
#5	Pre-centrifuge	1.6×10^3	4.4×10^3	3.8×10^3	4×10^3
	20,000 G	2×10^1	1×10^2	2×10^2	3.8×10^2

5. The Effect of Pre-Incubation and Pre-Soaking

On the basis of the experimental observations made thus far, several important phenomena related to processing soil samples for life detection are apparent:

1) Ease and speed of life detection in soils is a function of viable cell concentration.

2) Certain detection techniques (e.g., microscopy and turbidimetry) require soil microbe suspensions in a clarified menstrum.

3) Certain flotation fluids aid the process of clarification while permitting the retention of viable organisms in the supernate.

4) Most of the viable soil microorganisms are associated with small mineral particles which can be sedimented out at high centrifugal speeds, even in flotation fluids.

5) There is a significant difference between the sedimentation characteristics of native soil microflora and artificially prepared spore suspensions, probably because of the association of the former with heavy mineral particles.

Consequently, our further efforts were directed toward such problems as

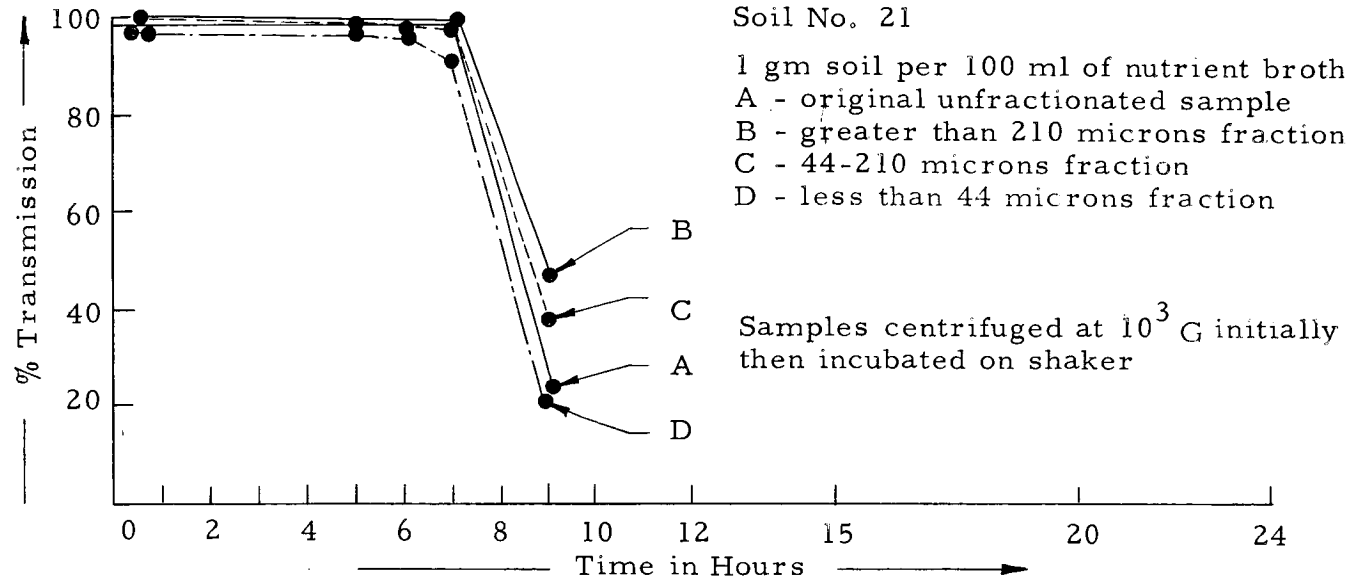
1) Dislodging organisms from the mineral particles, and

2) Providing soil suspensions in which native flora exist free and unattached.

The former approach will be discussed in Section F-6; the latter approach is described below.

When organisms grow in a broth, they usually distribute themselves in free suspension, even though the original inoculum may be sessile on a surface. This is the basis of turbidimetric sterility tests for surgical instruments. Consequently, it was thought that soil suspensions could be preincubated in a nutrient broth for several hours prior to clarification and turbidimetric analysis. In this way the broth would be enriched with young unattached cells that would not be as easily sedimented during centrifugation as the original attached soil flora. These young, unattached organisms which remained suspended during centrifuging could subsequently act as "large inoculum" in the clarified broth for the turbidimetry experiment and might significantly shorten the growth detection time.

Two of these experiments, using aqueous nutrient broth and 1000 G centrifugation are shown in Figures 3-15 and 3-16. It is quite apparent that the original hypothesis was valid. Whereas routine turbidimetry (i.e., mixing, centrifuging, incubating) permitted growth detection in



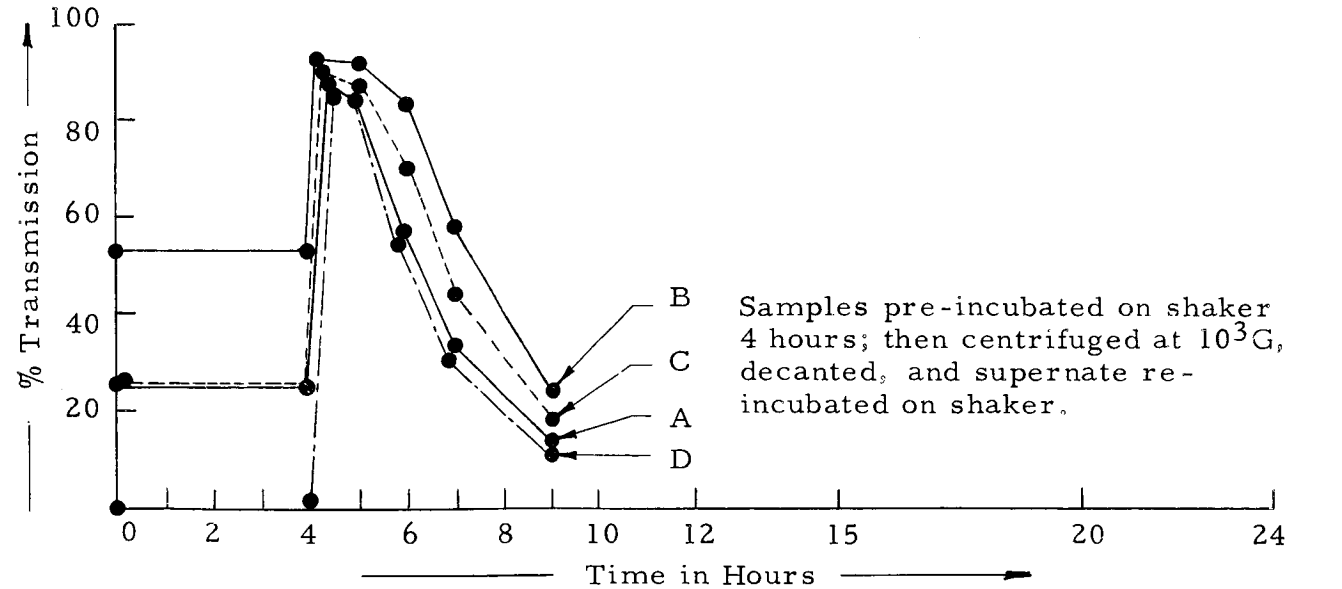


Figure 3-15. Influence of Pre-Incubation and Subsequent Centrifugation on Turbidimetric Growth Detection.

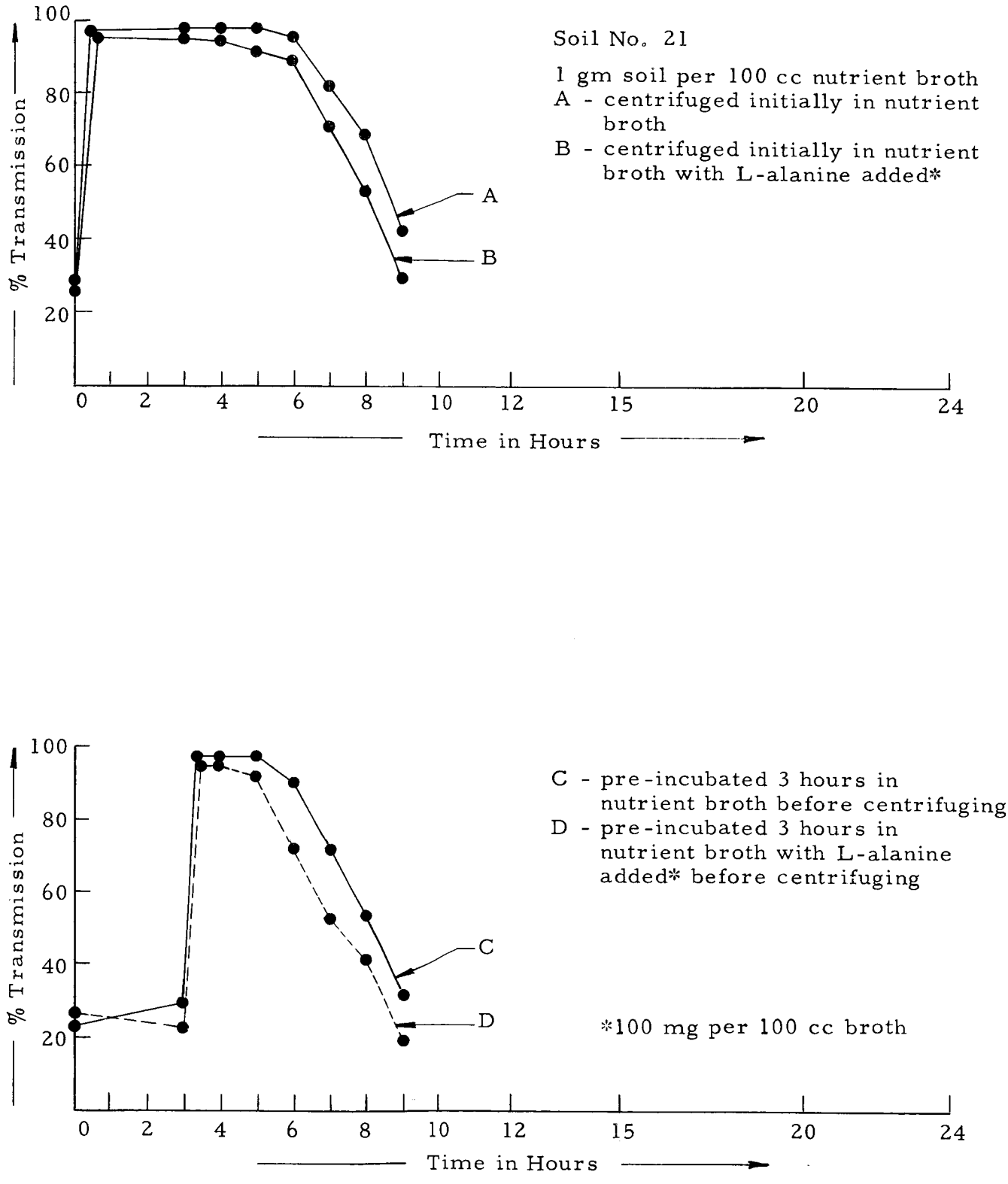


Figure 3-16. Effect of Pre-Incubation and L-Alanine on Turbidimetric Growth Detection

6-8 hours, preincubation followed by centrifugation and continued incubation permitted detection in the extremely short time of 5-6 hours. The addition of L-alanine as a spore germination stimulant aided detection slightly but not as dramatically as the preincubation itself.

