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ON
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FROM SOLIDS

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EFFECTS OF AEOLIAN EROSION
ON MICROBIAL RELEASE FROM SOLIDS

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ABSTRACT

Studies have shown that microorganisms can become encapsulated in selected spacecraft solid materials and under specific conditions survive to arrive on planetary surfaces. This study was initiated to determine the percentage of spores that would be expected to be released from the interior of the solid materials by aeolian erosion on a planetary surface. The information obtained from the study can be used in calculations to determine the probability of microbial release in the total planetary quarantine probability equation.

Methyl methacrylate and Eccobond discs were fabricated so that each disc contained approximately 4×10^4 *Bacillus subtilis* var. *niger* spores. The discs were placed in a specially designed sandblasting device and eroded. Exposure periods of 0.5, 2 and 24 hours were investigated using filtered air to accelerate the sand. A series of tests was also conducted for a 0.5 hour period using carbon dioxide. Examination of the erosion products showed that less than one percent of the spores originally contained in the solids was released by aeolian erosion.

1. Introduction

The basic planetary quarantine constraints for spacecraft have been established by the Committee on Space Research [1] and the National Aeronautics and Space Administration [2]. In order to comply with these constraints, the parameters for contaminating events have been identified and values have been assigned for the probability of contamination for each parameter. Originally, experimental evidence was not available for some of the parameters, therefore, very conservative values were chosen. One such value was assigned for the probability of the release of microorganisms from the interior of solid materials. This value was taken as unity [3].

The importance of microbial release from solids was referred to by Sagan, Levinthal and Lederberg [4] and by Horowitz, Sharp and Davies [5] in their discussions of the Martian contamination problem. These investigators identified several mechanisms that could release organisms from solids, including such factors as a hard impact on a planet's surface and environmental erosion. Experimental data have previously been reported for assessing the probability of microbial release due to hard impact [6]. Data on microbial release from solids by aeolian erosion is presented in this paper.

2. Technical Discussion

Internally inoculated solid materials were eroded with sand in a specially designed sand blasting device. Due to laboratory time constraints, the erosion process was accelerated as compared to naturally occurring rates.

These aeolian erosion studies were designed to provide comparative information on the release of microorganisms from two materials, three erosion rates, and two carrier gases. Six replicate samples were eroded for each test condition to provide statistically valid data.

The test materials were prepared by inoculating methyl methacrylate and Eccobond, an epoxy, with approximately 4×10^4 *Bacillus subtilis* var. *niger* spores per gram of material. The materials were then polymerized and fabricated into rods using a modification of the method reported by Angelotti, et al, [7]. Discs were machined from these rods which measured 1.27 cm in diameter and 0.71 cm long. Each disc weighed approximately 1 gram.

Aeolian erosion was accomplished by utilizing the Venturi principle in a specially designed sand blasting device (Figure 1). A controlled flow of gas was metered into the apparatus creating a pressure differential in the Venturi tube which picked up sand from a reservoir. The sand was then accelerated through the nozzle and directed at a test disc positioned in the apparatus. A cyclone separator and 0.45 μ membrane filter were used to trap the dust that was formed and any spores that were released from the disc. The flow of gas was adjusted so as to erode 0.25 ± 0.05 grams from each test disc for erosion cycles of 0.5, 2 and 24 hours. Air and carbon dioxide were used as carrier gases for the 0.5 hour cycles, while only air was used for the 2 and 24 hour cycles.

Following each erosion cycle, the sand blasting apparatus was disassembled and the contents analyzed for released viable spores. The remaining sand in the reservoir and the dust from the cyclone separator were placed in several petri dishes and mixed with Trypticase Soy Agar

