EVALUATION OF FUNGAL METABOLIC COMPOUNDS RELEASED TO THE AIR IN A RESTRICTED ENVIRONMENT

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ABSTRACT

The metabolic action of selected fungi species on common components of the interior of Space Station Freedom (SSF) will be tested. When present volatile organic chemicals will be collected on porous polymer adsorbent columns. Using thermal desorption the volatile compounds will be passed onto a gas chromatographic column for analysis.

The Space Station Freedom (SSF) modular complex will largely be individually self contained and the established air environment will not be easily adjusted. The development and maintenance of a safe working environment offers a considerable challenge. Present plans for use of SSF acknowledge periods of manned activities and alternate times when the station is unmanned. The obvious necessity for clean and safe air and water during periods of use have been pursued as fundamental systems to SSF success. Somewhat less obvious although perhaps of no less importance to the success of long term cyclic usage are those periods of inactivity. It is during these periods when spores from microorganisms may be afforded the best conditions to germinate and in the vegetative form react with the complex synthetic chemical polymers which compose the furnishings and hardware of SSF nodes.

Biodegradation could constitute a real hygiene problem , if the organisms form and release volatile organic chemicals. Similar problems have been documented in closed and improperly ventilated buildings and work spaces. Many of the metabolic products of fungi and bacterial growth create a variety of health problems.

Analytical chemical techniques will first be used to document the growth of Aspergillus, Penicillium,and Cladosporium fungal species on the potential substrates Nomex and Kevlar. Any volatile organics that are released will be measured using the spectrum of gas adsorption chromatography.

The level of microbial contamination that is necessary to produce such volatile compounds and the relative amounts expected to accumulate will be estimated.

INTRODUCTION

Air Quality is among the factors that will limit the ability of space explorers to participate in the extended operations of outer space.A great part of the concern is not with the obvious wastes from respiration, which is being dealt with effectively, but rather with the more discrete space travelers. The microorganisms upon being placed in environmental conditions that favor growth can sporulate, germinate, etc. and during subsequent metabolic processes release volatile organic compounds. Such volatile organics have been documented regularly and to have varying degrees of toxicity(3). There is a growing file of data for release of volatile products from the growth of both molds and bacteria on food stuffs(1,2). Food grains, such as barley, corn, oats, and wheat have all been used as substrates for growth of a variety of fungi. Organic compounds from the fungal growth include several alcohols and terpenes from species of Aspergillus, Fusarium, and Penicillium(1). Most of these volatile organics will pose no more than minimal risk, while other compounds including the terpenes are referenced as being moderately toxic when ingested or inhaled. Space Station Freedom(SSF) represents a largely closed environment that will require the treatment and recycling of its air. The growth and metabolic activity of groups of organisms such as fungi may add to the air quality considerations.

PROBLEM

This study was undertaken to address one of the air quality concerns for SSF. Three fungal genera were planted on media representing both natural and synthetic compounds. After allowing time for growth of the fungi the gas from the culture vessel headspace was tested for volatile organic chemicals.

EXPERIMENTAL DESIGN

Fungal cultures were obtained from the microbiology section of the Biomedical Research and Operations Branch at NASA/JSC. The particular fungi were chosen as representative of those previously isolated from surface swabs on Skylab and Space Shuttle missions(5) and because they have all been cited as producing volatile metabolites from a variety of substrates(2,7). A culture battery consisting of six 250 ml, 70mm wide-mouthed glass containers fitted with teflon lined lids and stainless steel luer lock bulkhead ports were used to contain the fungal cultures (Fig. 1).

The glass containers housed the media filled 54 mm glass petri plate cultures of the selected fungi. Each culture vessel was fitted with 7mm glass tubing and glass wool spore traps for both affluent and effluent air. The affluent air was humidified by passage of compressed air through a glass trap filled with sterile deionized water. The affluent air was further treated by passage through a tenax-gc 60/80 mesh organic adsorbent filter (Analabs Dist. for Chrom. Tech. Inc., Houston, Tx.). Collection of the headspace metabolites was by passing a measured volume(10ml/min.+/- 1.0 ml), filtered and humidified air over each of the cultures from a single air source. Each culture vessel was sampled continuously for a period of 12 days. The effluent air was passed through 6 mm x 2mm x 11.5 cm multi-bed carbon adsorbent glass filter tubes (Carbotrap 300 multi-bed thermal desorption tube, Supelco, Bellefornte,P.A.). A total of 144 L of air was sampled for each of the cultures and controls.

IDENTIFICATIONS

Effluent air adsorbed on the carbotrap tubes from six cultures and three controls were thermally desorbed and passed through the column of a gas chromatograph that is fitted with a mass selective detector (Hewlett-Packard Series II 5890 GC and HP 5971A MS, Sci. Div.,Palo Alto,CA.). A fused silica capillary column 60M x 0.25 mm I.D., film thickness 0.12 um (SPb tm-1, Supelco,Inc.) was used for resolution of the organic compounds. Helium gas of high purity was used as the carrier, the sample volume was 0.6+/- 0.1 ul and the injection temperature set at 200 C. The desorption products were cold trapped for three minutes in the first 1M of the column. Retention times and compounds from the first experiment are given in (Table 1).