A modification of the preincubation approach was the presoaking of dust samples in sterile distilled water before analysis. Under these conditions, the only nutrients available for growth (and subsequent increase of unattached cells) would have to be obtained from the soils themselves. Consequently, this approach would be one of the most feasible for enrichment of soils with unknown organisms and would have universal application to any dust sample, terrestrial or extra-terrestrial—as long as the organisms being searched for are water dependent. The results of this distilled water presoaking are presented in Table 3-21. There was a definite enrichment in total viable population, as measured by both plate count and phosphatase assay. Furthermore, when the presoak was combined with preincubation in broth for a turbidimetric assay, growth detection time was slightly, but definitely, shortened. It might very well be argued that the extra 16 or 18 hours of soaking in distilled water could best be spend in actual incubation in a nutrient solution. Indeed, if the extra time is considered part of processing, then the slight gain in detection time was inconsequential. However, it is important to note that a simple mixing of soil

Table 3-21. Effects of Presoaking on Viable Counts and Phosphatase Readouts

	No Treatment	Soaked at Room Temp for 16 hrs	Soaked at 37° for 16 hrs
Total viable count/ml (equal amts of soil and H_2O)	3×10^6	2×10^7	5.3×10^7
Phosphatase Readout (μgms phenol/5 ml steeping fluid)	1 hr	1.8	2.5
	2 hr	2.3	3.6
	4 hr	5.2	8.0
	6 hr	9.2	15.4

and water did enhance our ability to detect life by three basic techniques (plate count; phosphatase; turbidity); and this processing step is the simplest one to perform in an unknown environment and with unknown soils.

6. Processing by Ultrasonication and Detergent Treatments

Another approach to the problem of separating microorganisms from mineral particles involved ultrasonication. Eight different soil-Ludox suspensions were prepared and divided respectively into two portions. One portion was centrifuged in the continuous flow unit at 20,000 G, and plate counts were made of the original suspension and the clarified effluent. The other portion was exposed to ultrasonic treatment (300watts at 40 kc) and then centrifuged. Plate counts were made of each of the three samples: the untreated, the ultrasonicated, and the ultrasonicated-centrifuged effluent. These data are all shown in Table 3-22. An analysis of the results indicates that the ultrasonication had a slight but consistent effect in increasing the viable count/ml of fluid. However, the sedimenting effect of 20,000 G was much more significant, reducing the original counts by >90 percent (cf Table 3-20). Ultrasonication prior to centrifugation did appear to offer some slight advantage: the counts in these samples were usually higher than in the nonultrasonicated samples. However, the reduction of viable organisms in the ultrasonicated samples was also > 90 percent, and thus this treatment did not yield any significant gains.

Similarly the attempts to dislodge organisms from minerals by detergent treatment were unsuccessful—at least with the detergents and concentrations tested. Table 3-23 shows there was no advantage gained by soaking soils in Brij-35 (polyoxyethylene-Lauryl-ether) overnight, and that Calgon (hexametaphosphate) exerted a definite bactericidal effect.

Table 3-22. Effect of Centrifugation and Ultrasonication on Viable Counts in Ludox-Soil Suspensions

Soil	Viable Count/ml Supernatant			
	Unprocessed	After Ultrasonic (40 kcx10m)	After Centrifug. (20,000 G)	After Ultrasonic & Centrifug.
#1	1.2×10^3		4.5×10^2	
	1.3×10^3	1.3×10^3		4.1×10^2
#5	6×10^4		1.5×10^3	
	5.6×10^4	6.5×10^4		6×10^3
#6	7×10^4		5.5×10^3	
	8×10^4	1.9×10^5		1.5×10^4
#6	6.5×10^4		3×10^3	
	7.6×10^4	3.4×10^5		1.2×10^4
74-2	6.9×10^3		3×10^2	
	4.3×10^3	1.4×10^4		8×10^2
#21	1.6×10^5		1.6×10^4	
	1.3×10^5	3.8×10^5		2.8×10^4
#100	70		20	
	1.4×10^2	1.7×10^2		20
#22	5×10^4		9.5×10^3	
	7.2×10^4	1.5×10^5		2.7×10^4
ARD	8.3×10^2		42	
	1×10^3	1.2×10^3		50
ARD	1.1×10^3		$1.4 \times 10^{2*}$	
	9×10^2	1.1×10^3		$1.4 \times 10^{2*}$

*Centrifuged at 5000 G.

Table 3-23. Attempts to Dislodge Viable Organisms from Soil Particles by Detergent Action

	Viable Count/ml				
	No Treatment	Brij 35 0.01%	Brij 35 0.1%	Calgon 0.1%	Calgon 0.25%
Soil #1					
Immediate	1.7×10^5	2×10^5	2.1×10^5	1.4×10^5	1.5×10^5
22 hrs	4.3×10^6	4.6×10^6	4.0×10^6	8×10^4	7×10^4
Soil #6					
Immediate	2.4×10^6	3.0×10^6	---	---	---
22 hrs	8×10^7	8×10^7	---	---	---

7. Miscellaneous Processing Attempts

Certain efforts were made to flocculate the soil minerals by adding salts. These treatments invariably were toxic to the soil micro-organisms.

Other efforts involved a "dry-adsorption" technique. Starch, powdered sugar, echospheres (hollow glass particles) and polystyrene beads—all dry powders with particle sizes ranging from 10 to 100 microns—were added to soils in an attempt to adsorb the fine particles of the soil onto an excess of the inert powders. These powders, whose specific gravity was considerably less than the soil mineral particles could then be separated from the bulk of the soil by density flotation at high speeds and would act as "buoys" to the organism-rich small mineral particles that would normally be sedimented. In the case of starch and sugar, which are soluble in water, fluorochemical was used; in the case of the insoluble powders, Ludox was used.

Although preliminary studies demonstrated that the approach had certain merits, the advantages gained by this technique were insignificant, and our efforts were diverted to the more promising approaches described below.

8. Process Optimization

It has been shown above how certain processing-sampling techniques could enhance life detection in native soils. For example, selection of samples rich in small particles (either by pneumatic sampling or by sieving of classical samples) increased the total viable count/gm and increased the phosphatase readout; media clarification in high density fluids facilitated microscopic observation and turbidimetric analyses; presoaking and pre-incubating aided both turbidimetry and the phosphatase test.

A series of experiments was undertaken to evaluate the advantages of several of these processes when employed in combination with each other. Essentially we were interested in determining whether processing significantly accelerated life detection, using turbidimetry as a criterion. To accomplish this, two lots of soil (#1 and #21) were respectively sampled by classical and pneumatic means. The four samples were further subdivided into two subsamples each and were mixed with nutrient broth and high density (HD) nutrient broth respectively (1 gram/50 ml). The eight soil-broth suspensions were thoroughly shaken and again subdivided into two subsamples each. One series was pre-incubated on a shaker at 35°C for 3 hours and then centrifuged at 550 G for 10 minutes. The other series was centrifuged immediately. The sixteen clarified supernates were aseptically transferred to sterile bottles and incubated on a shaker at 35°C for 10 hours. At hourly intervals, aliquots were removed for turbidimetric readings and standard plate counts.

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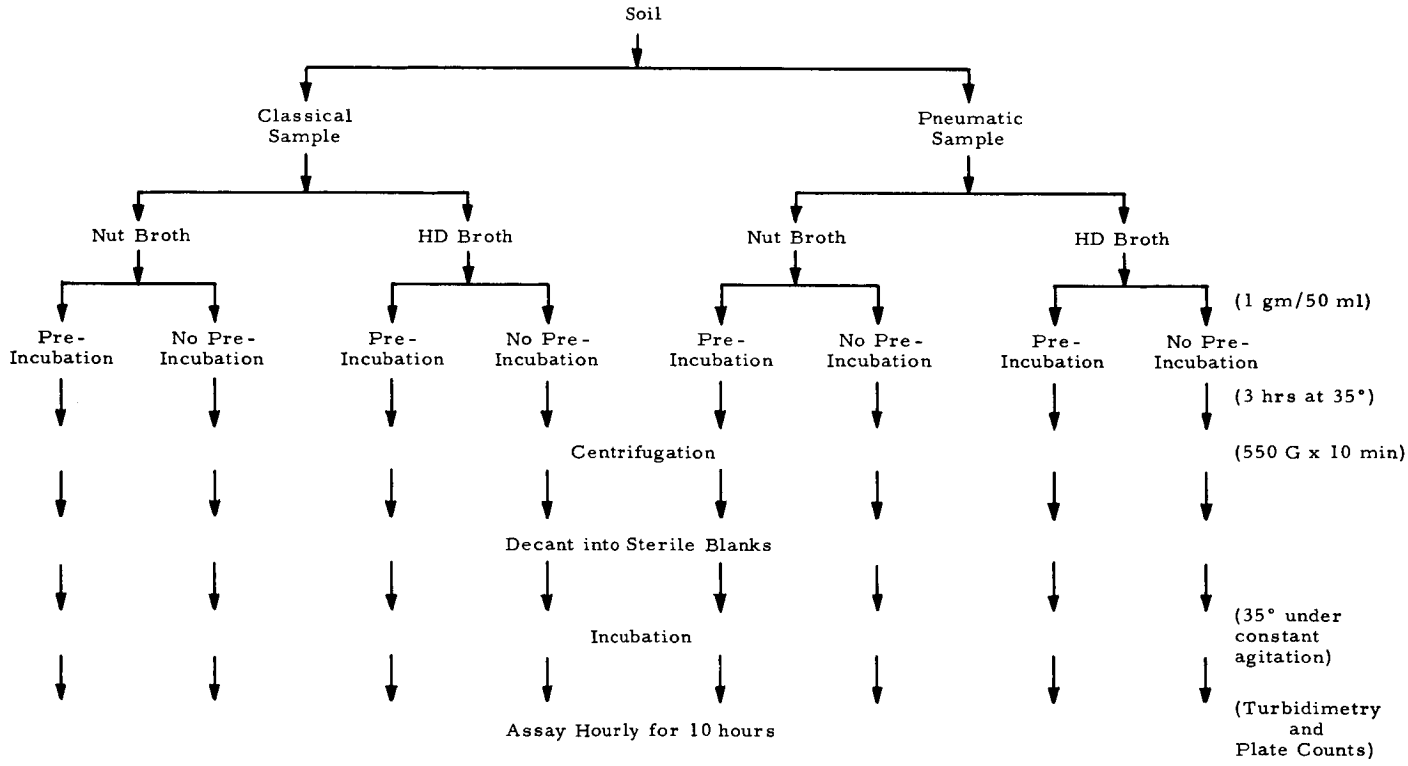
The experimental protocol is outlined below. Uninoculated (no soil) controls were run through the same sequence concurrently. The blank setting for the turbidimeter was either sterile nutrient broth or sterile high density broth where appropriate.

Experimental Protocol:



The results of these experiments are presented in Figures 3-17, 3-18, 3-19, and 3-20. The sterile controls showed no deviation from 100% Transmission and counts of < 300/ml during the ten-hour incubation period.

