TECHNICAL REPORT 4

Preliminary Sublimation Studies

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ABSTRACT

Preliminary studies were performed to determine whether viable microorganisms are released from the frozen state, when in an aqueous menstruum, upon sublimation. Data indicated that small numbers of viable bacterial spores are released from a frozen aqueous suspension and could thereby contaminate outer space. However, when microorganisms contained in skim milk were frozen and subsequently exposed to high vacuum, the process of sublimation was not associated with microbial transfer, whether using bacterial spores or vegetative cells. Thus, the residual protein matrix resulting from the sublimation process, when using skim milk, may provide a structure that contains the microorganisms and prevents their release. It is recommended that further studies be performed using a prototype of the Apollo sublimation apparatus.

INTRODUCTION

The recent removal of a microbial filter in the condensate line to the sublimator on the Lunar Excursion Module has been viewed with considerable concern because of the potential contamination of the lunar surface with viable terrestrial microorganisms from the condensate fluids. However, in view of further theoretical consideration, microorganisms within a frozen matrix should not be released upon sublimation since no kinetic energy source exists for their release and movement. Nevertheless, empirical evidence regarding this subject was required and the studies reported herein were conducted for that purpose.

RESEARCH OBJECTIVE

To determine whether bacterial spores or vegetative cells are released from various frozen menstra by the process of sublimation.

MATERIALS AND METHODS

Bacterial Suspensions: Bacillus stearothermophilus spores and Escherichia coli vegetative cells were used as test organisms. Spore suspensions at approximately 1×10^4 /ml were prepared in both distilled water and skim milk; vegetative cell suspensions at similar concentration were prepared in skim milk only.

Ice Baths: The ice bath surrounding the flask in which the sublimation process occurred contained water, ice, and urea. The purpose of the urea was to maintain a temperature of -2 to -9C. The condensing flasks were placed in a bath of dry ice and absolute ethanol; the temperature was approximately -67C.

Manometric Readings: The vacuum pump used provided a negative pressure of 28.1 to 30.0 inches of mercury; readings were made from a simple U-tube manometer.

Experimental Procedure: Six sublimation trials were conducted, five with bacterial spores and one with vegetative cells. All flasks and connecting tubes were sterilized prior to use. Each trial was conducted by placing five ml of the respective bacterial suspension into a 250 ml side-arm flask and subsequently freezing the suspension. In initial studies, the bacterial suspension was frozen at -20C in a deep-freeze but in later studies a quick-freeze process, using the dry-ice-ethanol bath, was substituted. After the

bacterial suspension was frozen it was placed in the ice bath previously described and the remaining equipment assembled aseptically as indicated in Figure I. In the majority of trials, the condensing flasks were changed at approximately 90-minute intervals and the sublimation process was continued until all the water vapor was released from the frozen suspension and transferred to the condensing flask.

Assessment of viable microorganisms was performed on all suspensions prior to freezing and after sublimation in order to obtain base-line data and establish whether the process of freeze-drying was deleterious to microbial viability. In addition, 1 ml aliquots were removed from all condensing flasks for purposes of microbial enumeration and the remaining condensates from each flask were added to 20 ml of appropriate bacteriological medium for recovery of small numbers of organisms.

RESULTS AND DISCUSSION

Consideration of the preliminary studies shown in Tables 1 and 2 indicates that small numbers of viable microorganisms may be released during the process of sublimation. It is important to observe, however, that the release is dependent on the nature of the frozen mass in which the bacteria are suspended. Bacterial spores frozen in distilled water (Trials 2 and 3) were pulled free by the vacuum and carried over to the condensing flask whereas those frozen in skim milk (Trials 4 and 5) were not pulled over by the vacuum and adhered to the protein residue. Moreover, when vegetative cells were frozen in skim milk (Trial 6) and then exposed to high vacuum, no release of microorganisms was indicated.

Preliminary results suggest that the process of bacterial release from the frozen state may also be associated with the degree of negative pressure utilized. In Trial 1 where 28.1 inches of vacuum were used on spores frozen in distilled water, no transfer of spores occurred. In comparison, in Trials 2 and 3 where 29.8 to 30 inches of vacuum were used on similar preparations, a release and transfer of spores was observed. Moreover, although the difference in vacuum between Trials 2 and 3 was only 0.2 inches, a greater than tenfold increase in microbial transfer was observed with the higher vacuum. These observations have direct implications to the high vacuum conditions of the lunar surface.

Data presented in Table 2 shows that the process of freezing used in Trials 1 and 2, whereby cultures were frozen slowly at -20C, is associated with a significant decrease in spore viability; recoveries ranged from approximately 10 to 30%. In contrast, when suspensions were quick-frozen at -67C, as was the procedure in Trials 3 through 6, little loss in viability was encountered.