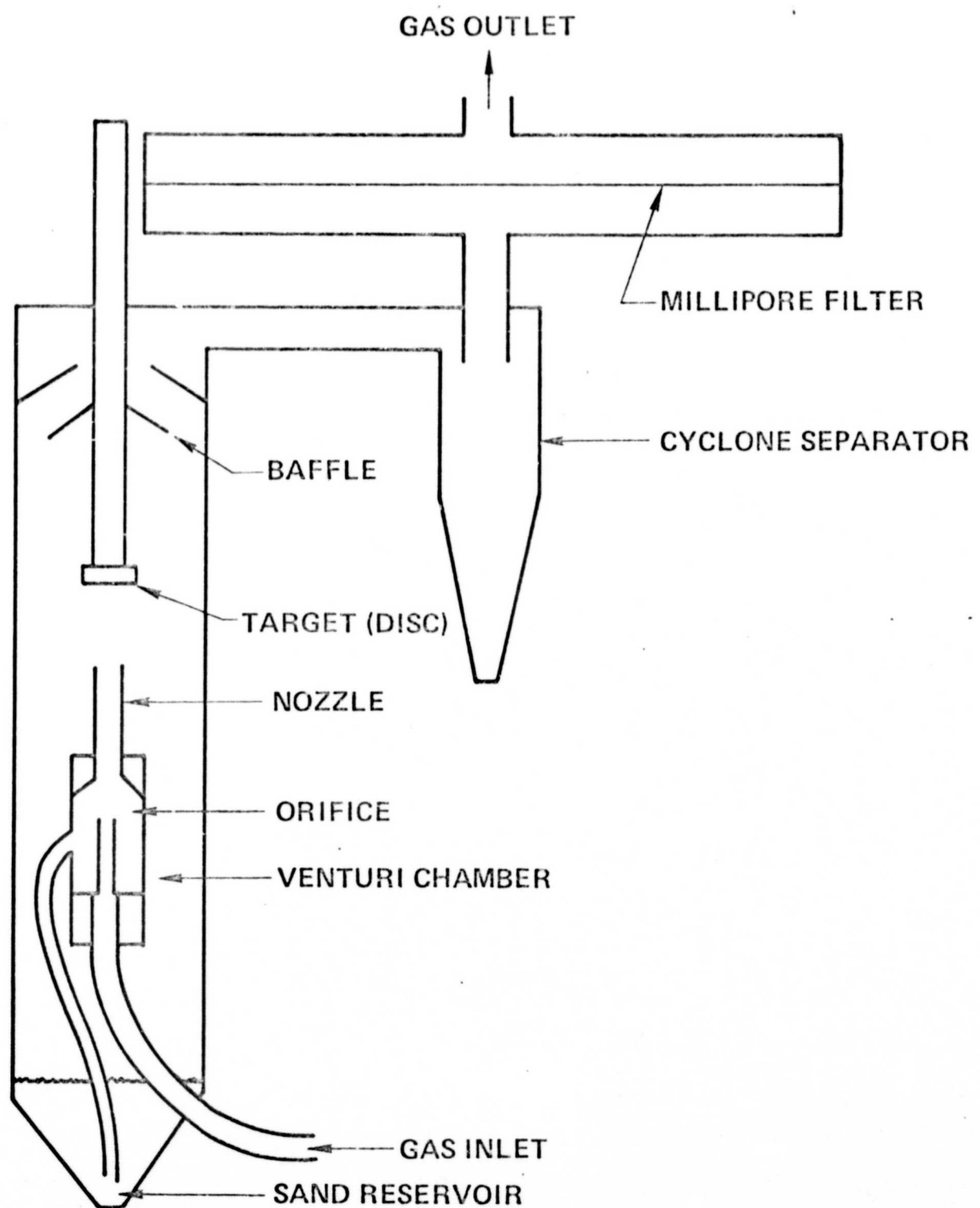


Figure 1: SCHEMATIC OF SAND BLASTER

(TSA). The apparatus was then rinsed with sterile distilled water to assure that all material had been removed. Any viable spores in the rinse water were recovered by membrane filtration. The eroded disc and the membrane filter from the apparatus were overlayed with TSA. All of the agar plates were incubated at 30°C for 2 weeks. Each colony counted on the plates was recorded as one spore released from the disc.

Non-eroded discs were also assayed in order to determine the total spore concentration initially present within a disc. Randomly selected methyl methacrylate discs were dissolved in acetone and the viable spores recovered by membrane filtration. Since a suitable solvent is not available for Eccobond, these discs were assayed by a wet grinding procedure from which agar pour plates were prepared to determine the initial viable spore level. These Eccobond counts were adjusted with an experimentally determined constant to compensate for discrepancies inherent in the grinding technique [8].

3. Results and Discussion

Table 1 shows the gas flow rates as metered into the erosion device. The velocity of the gases through the orifice was approaching sonic velocity; however, the sand velocity was somewhat lower due to the size of the nozzle (4.7 mm inside diameter), air drag on the sand and a slight positive pressure in the apparatus due to the membrane filter.

Table 1: GAS FLOW RATES

EROSION CYCLES (HOURS)			FLOW RATE (LPM)
0.5	-	CO ₂	105.2
0.5	-	AIR	134.4
2	-	AIR	119.3
24	-	AIR	66.2

The percentages of spores released by aeolian erosion are shown in Table 2. A statistical analysis of the data show no significant difference of the percent spores released between the four erosion cycles or between the two materials. The data also show that an average percentage of 0.03 viable spores was released from the discs by erosion under all test conditions.

Table 2: PERCENT SPORES RELEASED BY EROSION
BASED ON 1×10^4 AVAILABLE SPORES

EROSION CYCLES (HOURS)	PERCENT SPORES RELEASED	
	METHYL METHACRYLATE	ECCOBOND
0.5 - CO ₂	0.11 ^{A)}	0.03
0.5 - AIR	0.02	0.002
2 - AIR	0.01	0.02
24 - AIR	0.02	0.01

A) MEAN OF SIX REPLICATES

4. Summary

The aeolian erosion data for all test conditions show an average microbial release of 0.03 percent which is a 3 log reduction in the initial microbial population. It is of significance to note that no apparent differences were observed for the release data between carrier gases, materials or erosion rates. The data obtained during this investigation indicate that the planetary quarantine probability for microbial release due to aeolian erosion should be re-evaluated and the quantitative value reduced.

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