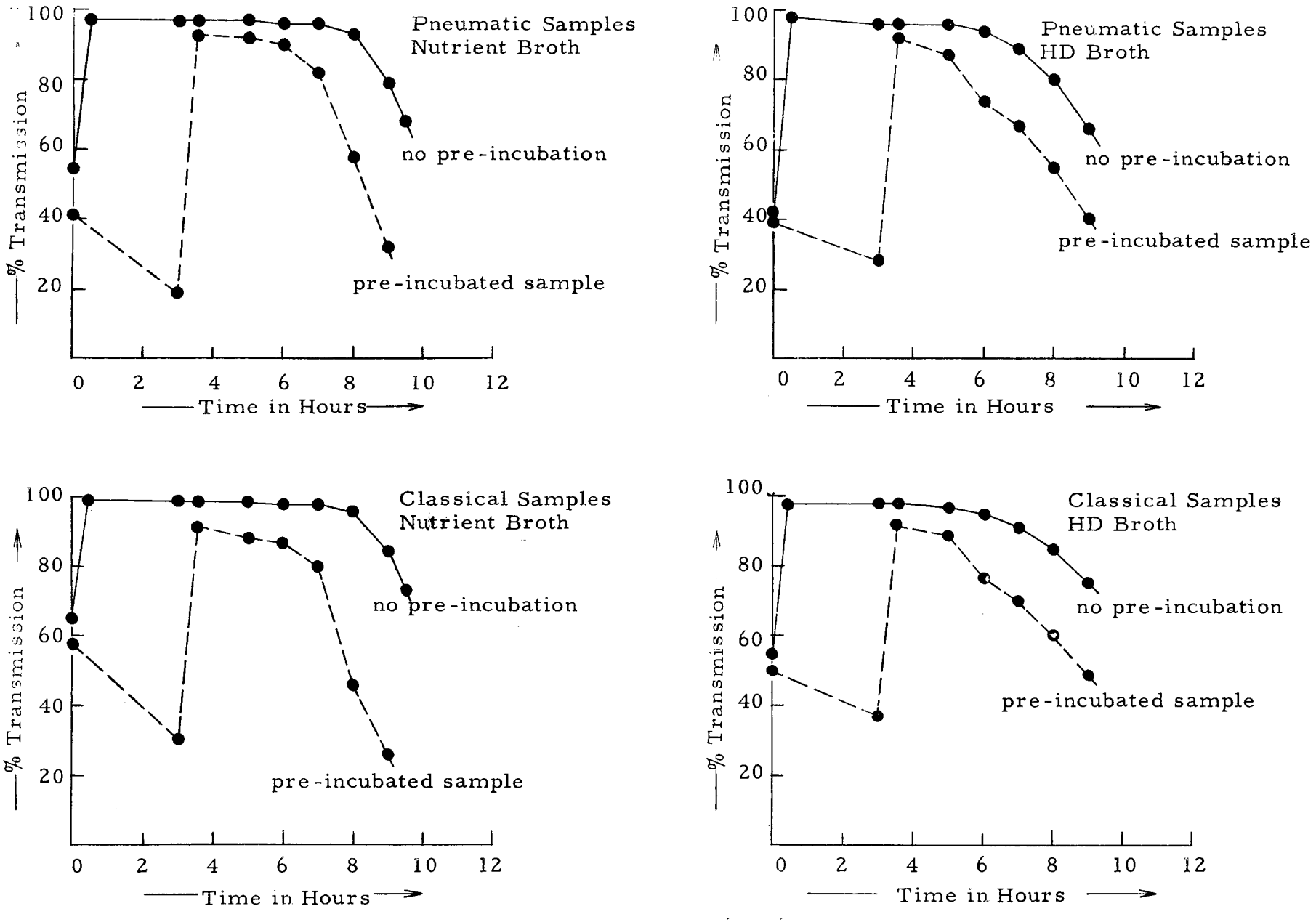


Figure 3-17. Turbidimetric Growth Curves on Soil #1 Relating Effects of Sampling Procedures, Density of Broth, and Pre-Incubation

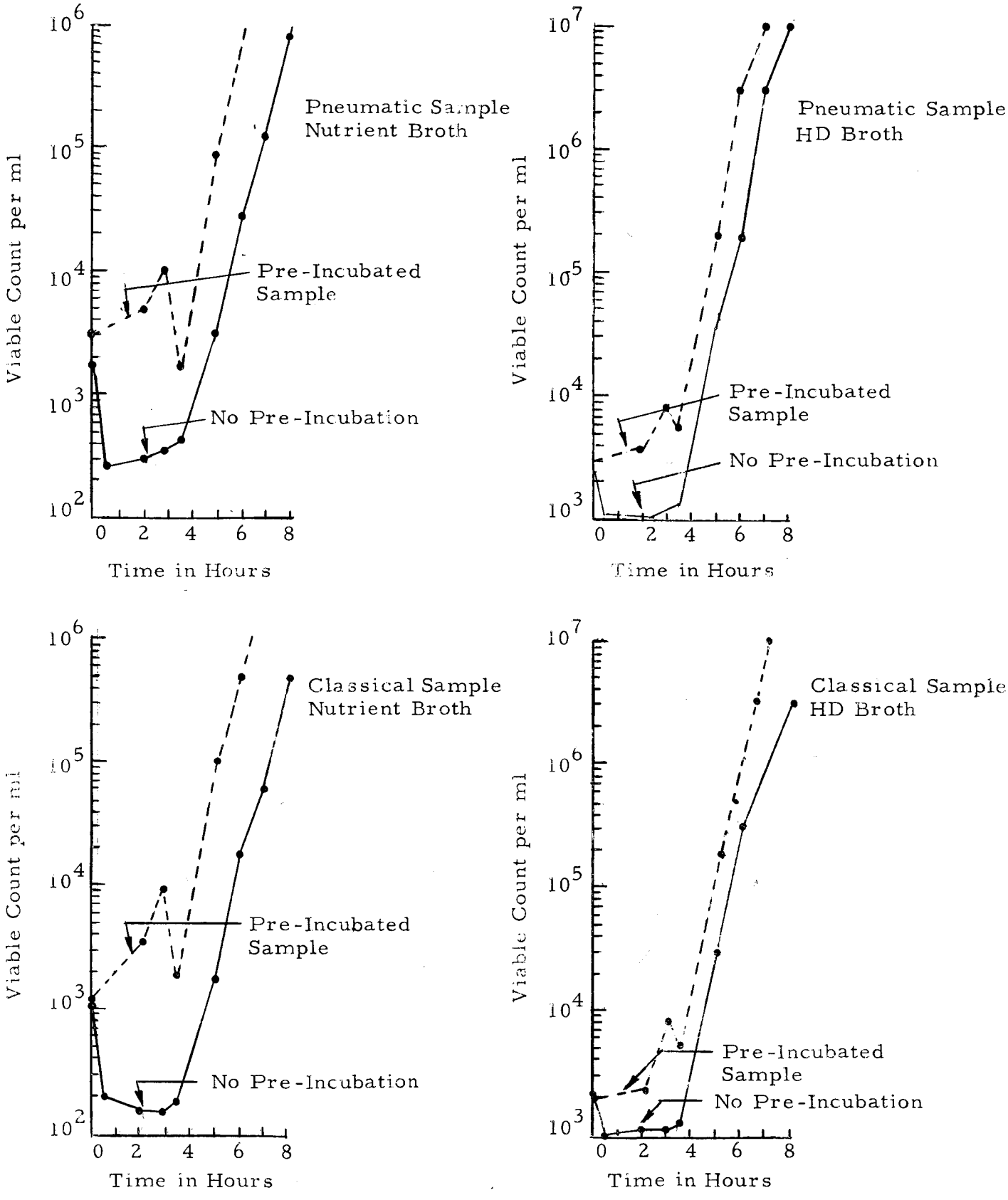


Figure 3-18. Viable Count Growth Curves on Soil #1 Relating Effects of Sampling Procedure, Density of Broth, and Pre-Incubation

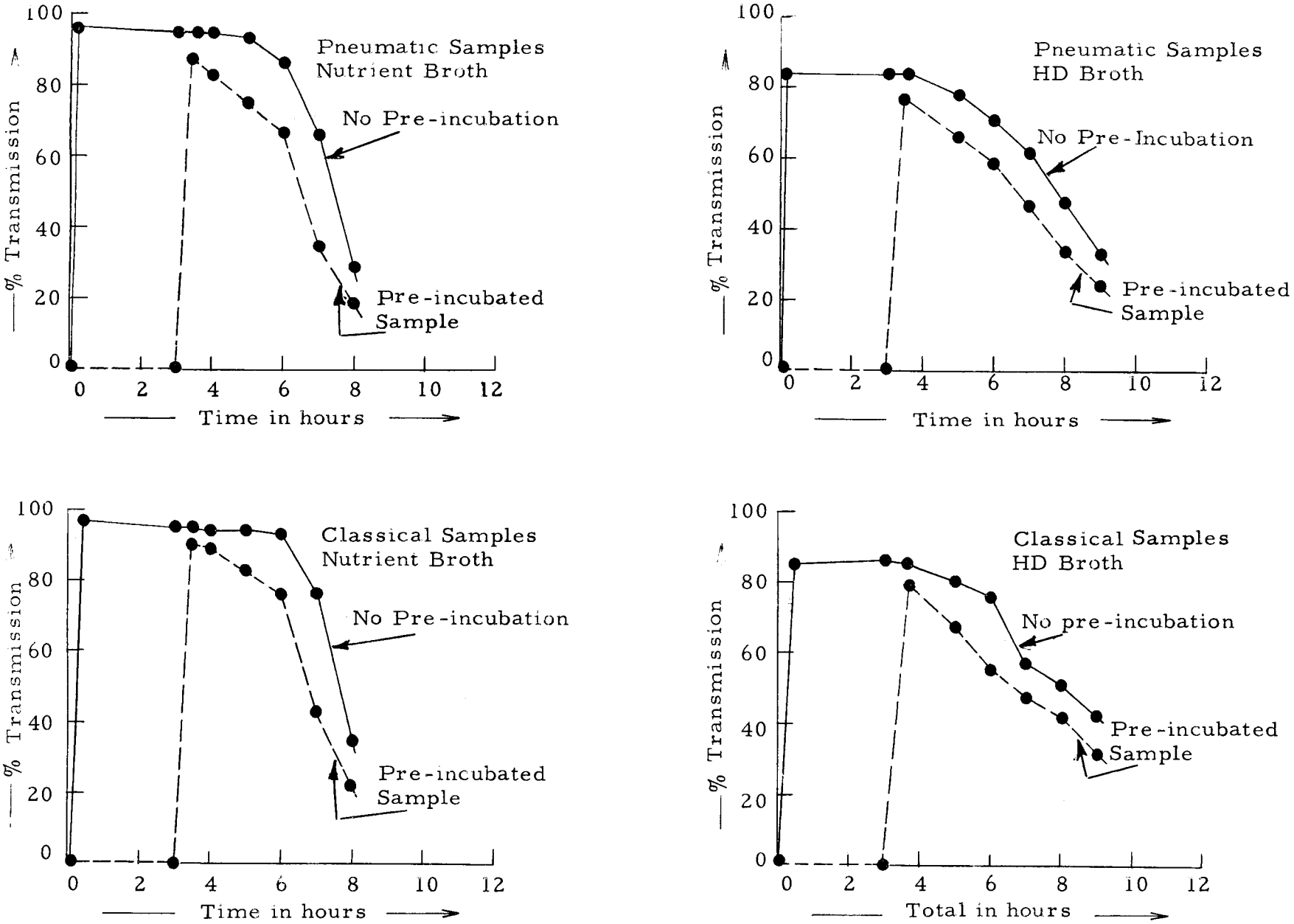


Figure 3-19. Turbidimetric Growth Curves on Soil #21 Relating Effects of Sampling Procedure, Broth Density and Pre-Incubation