CONCLUSIONS

These studies, although preliminary, suggest that the release of bacteria from the frozen state depends on both the degree of vacuum and the suspending solution used. Thus, when bacterial spores or vegetative cells are suspended in a protein solution such as skim milk, frozen, and then freeze-dried under high vacuum, the water vapor may be sublimed without release of microorganisms. The forces which prevent the bacteria from being carried off by the process are unknown, but it is theorized that the protein matrix may provide a structural barrier or support for the residual microorganisms.

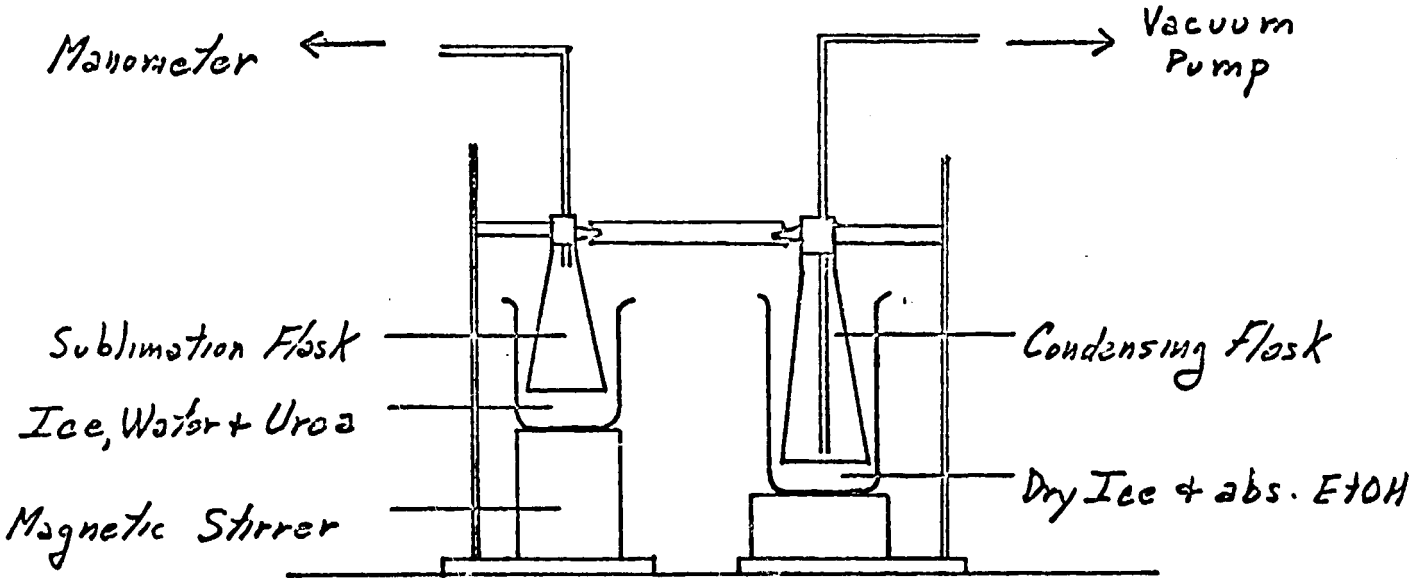

Figure I: Schematic of Sublimation Procedure

Table 1. Transfer of Viable Bacteria by Sublimation Process

Trial	Suspending Solution	Evacuation Procedure		Water Vapor and Bacteria Recovered in Respective Condensing Flasks					
		Vacuum (inches)	Time (hrs)	% Initial Volumes			Number Bacteria		
				I	II	III	I	II	III
1[a]	Water	28.1	4.0	20[c]			0		
2[a]	Water	29.8	4.5	30	30	40	71	30	9
3[a]	Water	30.0	4.5	40	20	40	200	340	940
4[a]	Skim Milk	30.0	3.0	60	24		0	0	
5[a]	Skim Milk	30.0	3.0	76	24		0	0	
6[b]	Skim Milk	30.0	2.5	88	12		0	0	

(a) Five ml Bacillus stearothermophilus

(b) Five ml Escherichia coli

(c) Only one recovery flask used; i.e., 20% initial volume transferred

Table 2. Recovery of Microorganisms in Sublimation and Condensation Flasks

Trial	Total Number Bacteria Recovered		
	Sublimation Flasks		Condensation Flasks
	Before (x 10^5)	After (x 10^5)	
1	2.1	0.7	0
2	1.0	1.1	101
3	2.1	0.9	1480
4	1.8	1.9	0
5	1.0	1.2	0
6	1.9	1.7	0