DISCUSSION

All of the fungi exhibited good growth in the experimental environment chosen as evidenced by increase in mycelial mass and microscopic observation of the hyphae. The penicillium was the only fungi that evidenced any significant release of organic compounds. Traces of 2-butene, n-propanol, and octanol were found from the sampling of Penicillium pupurogenum growing on malt extract agar(MEA). Additionally a significant quantity of ethanone - 1 phenyl was released from the penicillium culture on MEA plus Nomex fabric; the GC-MS scan for this is included (see appendix). Additional analysis will be needed to support this finding. Aspergillus flavus exhibited traces of ethanol and 2-propanol. The Cladosporium species gave trace amounts of octanal but not on MEA plus Nomex. All of the fungi used exhibited hardy growth characteristics. Additional experiments with the Penicillium pupurogenum and with other penicillium species are planned for the fall of 1991 and spring of 1992.

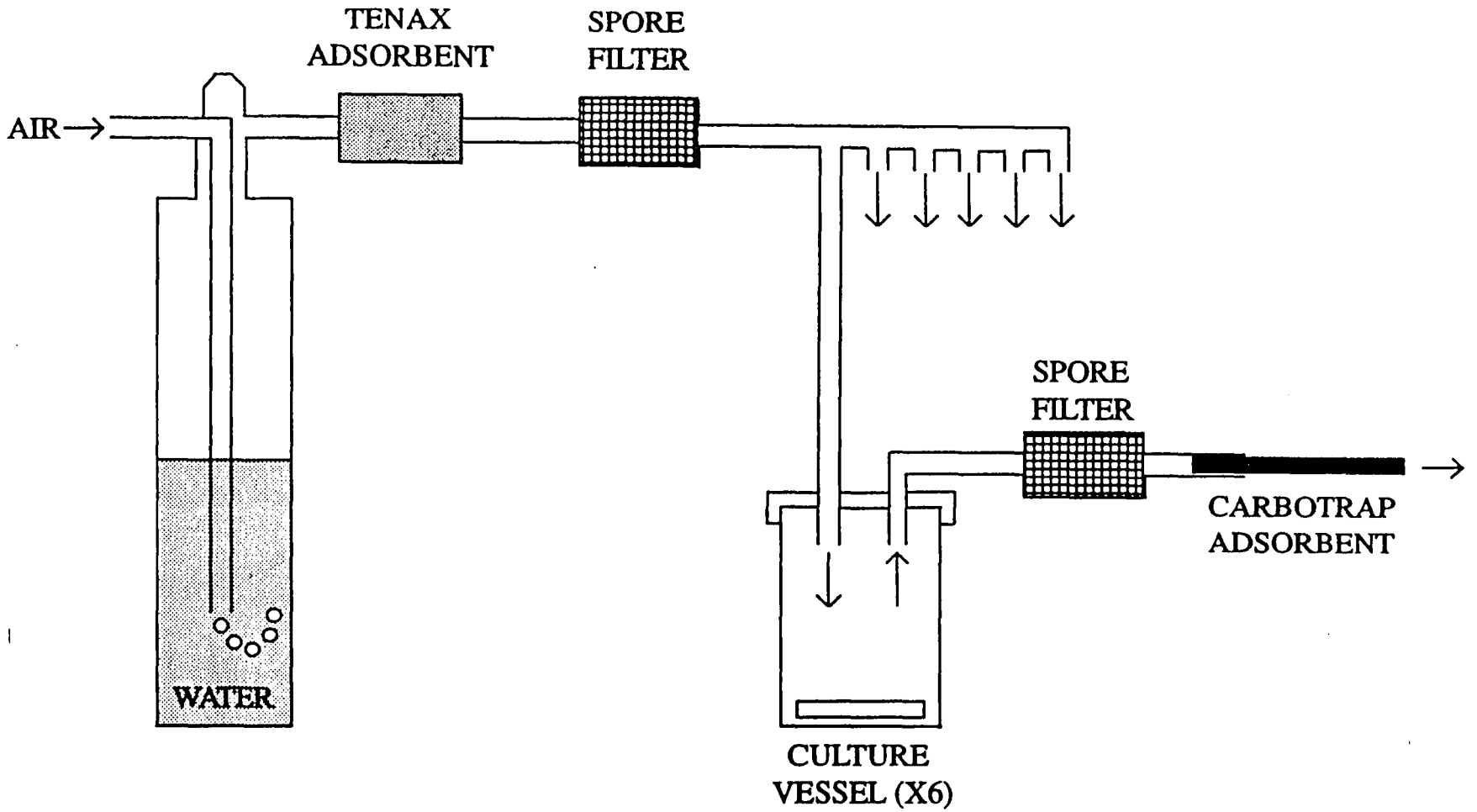

FIG. 1 - CULTURE SYSTEM FOR COLLECTING VOLATILE ORGANICS

Table 1. Volatile Metabolites - 12 Day Cultures of Fungi

Organism/Std	Retention Time	Compound	Reliability%
Standard	7.40	Ethanol	72
"	8.80	Tert-Butyl Alcohol	-
"	10.10	N - Propanol	-
"	16.66	1 - Octanol	-
"	18.00	D - Limonene	83
"	20.60	α - Pinene	91
"	21.67	ß - Pinene	91
Penicillium purpurogenum	9.30	2 - Butene	80
"	9.92	N - Propanol	-
Penicillium + Nomex	11.18	Ethanone 1-phenyl	95
Penicillium	25.90	Octanol	96
Aspergillus flavus	7.60	Ethanol	64
"	12.00	2 - Propanol	72
Aspergillus + Nomex	_	No compounds	-
Cladosporium sp.	27.4	Octanal	87
Cladosporium sp. + Nomex	_	No compounds	_
MEA Control	-	No compounds	-
NOMEX Control	-	No compounds	-

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