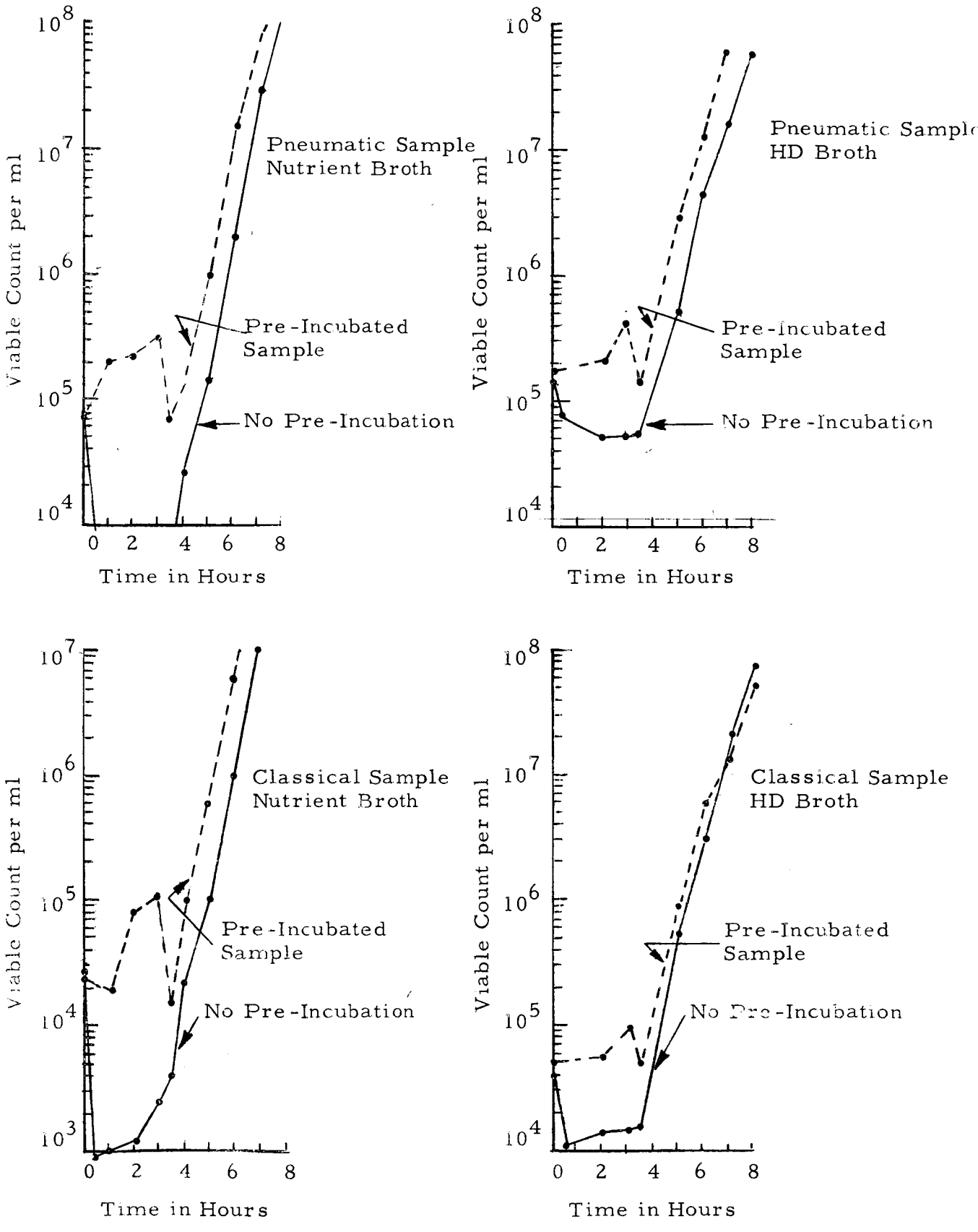


Figure 3-20. Viable Count Growth Curves on Soil #21 Relating Effects of Sampling Procedures, Broth Density, and Pre-Incubation

According to our hypothesis, the greatest enhancement (i.e., the fastest detection) would occur when a pneumatic sample would be pre-incubated in high density broth, and the least enhancement (i.e., the most delayed detection) would occur in a classical sample, centrifuged immediately after mixing, in low density broth. This hypothesis is predicated on the following assumptions:

1) Pneumatic samples contain a larger proportion of microbial-rich small particles.

2) High density broth retains the smaller particles and lighter (i.e., organic) particles in the supernate and permits the sedimentation of larger, denser particles, whereas low density broth would not discriminate between organisms and minerals.

3) Pre-incubation encourages the proliferation of unattached cells which are not sedimented as easily during centrifugation as the cells attached to mineral particles. Thus, a larger inoculum is retained in the supernate to initiate growth after clarification.

This hypothesis seems to be borne out by the data. In soil #1, most rapid detection of growth (5-6 hours) took place in the HD samples which had been pre-incubated, whereas slowest detection (8-9 hours) took place in the non pre-incubated low density broth. There did not appear to be any significant difference between the classical and pneumatic sample. Similarly, in soil #21, most rapid growth detection (3-5 hours) occurred in the pre-incubated HD broth, whereas slowest detection (6-7 hours) occurred in the low density non pre-incubated sample. In this soil there was a slight but inconsistent difference between the pneumatic and classical samples, with growth detection being favored in the former.

The viable count data for soil #1 also support the hypothesis. Using a 6-hour endpoint as an arbitrary criterion, we observed the highest count (3×10^6/ml) in the pneumatic pre-incubated HD broth sample, and the lowest count (2×10^4/ml) in the classical non pre-incubated low density broth sample. Furthermore, when the viable

count reduction occasioned by centrifuging is compared in the different experimental conditions, the assumptions underlying the hypothesis gain validity. In low density broth, centrifuging effected identical count reductions in both the pre-incubated and non-pre-incubated samples, suggesting no discrimination between attached and unattached organisms. On the other hand, in HD broth, centrifuging lowered the count in the non-pre-incubated samples more than it did in the pre-incubated samples, suggesting a difference in the density of the viable population (i.e., the probability that there were more unattached cells in the latter).

The viable count data for soil #21 suggest that this soil contains a large number of small low density clay particles to which the viable organisms are attached. Thus in low density broth, there is a considerable difference between pre-incubated and non-pre-incubated samples after centrifuging, whereas in the high density broth this difference is considerably diminished. Furthermore, these data illustrate the interaction between sampling technique, pre-incubation and density flotation. The pneumatic samples (which contains more small particles) all had higher counts after 6 hours incubation than did their classical counterparts. Similarly, the pre-incubated samples all had higher counts after 6 hours than did their non-pre-incubated counterparts. However, no real advantage was gained by combining pre-incubation and HD broth, when the 6-hour endpoint is considered. This might be due to some slight differences in growth characteristics exhibited by the organisms in high density and low density broth respectively. It appears that at the end of 4 and 5 hours, HD broth showed higher counts, but the rate of growth in normal broth was slightly faster, and by 6 hours the counts were equal.

G. Related Biological Experiments

During the course of this contract, a number of ancillary investigations were made which were generally related to the problems delineated in the contract Work Statement, but which were not strictly associated with sampling or processing as such. Among these investigations were the quantitative relationship between soil particle size and microbial concentration (alluded to in Tables 3-9 and 3-10); the fastest and most unequivocal method for life detection in soils with extremely low viable counts; and the characteristic "growth curves" of total soil organisms in mixed culture.

1. Relationship of Particle Size and Viable Count

Three soils which differed from each other in total viable count and organic carbon concentration were classified by sieve shaking into seven respective size fractions. Each fraction was plated and the total count plotted against the mean particle diameter of that fraction. These data are shown in Figure 3-21. They support fairly well the contention that the positive biological bias observed in pneumatic samples was not an artifact, but rather was due to the discrimination of that technique against soil fractions relatively poor in viable organisms.

It could be argued, with logical justification, that the soil plating technique itself creates an artifact which would generate such data as shown on these charts. This argument is based on the recognition that classified soil fractions suspended in water (i.e., dilution blanks) would sediment out at different rates due to gravity settling. Thus at any given time, in a dilution blank containing the >210 micron fraction, there would be fewer soil particles suspended in the body of the liquid than in a dilution blank containing the <40 micron fraction. Since pipette transfers are made from the body of the dilution water rather

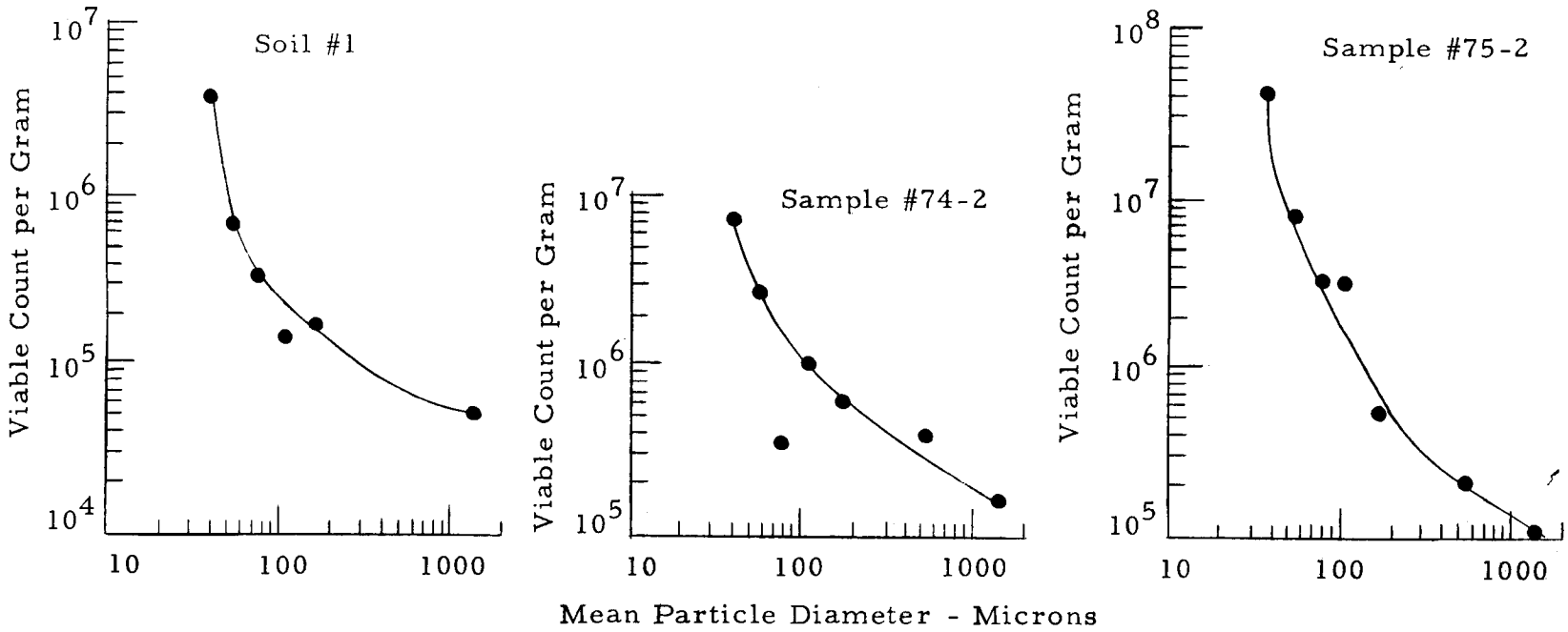


Figure 3-21. Relationship of Total Viable Count to Soil Particle Size

than the sedimented layer, the ultimate number of soil particles delivered to the plate from the former fraction would be less than from the latter fraction. And further, since most organisms are attached to the soil particles, the "apparent" total count representing the larger size fraction would be less than the count representing the smaller sized fraction. Despite the fact that the original soil suspensions are shaken before pipette transfers are made, the bottles do remain undisturbed for certain time periods, and the sedimentation described above may occur.

In order to ascertain the merits of this argument, a plating experiment was undertaken in triplicate, using three classified soil fractions, each diluted and plated in three different fashions. One treatment was classical: pipette transfers from dilution blanks were hand shaken and allowed to stand undisturbed. Two treatments employed pipette transfers from dilution blanks kept constantly agitated: one series involving test tubes in a vortex agitator, and another series involving sterile Waring blender heads operating at high speed. The data from this experiment are shown in Table 3-24.

Although the constant agitation series showed consistently higher counts than the classical series, it was evident that even when the particles were not permitted to settle, the viable count/gram was still inversely proportional to the particle size of the given soil fraction.

Table 3-24. Effect of Method of Suspension on Viable Microorganism Count

(Soil No. 21)

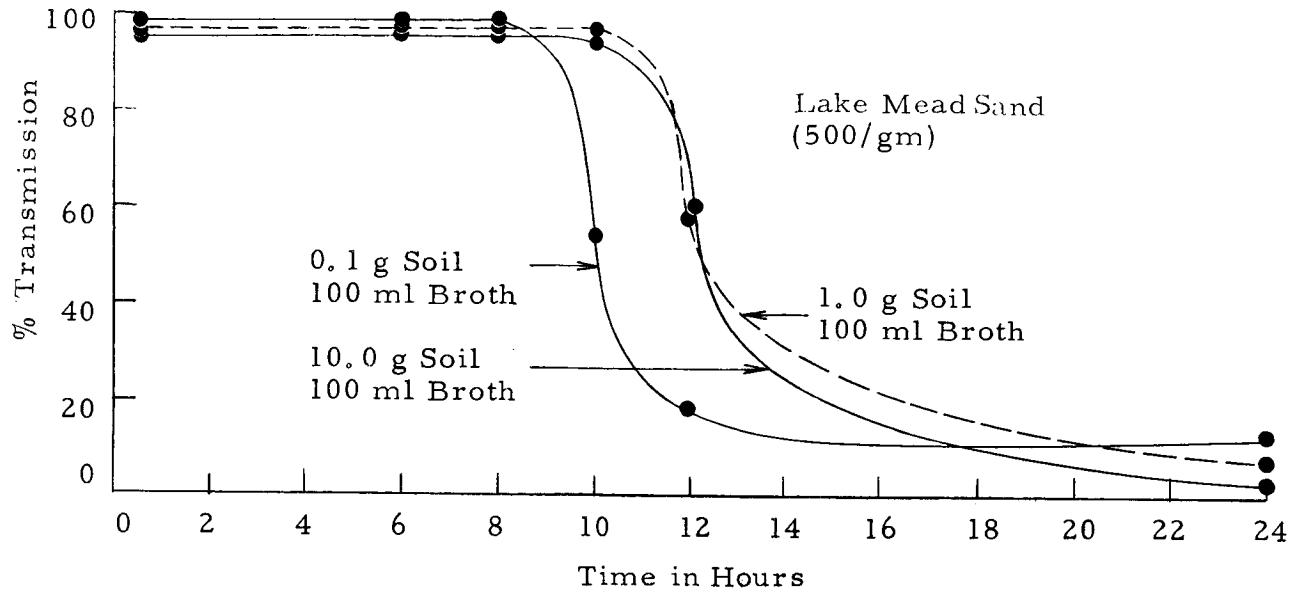
Particle Size Fraction	Method of Suspension		
	Hand	Vortex	Waring Blender
	(Viable Count per Gram		
<44 microns	6.0×10^6	8.0×10^7	1.0×10^7
44 to 210 microns	1.4×10^6	8.5×10^6	6.5×10^6
>210 microns	7.0×10^5	5.2×10^6	1.4×10^6

2. Life Detection in Soils with Low Viable Counts

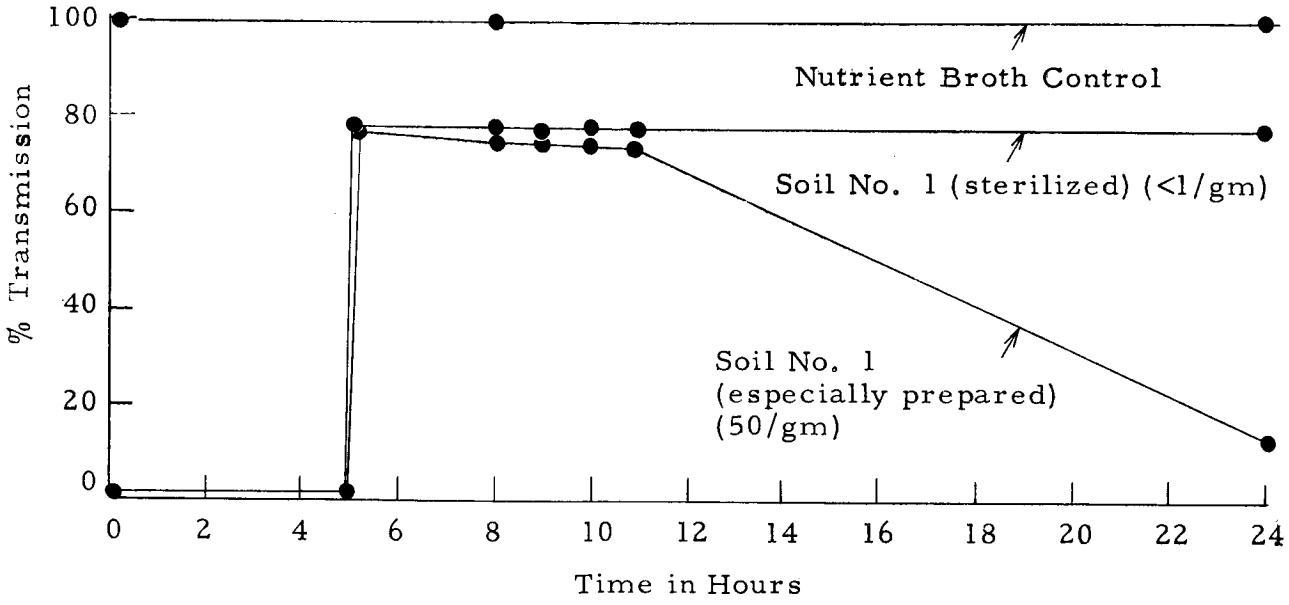
Two soils with particularly low viable counts were subjected to a more intense scrutiny than usual because of our understandable interest in samples whose response to life detection assays might be equivocal. One soil was Lake Mead sand with a viable count of ~500/gram. The other soil was a specially prepared mixture of sample No. 1 (0.01 gram raw soil and 100 grams of sterile soil) with a viable count ~ 50/gram.

Both samples were assayed for life by turbidimetric tests, ΔpH, phosphatase readout, and plate count. Neither the phosphatase test (17 hours) nor the ΔpH test (24 hours) indicated the presence of viable material in either sample. On the other hand, the standard plating technique and turbidimetry techniques detected growth. Figure 3-22 shows the turbidimetry results. It appears that the Lake Mead sample had, in addition to a low count, a growth inhibitor associated with it. As the size of the soil inoculum was increased, the time lag before growth could be detected increased. Nonetheless, the significance of this experiment lay in the fact that unequivocal growth could be detected in less than 12 hours by turbidimetry and not by any other method tried.

The "low count" sample 1 required a certain amount of special "coddling". Large inocula were used (100 grams to 100 ml broth), and were preincubated for 5 hours. Relatively low G forces were employed for clarification. Even so, growth was not evident until after 11 hours of incubation. This time was shortened in subsequent trials to 9 hours when Ludox was used in place of water.



A. Lake Mead Sand; No pre-incubation; centrifuged at 1000 G; Shaker incubation.



B. Specially prepared Soil No. 1 mixture: 5 hrs pre-incubation; Centrifuged at 550 G; shaker incubation. (100 g soil + 100 ml broth)

Figure 3-22. Turbidimetric Detection of Viable Material in Soils with Low Viable Counts

3. Soil Growth Curves

Most of the candidate detection techniques for Martian life forms have a characteristic threshold value which is a function of the specific detection device. If the original dust samples taken do not provide a high enough original population to meet the threshold value (which might range from several hundred to several million cells), an incubation step must be employed. During this period the microflora, if present, will be encouraged to propagate themselves and to reafh a sufficient number of cells compatible with the life detection experiment. Similarly, if microbial products are being sought, incubation will be necessary to permit growth and concomitant accumulation of biochemical products. Consequently, a fundamental factor determining success or failure of life detection experiments will be the growth characteristics of dust and soil organisms. Among these characteristics are the length of lag phase, the generation time during active multiplication, the duration of the active multiplication phase, and the ultimate maximum population attainable. These characteristics, for terrestrial organisms at least, are functions of the individual organism and the environment in which it finds itself.

To gain information about the growth characteristics of terrestial soil microflora the following set of experiments was undertaken: Eight different samples of soil were inoculated into nutrient broth (1 gram soil to 100 ml), were shaken to distribute the suspension, and were subdivided into two parallel series. One series was left undisturbed, the other was centrifuged at 1000 G for 15 minutes, and the supernates decanted. At hourly intervals for eight hours, aliquots were removed from the 16 bottles and plated on TGE agar. The plots of the standard plate count are shown in Figure 3-23.

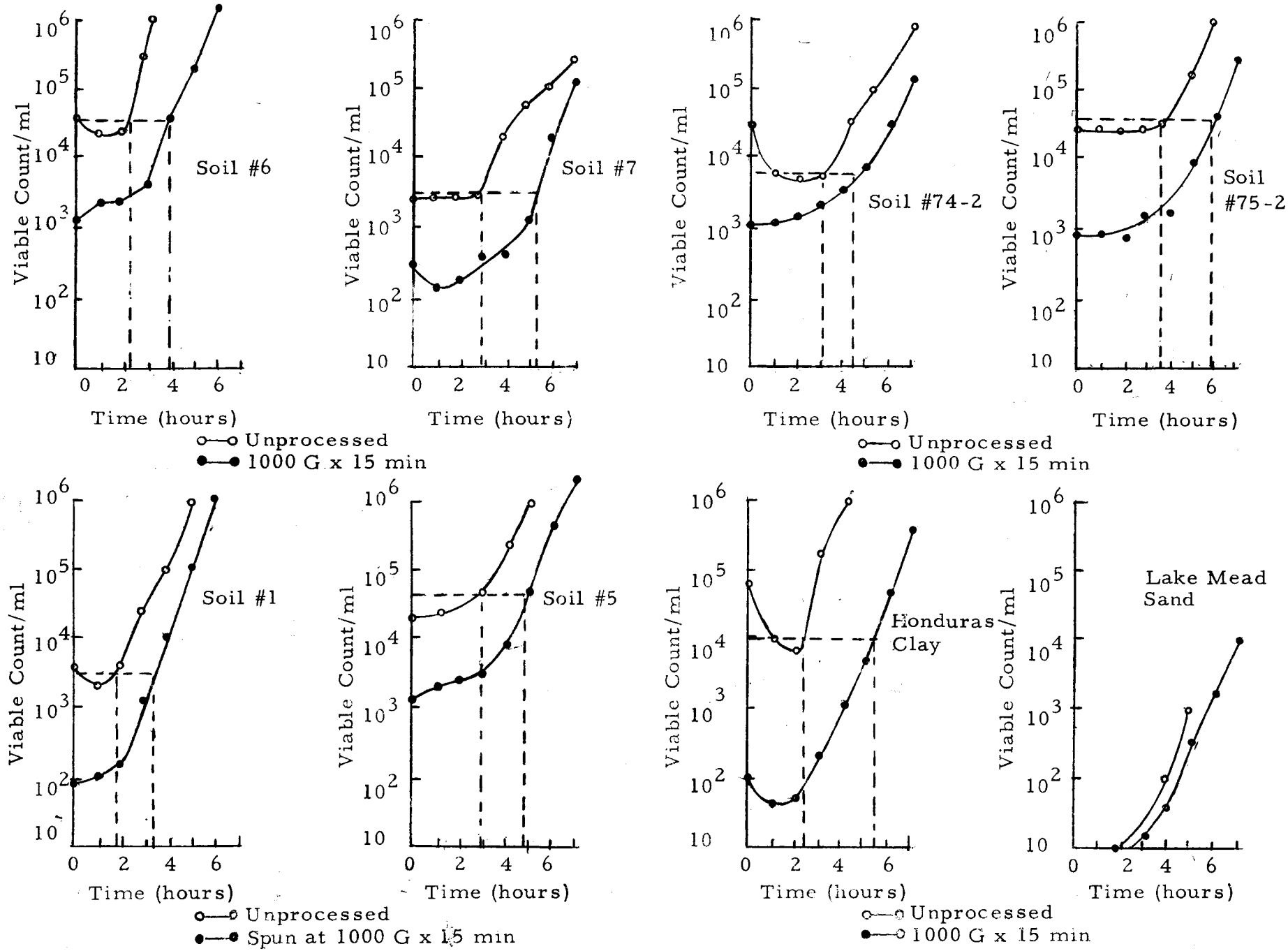


Figure 3-23. Growth Curves of Soil Organisms from Unprocessed and Centrifuged Samples

Three phenomena were observed:

1) The "growth curves" are more or less characteristic for a given soil and vary considerably from soil to soil.

2) The growth curves from the centrifuged sample and the undisturbed sample are quite similar—suggesting a qualitative similarity between the organisms of the two samples.

3) The time which elapsed before the centrifuged sample attained the original count (or the period of positive acceleration) in the undisturbed sample, was typically about 2 hours, and ranged from 1.5 to 3 hours.

Essentially, this experiment indicates that the advantage gained by clarifying soil samples is paid for by about 2-3 hours of detection time. Thus if time is a critical factor in detection, then techniques should be chosen which will work in a turbid menstrum. On the other hand, if several extra hours are not critical, then detection can be facilitated or imporved by centrifugation.

IV. SUMMARY AND RECOMMENDATIONS

The sample acquisition studies evaluated a pneumatic sampling method to obtain surface material for biological experiments on Mars. The program included investigation of soil aerosolization methods, pneumatic transport of particulates, and the collection of the transported particles. An air ejector was also investigated for use as the air mover for the pneumatic system.

The evaluation of the concepts was carried out at standard atmospheric conditions and at simulated Martian atmospheric conditions. An environmental chamber to produce low pressures and temperatures was built for that purpose. Natural soil samples were obtained for testing in the chamber .

Particulate pneumatic transport studies were conducted over a range of atmospheric pressures from 5 millibars to 980 millibars and at temperatures as low as -45°C. Glass beads and soil particles of 100 microns diameter were used in the studies. For horizontal transport at low pressures an air velocity of approximately 2000 ft/min or more was required for 100 percent transport efficiency through small diameter, smooth-walled tubing. Rough-walled tubing required considerably higher air velocity for the same transport efficiency. Vertical transport can be accomplished with a lower air velocity than the horizontal transport due to settling of the particles in the horizontal section of the tube.

With the data obtained in the transport studies, a pneumatic field sampling unit was designed and built to study the aerosolization of soils. Tests were run on native Minnesota soils in the field and in the environmental chamber. A high velocity air jet was adequate for loose soils, such as sand, while additional mechanical agitation of the surface was required for hard materials.

An air ejector for use as the air mover in a pneumatic sampling system was investigated for use in a Martian atmosphere. The effects

of mixing tube length, the ratio of primary nozzle area to the secondary nozzle area, the ratio of primary air pressure to ambient air pressure, and the diffuser length and angle were studied. It was found that a small air ejector could be built to operate satisfactorily in a pneumatic sampling system at ambient air pressures as low as 5 millibars.

The biological support phase of this study was primarily concerned with evaluating various sampling and processing concepts with regard to their enhancement of or interference with life detection in native soil samples. The sampling concepts evaluated included pneumatic techniques and a variety of classical methods. Clarification by centrifugation (both batch and continuous), density flotation in a variety of fluids, pre-incubation and presoaking, ultrasonication, and detergent treatment were included in the processing steps evaluated.

To evaluate any biasing effects of sampling and processing techniques, a battery of tests was employed. Among these were moisture analysis, specific gravity, bulk density and organic matter determinations, particle size analysis, direct microscopic observation, total viable counts, pH and Eh change, ATP and phosphatase determinations, micro-respiration, and turbidimetric growth analysis. Among the various soil types studied were sands, gravels, limestone debris, hydrous iron oxide, volcanic ash, loams, silts and clays.

The method of sampling introduced a significant quantitative and qualitative bias that was reflected in a sample's physical, chemical, and biological characteristics. Pneumatically obtained soil and dust samples had a larger proportion of their mass associated with small particles than did any classical samples. This in turn was usually reflected in a higher organic matter content, a higher alkalinity, a slightly higher plate count, and a faster phosphatase readout. On the other hand, there was no essential difference between pneumatic and classical samples in qualitative microbial distribution, turbidimetric growth detection, and microrespiration. In general, it was found that an inverse relationship

exists between sample particle size and microbial content; sampling techniques which discriminate in favor of fine dusts usually provided microbially-rich samples. Both classical and pneumatic soil sampling techniques were compatible with microrespiratory, turbidimetric, and enzymatic means of life detection, as well as with most classical chemical and microbial analytical procedures.

With regard to sample processing, there was a close dependency between the life detection criterion chosen and the need for processing. Certain detection techniques (e.g., microrespiration) could be employed with crude, unprocessed samples. Other techniques (e.g., microscopy, turbidimetry) required some type of clarification and microbial purification before results could be read out.

The most useful processing procedures involved the combination of preincubation and density flotation in a Ludox broth. Other processing steps, such as ultrasonication, detergent treatment, mineral flocculation, and dry adsorption did not show too much promise in facilitating life detection. Similarly, a variety of other high density fluids were evaluated and were ruled out because of such objectionable features as visosity, toxity, and immiscibility with nutrient solutions.

To extend this project toward a logical goal, it is now necessary to proceed in four directions: 1) Evaluate other sampling concepts and devices from a biological point of view; 2) Evaluate candidate life detection concepts and devices with regard to the need for processing steps and the concomitant complexity involved; 3) Test a larger variety of terrestrial soils and dusts by the battery of tests used in this project; 4) Build a pneumatic sampling system for use with any life detection device.

1) It is now possible to state that pneumatic sampling is compatible with a variety of life detection techniques and that any bias introduced by pneumatic sampling will probably facilitate or accelerate life detection.

Acceptance or rejection of pneumatic devices for Martian experiments can now be based on engineering, geological, and logistic considerations alone. Since a variety of other candidate sampling concepts have been fabricated or proposed, it should now be possible to evaluate them in a similar fashion. If a sampling device or concept is incompatible with, or influences, biodetection, this should be known before any further effort is expended on its development.

2) A number of candidate Martian life detection experiments have been suggested and are being developed. These should undergo a preliminary phase of evaluation, using native soils and dusts. Feasibility of any candidate experiment will depend less on its ability to detect pure cultures of laboratory organisms than on its ability to work with crude soils. If processing steps are needed to prepare the soil for analysis, this processing must be considered as part of the experiment. Therefore, acceptance or rejection of a life detection device might in part depend on the added weight, power, and engineering involved in processing.

3) The tests employed in the present project were chosen because their principles represented a cross section of Martian life detection concepts, and because they could be performed in the laboratory. On the basis of this work, we became aware of the advantages and limitations associated with each test. However, only a limited number of soil types were studied. It should now be possible to gather hundreds of soil samples and evaluate them in the laboratory by a combination of these tests. If, on the basis of this broad spectrum, it is shown that life detection is unequivocal (i.e., all techniques agree with each other), then candidate life detection experiment can be chosen on engineering and logistic bases. On the other hand, if life detection is equivocal (i.e., the techniques disagree with each other), we will have a better basis on which to evaluate candidate life detection concepts.

4) To facilitate the evaluations proposed in 1) and 2), a suitable pneumatic sampling system should be available for testing. It will then be possible to determine in each life detection experiment what, if any, bias is introduced into that experiment by pneumatic sampling as compared with other sample acquisition concepts.

In essence, before any attempt is made to detect life on Mars, a great deal more should be known about the influence and interaction of sampling technique, processing technique, and detection technique on life detection in terrestrial soils.

APPENDIX

SAMPLE ACQUISITION - A PROBLEM IN EXOBIOLOGICAL RESEARCH

Seminar Presented at Jet Propulsion Laboratory
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by
Dr. V. W. Greene
Applied Science Division
Litton Systems, Inc.

APPENDIX

SAMPLE ACQUISITION: A PROBLEM IN EXOBIOLOGICAL RESEARCH

I. INTRODUCTION

In the short span of six years since the passage of the National Aeronautics and Space Act (1958), the scientific and technological activities of NASA have had an exciting influence on nearly every branch of American scientific endeavor. This influence has been particularly noticeable in the biological research community wherein NASA has stimulated investigations in biophysics, spacecraft sterilization, life support systems, environmental biology, and exobiology.

Although the relative level of research in exobiology is small when compared to the overall NASA program and budget, the impact of this program on basic and applied biological investigation cannot be underestimated. Ranging in scope from molecular genetic studies to balloon and rocket explorations of our own biosphere, exobiology has already exerted an orientation and an influence on classical biology well beyond its budget and "youth".

The most intriguing mission of the exobiology program is the search for life on other planets. This program involves technological and theoretical problems never before encountered by man. Among these problems, techniques for sample acquisition and analysis pose prime challenges. This is particularly true if the initial probes involve unmanned, automatic, instrumented approaches.

If the first landing on a planet is made by a manned craft, sample acquisition and analysis could obviously be performed by people. On the other hand, if the first landing is made by an instrumented probe,

mechanical devices will have to substitute for man. Furthermore, if the first landing is delayed until a large complex automated laboratory can be employed, the problems of sampling and detection will be considerably different than on a probe where weight and power are extremely critical and limited. The whole question is further complicated by the availability during the next two decades of only a few optimal opportunities to launch a probe toward Mars and the consequent need to advance the state of the art in an area with a very poor backlog of basic knowledge.

The choice between manned vs. instrumented landings, and the tradeoffs between complex laboratories vs. simple experiments are functions of cost, philosophy, and politics as well as technology. Technology may not wait, however, until the aforementioned considerations are resolved. The possibility exists that an instrumented life detection probe may be directed toward Mars in the next few years. Many suggestions have already been made regarding the type of detection device(s) that should be included. The purpose of this discussion is to ascertain and to elaborate the problems of sample acquisition for these automated probes.

The subject will be approached by a brief review of the interfaces that exist between sampling and life detection. This will be followed by a more detailed discussion of the biological, mechanical, and statistical considerations involved. Finally, some suggestions will be made about the path that should be followed in resolving, experimentally, some of the problems raised.

II. SAMPLING AND LIFE DETECTION

It is possible to conceive of a "life detection" program without sampling, and indeed without any laboratory analyses at all. This conception visualizes macroscopic life forms as we know them on this planet, i.e., trees, animals, birds, fish, or even mosses and algae. The detection of this type of biota requires such apparatus as cameras or microphones or long-range reflective spectroscopes. If Mars is populated by visible or audible creatures, the problem of NASA's exobiology program will be greatly eased (though still intriguing). It is toward the realm of microbiology that extraterrestial life detection has become oriented, and it is in this realm that the technology becomes imposing.

There are vast stretches of our own planet which appear barren and sterile to the casual observer, and which are nonetheless inhabited by countless numbers and types of microscopic living creatures. It has become axiomatic that there is no area of our planet which is sterile—that wherever macrobiota exist, there are also present microbiota, and further, even where macrobiota are not found, microbial representatives are always present. Although this generalization is made on the basis of some very limited observations (certainly not enough is known about the biology of the Himalayas and the arctic wastes to talk about "no area of our planet. . ."') the fact remains that if the search is sufficiently diligent, microbes will be found in nearly any body of water, sample of dust, or volume of air. Consequently, it has been reasoned, a search for life on Mars should take the form of a microbiological experiment. If anything living exists on the planet, they will either be microbes or have microbes associated with them.

The crux of the problem, glossed over rather glibly above, are the words "if the search is sufficiently diligent". It is no great accomplishment to find bacteria in one's backyard, or now that it has been done,

in storage tanks of jet fuel, or in Old Faithful. It is not even very difficult to find them in the air, or on hospital walls or on "sanitized" toilet seats if one becomes familiar with the good public health techniques. It does become a little more challenging to find them at altitudes of 20 km, or at the top of Mount Everest, or in the Phillipine trench. It may be fairly said that the difficulty of the challenge is a direct function of the following variables: Getting to the desired location, getting a representative sample, and choosing the best means of analysis.

These variables are three of seven basic steps involved in any microbial detection "experiment", whether it is conducted on Mars or on earth, whether it involves Sahara desert soil or noctilucent clouds. The complete environmental microbiology operation consists of the following identifiable processes:

1) To reach the environment in question with sampling equipment that will not bias the result.

2) To acquire or study a representative and sufficiently large sample which will yield a meaningful inference about that environment.

3) To transport the sample to a suitable location for analysis or to an analysis apparatus.

4) To process the sample preparatory to analysis.

5) To analyze by a process or instrument whose readout will be unequivocal for the microflora and/or microfauna of that environment.

6) To read out the information and interpret it quantitatively and/or qualitatively.

7) To communicate this information to those interested.

In certain instances several steps (e.g., 3 and 4; 2 and 3; 4 and 5) may be combined, such as by layering an indicator or medium directly over the area to be studied; similarly steps 6 and 7 are often combined. However, the basic seven operations are essential for the majority of

environmental biology experiments. In case some doubt is entertained about step 4, it should be recognized that diluting dust in water, or simply leveling out a pinch of dust on a microscope slide should be considered "processing".

These steps are performed in the simplest of soil bacteriology experiments and will probably have to be performed in the most esoteric exobiological probes. In the first instance, we usually send a technician out to do the job. She (1) goes out with a sterile spatula or trowel, (2) gets a scoop of dust weighing several grams, (3) brings the dust back to the lab, (4) weighs it and makes the necessary dilutions, (5) plates it out on one or more appropriate media, (6) incubates and counts the plates, and makes some cursory examination of colonial morphology, and (7) tells you about it when she is done. If she is really just a "sterile-nonsterile" detector, she will (4) aseptically add some dust to sterile broth, (5) incubate it for a day or so, (6) observe for turbidity and (7) tell you about it.

Unfortunately, on an unmanned Martian probe we will not have this indispensible technician. Each of these steps will have to be studied, designed, engineered, programmed, tested and evaluated so that they can be carried out by instruments. The techniques of "getting there" and choosing the area of study is the concern of the propulsion and navigation experts. Similarly, the problem of transmitting the data belongs to communications experts. Interpretation will belong to the philosophers. Detection and analysis is presently being approached in a number of different laboratories by a variety of methods and concepts at different stages of development. It is in the area of sample acquisition, sample transport, and sample processing that no comprehensive and systematic program has as yet been undertakne. And it is to this area that the following remarks are directed.

Since sample acquisition on earth is relatively simple compared to sample analysis, it might be thought that the former may be neglected

or postponed for exobiological probes until the latter is solved. This reasoning is fallacious. Ultimately the type of detection device used might depend on the type and quantity of sample obtainable. The best life detection instrument cannot perform without a sample or with an inadequate sample. As indicated above, our best terrestrial laboratories with all of their instruments and trained technicians can know very little about the microbiology of the Phillipine Trench or the 20 km stratosphere until someone brings them a good sample of those environments. (And getting there is only the first step.) Indeed, the power and weight limitations imposed on sample acquisition might become as critical to the success of a Martian probe as the power and weight quotas assigned to detection, readout and communications. It cannot be emphasized too strongly that whereas the drama and breakthroughs of life detection are occupying a great deal of time and money, the success or failure of these experiments might depend on the time and money spent on studying sampling.

III. SAMPLE ACQUISITION AND CANDIDATE EXOBIOLOGY EXPERIMENTS

A variety of techniques are being considered for life detection on Mars (Table A-1). One of these (Gulliver) has reached the prototype experimental model stage; the others are at various levels of contemplation and/or development. Even though all of these concepts must ultimately be mated with some type of sampling-transport device, and some must consider additional processing between acquisition and analysis, the approach to sampling has been so fragmented among the different investigators that no clear-cut picture of feasibility can be gained.

Thus, Gulliver and Marbac depend on the "sticky-string" approach. Most of the others depend on some type of pneumatic device. Several have not yet reached the stage of development where sampling is important. Among the investigators in exobiology, two schools of thought have arisen relative to the interface between analysis and sampling: One group holds that the complete experiment be considered as an integrated concept, i.e., that the detection device includes the sampler, transporter, processor, analyzer and readout instrumentation. The other group is willing to segregate analysis from the other procedures and to concentrate on the detection work per se—leaving acquisition and transport to researchers especially qualified in that field.

Both arguments have merit, although at times the merit becomes clouded by proprietary interests. It should be pointed out, however, that the technology of sampling on Mars is a sufficiently complex enterprise, involving mechanical, biological and statistical inputs, so as to merit more than what time and money is left over from "pure" life detection experiments. We submit that a comprehensive program to study and develop sampling instruments would on the one hand liberate qualified workers to upgrade detection systems, and on the other hand, would provide information (and ultimately samples) to any specific candidate experiments chosen for flight.

Table A-1. Candidate Experiments/Concpets for Martian Life Detection

	Name of Experiment	Laboratory	Basic Technique	Type of Sample Required	Amount of Sample Required
1.	Gulliver	RRI	CO_2 evolution (respiration)	dust	100 mgm
2.	Minivator	JPL	Turbidimetry	dust	10-100 mgm
3.	Wolf Trap	Univ. of Rochester	Turbidimetry pH	dust	5-10 mg
4.	Marbac	Marquardt	Eh	dust	10-100 mgm
5.	Gas Chroma-tography	JPL Ames	Chromatography of pyrolized end products	dust	100 mg-10 gm
6.	Microscope	JPL	Visual and UV obser-vation	dust	10 mg
7.	APT Assay	RRI	Bioluminescent of luciferin-luciferinase system using ATP as catalyst	dust	?
8.	Fluorescence	JPL	Primary and secondary fluorescence of organic matter	dust	10 mg
9.	Multivator	Stanford	Assay for enzyme and growth	dust	1-10 mg
10.	Optical Rotary Dispersion	Melpar	Polarimetry of bio-chemicals	dust	?
11.	J Bands	Aeronutronic	Binding of dye by or-ganic compounds	dust	?
12.	Macromolecule Analyzer	Florida State	Chromatography of hydrolyzed organic compounds	dust	100 mg-1 gm

IV. BIOLOGICAL CONSIDERATIONS IN SAMPLE ACQUISITION

It might be assumed that the first instrumented explorations searching for Martian life forms will be relatively crude and qualitative. The most important question to be answered should be of the "yes" or "no" variety rather than "how much" or "what kind". Once the answers regarding presence or absence of life will be available, subsequent experiments will have to be designed to determine something about relative abundance of life, and the characteristics of the living creatures present. However, the cost and effort involved in any given probe, as well as the limited celestial opportunities to launch a probe require exobiologists to try and gain as much information as possible from any given experiment. Thus, even the initial probes should try to ask quantitative and analytical questions at the same time as they try to detect life.

It is necessary, therefore, to think of sample acquisition from a semi-quantitative point of view if at all possible. Equally as important, even "yes or no" detection experiments may be biased if insufficient attention is paid to quantitative sampling. As a simple example we may consider three soils containing respectively 10^6, 10^4, and 10^2 organisms per gram (such distributions are not uncommon in natural soils). A sampling device which acquires a 10 mgm sample and provides it to a foolproof "detector" will be quite satisfactory for the first two soils, but could bias the results from the last soil. In other words, living matter exists and may even be abundant, and the detector itself may be reliable and unequivocal—but because of a sampling inadequacy, the "yes-no" answer will be equivocal.

We have encountered this frustration in our stratospheric biology program. Whereas at ground level the airborne microbial count is somewhere in the order of 1-100 organisms/ft^3 and 1 cfm samplers are adequate for microbial detection, at higher altitudes the airborne viable concentration decreases considerably (though at an unknown rate).

Explorations to ascertain the mere presence of viable organisms above the tropopause succeed or fail simply on the basis of the sample volume they are programmed to acquire. If the count is 10^{-4} organisms/ft^3 and the sample volume is 10^3 ft^3, the probability of detecting one organism is 1:10. If power and weight and cost limitations permit fabrication of a sampler which can sample 10^4 ft^3, or the fabrication of ten samplers which can sample 10^3 ft^3, which alternative shall be chosen? This situation is presented simply as a realistic exercise in sampling logic and illustrates the fundamental importance of the quantitative aspects of sample acquisition in explorations of unknown environments. Again it emphasizes the interplay between sampling and detection, in which success of detection techniques depend as fully on the sample obtained as does the overall success of the probe depend on adequacy of analytical process.

It is quite possible that the "sampling tail" will ultimately wag the "detector dog": The type of sampling device chosen might dictate the type of analytical instruments selected. If, for example, the sticky string approach is compatible with only CO_2 evolution experiments, and the pneumatic sampler can provide multiple and quantitatively measurable dust aliquots to a variety of detectors, practicality might dictate a redesign of "Gulliver" so that it too can handle a pneumatic sample. There is little question that the probability of success of a life detection experiment is considerably improved if several detectors can serve as "backups" or verifiers of each other. Consequently, any device which can acquire fairly large samples that can be subdivided into replicates (each to be analyzed independently) would be the sampler of choice, and the analytical devices launched would be designed to take advantage of this approach.

Just as sampling technology may influence quantitative microbiology, so must it also concern itself with certain aspects of qualitative bias. This is probably not very significant in an extraterrestrial

situation, where the problems of detecting unknown life forms are overwhelming compared to sampling them. However, in terrestrial situations, it is known that the sampling technique per se can influence the type of organisms recovered, as well as their numbers. Thus certain types of aerobiological samplers are deleterious to moisture sensitive strains of bacteria and viruses; certain types of samplers have a built-in bias for or against particles of a certain size (and consequently the type of organisms characteristically associated with that size of particle); and certain techniques of sample processing may promote the development of some types of organisms while inhibiting the development of others.

An extremely important sampling consideration exists at the interface of landing technology and biology. If the capsule is decelerated by some type of parachute system, samples for life detection may be taken anywhere in the vicinity of landing. If, however, retrorockets are employed to ease the impact, the possibility exists that the soil in the immediate vicinity of the capsule will be heat sterilized. This possibility implies that a sample for life detection will have to be acquired at some distance from the capsule, and transported back for analysis. Some work should be undertaken to ascertain the sterilizing effect of retrorockets on soil, and in particular, the potential "non-productive" area should be delineated. Ultimately, the solution to this problem (i.e., acquisition and transporting a soil sample "x" meters from the capsule) might be one of the more critical in determining the success or failure of a life detection experiment.

Biological considerations will also be involved in the decision about what to sample. It is generally assumed that a surface sample of dust will be adequate. However, it may be more desirable to obtain a subsurface sample, in which any microbiota would have been protected from dessication and lethal radiation. On earth it is not uncommon to find greater microbial concentrations at 10 cm below the surface than directly at the surface itself. The same situation could also exist on Mars.

A great deal of effort is presently being devoted to problems of spacecraft sterilization. These endeavors are obviously of first ranked importance for a life detection experiment. Is is patently obvious that any contaminants introduced by the equipment designed to search for life will irrevocably ruin not only that particular experiment, but possibly any subsequent trials. Until the techniques for spacecraft sterilization and aseptic launching can be completely solved, any life detection experiments are beyond the state of the art.

V. MECHANICAL CONSIDERATIONS IN SAMPLE ACQUISITION

In theory, there are six fundamental approaches to the acquisition of soil and dust samples for microbial analysis by inanimate or mechanical devices:

1) The shovel, claw or drill concept.

2) The electrostatic or magnetic concept.

3) The "fly paper" or sticky-string concept.

4) The pneumatic-aerosolization concept.

5) The explosion-aerosol sampling concept.

6) The liquid expulsion and suction concept.

In addition, there is a seventh approach alluded to earlier. This concept actually does not involve sample acquisition as such, but rather attempts to carry out a life detection experiment in situ, on the undisturbed soil surface. Thus, any problems of sampling, transporting and processing are eliminated and replaced by the mechanical problems of getting the life detection apparatus to the sample rather than vice versa.

Before reviewing these seven approaches in greater detail, certain generalizations may be made that are applicable to any discussion on mechanical sampling problems. It must be understood that two major parameters whose dimensions are today still unknown will largely determine the choice of life detection experiments for a Martian mission, and consequently the type of sampling machinery that is necessary. Until we have some reliable knowledge about the surface and topography and atmosphere of Mars on the one hand, and some more detailed input about the permissible weight, power, space and cost limitations of the capsule on the other, we are dealing in metaphysics rather than microbiology. Much good research on sample acquisition may be done (and indeed should be done) under terrestrial conditions. But the ultimate

tradeoffs that must be made between reliability and complexity, weight and power, mission objectives and mission budget will have to wait for some realistic assessments of the Martian physical environment and the capabilities of the launch vehicle.

Nonetheless, some guidelines may be contemplated now based on some educated guesses and astronomic observations. We should be prepared for a dry, hard, windswept surface. The atmospheric pressure will probably be in the order of 30 mb. The power available for detection will be in the order of 10 watts, the weight of life detection and sampling devices will be in the order of 10 lb and will occupy in the order of 1 cubic foot. Sampling and detection should take place within hours rather than days, and the equipment should have as few moving parts as possible. Most of the energy available should be used for readout and interpretation rather than sampling and detection, and as many things as possible should be learned from the first sample acquired in case environmental or technical conditions preclude a sequence of sampling.

Some other generalizations may be made about the type of sample that should be obtained during the first probe of an unknown planet. It is obviously highly desirable to gain insight about as large an area as possible. If the sampler is mobile, this means that several spot samples of surface and subsurface material should be taken from different locations. If, however, mobility is restricted to one sampling site, then as large a quantity of fine surface dust should be obtained as is practically feasible. Under these circumstances, dust would be much more representative of a large area than would a single core of soil, or a large piece of gravel. Quite often, a sample of dust represents both the local site and also wind-borne fallout from other areas. Furthermore, it can be shown that on earth, at any rate, there is an inverse relationship between particle size and microbial concentration. A gram of soil in which 80 percent of the particles are < 200 microns contains 10-100 times as many viable organisms as a gram of the same

soil in which 80 percent of the particles are > 200 microns. Since the objective criterion of sampling efficiency is the weight of sample acquired for a given expenditure of energy, it would be well to concentrate on those techniques which acquire the bulk of their sample as fine particles.

On the basis of the above mentioned projections: Economy of weight, power, space and cost; operability and versatility in unknown and inimical environments; and the desirability of large samples of fine dust—it is possible to evaluate the seven basic approaches to mechanical sample acquisition. These comments, however, should be prefaced by the acknowledgement that few of the techniques have been systematically studied and that consequently any criticism is not directed at the techniques themselves but mainly at our ignorance about what they can do or be made to do.

1) The shovel, claw, or drill approach would certainly acquire the largest sample and could be made to operate on a mobile platform under the adverse Martian environment. This approach essentially duplicates the classical techniques of terrestrial soil sampling and, as such, would obviate certain questions about biological bias and data interpretation. However, to meet the weight and power limitations of an early mission, samplers of this type would have to be drastically miniaturized, and there is some question as to whether these midget draglines could to an adequate job.

2) The electrostatic or magnetic approach has some theoretical merit but very little research and development. Essentially the concept envisions a rolled up "tongue" which would be stretched out on a soil surface and electrically charged, would attract small dust particles electrostatically or mineral particles magnetically, would be re-rolled into the capsule (like a party favor blower) and would have its soil sample scraped off. This concept could accomplish both sampling and sample transport with little energy expenditure, and can probably be mated to a mobile sampling vehicle. However, the problem of recovering the sample from the sampler, and the problem of sampling at any distance from the capsule might be awkward to overcome.

3) The flypaper or sticky-string concept is similar to the above, but has already reached a fairly sophisticated level of development. It involves the extension of an adhesive coated cord at some distance from the capsule by a small ballistic projectile, and then reeling in the cord to which dust will adhere. In its present application the sample does not have to be recovered from the sampler. Instead, the life detection experiment is performed directly on the contaminated string. The approach is ingenious in its own right, and the fact that it works places it high up on the scale of candidate devices. Weight and power for sampling is negligible and any present mechanical difficulties would be simple to overcome. The major drawbacks to this technique, however, have been suggested above: it is not compatible at present with more than a few of the proposed detection experiments; the acquired sample cannot be easily subdivided for replicate determinations; and the sample itself would represent only a very small surface area that could not be easily re-sampled.

4) The pneumatic-aerosolization approach is essentially a "vacuum cleaner" with some type of agitation device for mechanical dislodging of surface dusts. During the past year some essential data has been acquired to verify the feasibility of this concept under "Martian" conditions. There is also some evidence that on terrestrial soils any biological bias is in favor of life detection (i.e., the pneumatic sampler acquires a biologically enriched sample). This approach lends itself to mobile sampling, repeated sampling, subdivision of sample into replicate subsamples, and some semiquantitation. However, there could be weight and power requirements involved which, though not as imposing as those facing the dragline, may still be critical.

5) The explosion-aerosol concept involves conversion of a soil or rock surface into an aerosol by explosive fragmentation and then sampling the aerosol. There are so many unanswered questions related to this approach that even cursory evaluation is impossible. How does explosion influence the biota? How will the aerosol be sampled, and

how will the dust be recovered from the aerosol sampler? Will the components of the explosive interfere with or confound life detection experiments? Can explosives be sterilized? Nonetheless, this approach might be a simple one to solve and might yield an easy answer to sample acquisition from a completely unknown topography.

6) The liquid expulsion and suction concept permits sampling, processing, and transportation integrated into one device. Essentially it visualizes the washing of a hard surface with a sterile fluid and recovering the fluid and any contaminants for analysis. If the problems of confining the fluid on an irregular topography can be solved, and if the mechanics can be made compatible with weight and power limitations, the technique might be meritorious for quantitative surface sampling. However, the temperatures and atmospheric pressures of Mars seriously limit the choice of fluids that may be used, and the life detection experiments are almost all predicated on aqueous solvents—creating a dilemma before we get this concept off the ground.

7) In situ life detection deserves a discussion beyond the scope of this document. It can be approached on the simplest level by high magnification observation of undisturbed soil. But here readout and optics are critical limitations. It can be approached (and indeed has been attempted) by inoculating a surface with some "life detection fluid" such as C^{14} labeled sugar or a phosphorylated substrate. Here we have the problem of transporting the readout instrumentation directly to the sampling site. Certainly not enough thought or work has been devoted to this concept to generate any serious evaluations.

VI. STATISTICAL CONSIDERATIONS IN SAMPLE ACQUISITION

At first glance it may seem premature to raise the question of statistics at the present state of the mission. It could be argued that the classical statistical concerns of randomness, bias, replication, and tests of significance should wait until data have been acquired. Indeed, there are so many biological, mechanical and operational barriers to overcome before any information will be made available that it would be difficult to justify any diversions into the realm of statistics at this time. It could further be argued that since the probability of success of the first few probes, at any rate, is sufficiently low, and since meaningful statistics must deal with multiple bits of information, we would be presumptious as well as premature in even raising the question. Above all, it is well recognized that the overwhelming considerations in a sample acquisition program will be cost, reliability, weight and power—and statistical requirements will yield to the above.

However, it is to these very problems that statistical forethought can be brought to bear. Such simple questions will have to be answered:

1) How many different environmental samples should and can be taken?

2) What frequency of sampling do we envision over the course of an hour, a day, a week?

3) Should we take many small samples of an environment and test them individually, or should they be pooled, or should one large sample be taken and subdivided for replicate testing?

4) If there is a tradeoff between one sophisticated experiment and two less sophisticated "backup" experiments, which should be chosen?

The recurring nightmare of exobiologists is that data will be obtained from the first probe which will not be interpretable. Or that

duplicate experiments will be equivocal—one saying yes and the other saying no. What will we do with a disagreement of results? All of these are essentially statistical questions, and we should, if possible, start gaining some inputs from terrestrial work of the same nature before we plan to do anything on Mars.

Ironically, the workers in those branches of microbiology which deal with air and surfaces have only recently become aware themselves about the impact of statistics. There was no great requirement for the interplay when the analyses were being made mainly of heavily contaminated environments. But such fairly recent problems as stratospheric biology and sterility of operating rooms have brought into focus a new effort to sample and to interpret data from areas where results are not clear cut and where autocontamination might be significant. In these fields it is well known that two samples are better than one. But little is known about how many more than two are needed to gain a reliable insight. A recent thesis from the University of Minnesota (Keenan, 1964, Some aspects of a statistical approach to environmental microbiological sampling) raises some of these intriguing questions—and unfortunately raises more questions than it answers. It is apparent that until we answer some of these questions here on earth—questions which deal with reliability of small samples from large environments, questions that deal with interpretation of microbiological data from low density contamination surfaces—we will be handicapping ourselves unnecessarily in trying to answer questions about Mars.

Perhaps the most useful analogy between microbial sampling on Mars and microbial sampling on Earth can be found in the field of sterility quality control. Any pharmaceutical house or surgical supply house which manufactures large numbers of sterile products is faced with the problem of ascertaining yes or no answers on selected samples from an assembly line. These people have developed some refined statistical techniques whereby a small number of samples provides

inference about an extremely large population. Exobiologists will also be trying to provide inferences about a large population (i.e., the surface of Mars) based on yes or no answers from some small samples (i.e., a few grams of dust). The analogy breaks down, of course, upon careful scrutiny—but it is a start. Perhaps we will find that statistical approaches to our problems are completely meaningless. Perhaps the first flight or two should be completed before going off on this tangent.

Nonetheless we must recognize that the first flight might work according to program and the subsequent ones might fail. (This happens in the balloon business.) We might not enjoy the relative luxury of "preliminary trials". We should certainly preclude the potential embarrassment of acquiring data from Mars and not knowing how to exploit it fully. Instead of playing "catch up" later, this is a good time to start asking some basic questions about sampling statistics in unknown environments.

VII. SUMMARY AND RECOMMENDATIONS

The field of sampling and the biological, mechanical and statistical considerations involved in it have been neglected. Yet the fundamental problems of sampling are as pertinent to the success of a life detection mission as are problems in propulsion, navigation, biological and biochemical assay, readout and communication. The multiple interfaces of sampling and detection, sampling and power requirements, and sampling and weight limitations might be extremely easy to solve—but they should not be relegated to low priority projects until it can be shown that they are easy to solve.

Above all, some effort should now be devoted to ascertaining the feasibility of the several sampling methods available under simulated Martian conditions, and over a wide variety of terrestrial topographical features. This work should involve studies of the biological, mechanical and statistical advantages and disadvantages of these techniques. Ultimately the most feasible methods should be brought to the prototype instrument phase and studies of mating these instruments with detection instruments should be initiated. Until sampling reaches the state of the art of the other phases of the exobiology program, the whole program will lag behind.