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NEW RESEARCH ON BIOREGENERATIVE AIR/WATER PURIFICATION SYSTEMS

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INTRODUCTION

For the past several years, the Science and Technology Laboratory at Stennis Space Center (SSC) has been involved in the development and application of air and water purification systems. This technology is based on the combined activities of plants and microorganisms as they function in a natural environment. Early efforts dealt with the use of artificial or constructed wetlands for wastewater treatment. Numerous communities as well as corporations have adopted this technology. In fact, all of the wastewater at SSC is treated using these types of systems. More recently, researchers have begun to address the problems associated with indoor air pollution. Various common houseplants are currently being evaluated for their abilities to reduce concentrations of volatile organic compounds (VOCS) such as formaldehyde and benzene.

With development of the Space Exploration Initiative (SEI), there will be significant increases in mission duration. Problems with resupply necessitate implementation of regenerative technology. Although the final system may primarily be based on physicochemical processes, it is feasible to consider the application of bioregenerative technology for the air/water purification.

Aspects of bioregenerative technology developed at SSC have been included in a prototype habitat known as the BioHome (Figure 1). A 650 SF structure, the BioHome functions as a pilot system to facilitate analysis of bioregenerative technology in a semi-closed environment. The ultimate goal is to employ this technology in conjunction with physicochemical systems for air and water purification within closed systems.

The BioHome is divided into two regions. one is designated as a living area while the second contains the wastewater treatment system. This system is a modified version of an artificial wetland, relying on vascular plants and microorganisms to effect the treatment process. The system is housed within 6 - 8 inch segments of polyvinylchloride (PVC) pipe ranging in length from 10 to 12.75 ft. and contains plants such as canna lilies (Canna) and bulrush (Scirpus). In addition, there are various types of porous substrate included such as activated carbon. Due to increased surface area, the substrate material promotes biofilm development, a process integral to successful treatment of wastewater (1). In addition, biofilms also play a role in the presence or absence of bacterial pathogens (2).

Prior to inclusion of bioregenerative air or water purification systems in a closed environment, it is necessary to fully assess the associated risks. It is expected that wastewater will have a characteristic microflora, some of which will be pathogenic. Similarly, biological contaminants may be airborne. The bulk of the latter group will probably originate in the abundant plant material present. There is a potential problem in closed systems with build-up of airborne microbes that may be attributed to the lack of ozone and ultraviolet rays. These elements are present outdoors and comprise what is known as the "open air factor" (3). Consequently, there is a tendency for microbial survival to be enhanced indoors due to the absence of this

effect. Devices such as HEPA filters may be used to reduce some biological airborne contaminants, however they will not alleviate the problem. Similarly, chemical contaminants may occur in ambient air. They stem from a variety of sources including building material, plants, and humans.

Earlier preliminary studies have dealt with partial assessments of biological contaminants in the BioHome. Data indicated that the wastewater treatment system exhibited tremendous potential for reduction of bacterial pathogens such as Salmonella (97.53%) and Shigella (98.52%) (4). Similarly, the biological oxygen demand (BOD) and fecal coliform counts were significantly reduced (Tables 1, 2). Studies analyzing ambient microflora revealed relatively low levels of bacteria and fungi present. Bacterial genera included Bacillus, Escherichia, Flavobacterium, Klebsiella, Micrococcus, and Staphylococcus. Fungal isolates were identified as members of the genera Aspergillus, Mucor, and Penicillium.

The purpose of this study was to continue the risk assessment of bioregenerative technology with emphasis on biological hazards. In an effort to evaluate the risk for human infection, analyses were directed at enumeration of fecal streptococci and enteric viruses within the BioHome wastewater treatment system.

MATERIALS AND METHODS

Fecal Streptococci Analysis: For a period of ten weeks, weekly water samples were taken from both effluent (segment 1) and effluent (segment 6) sites of the treatment system. Using the membrane filtration technique, appropriate volumes of sample were analyzed using Gelman GN-6 0.45 m sterile filters. Following filtration, the filters were aseptically transferred to KF agar and incubated at 35°C for 48 hours (5). The density of fecal streptococci/enterococci per 100 ml was calculated using only those plates with colonies numbering in the desired range (20 to 60). Verification of isolates was accomplished according to the protocols outline in A.P.H.A.'s Standard Methods (5).

Enteric Virus Analysis: Measured quantities of wastewater were pumped through 90 mm 1MDS Virosorb membranes for a total of 27 samples. The majority of samples were taken from the effluent sampling port. Additional samples were obtained from segments 3 and 4 as well as the septic tank. 90 mm membranes were eluted using 80 ml of 0.1 M glycine, pH 10.5. The eluent from this step was then passed through a 47 mm Virosorb membrane and eluted with 5 ml of 0.1 glycine, pH 10.5. Next, 10% PSF and 0.1 (10X) gentamicin was added, sample pH was adjusted to 7.0, then the sample was incubated at 35°C for one hour. Samples were then centrifuged at 1900 X g for 20 minutes, filtered (0.20 micron), and distributed into 1.5 ml aliquots for storage at -70°C. For purposes of inoculation, Linbro plates were prepared from stock MA-104 cells and allowed to settle for 24 hours. Next, the growth medium (L-15) was removed by aspiration and each monolayer inoculated with 0.1 ml of undiluted sample. Following an incubation period of one hour at 35°C, monolayers were covered with 1 ml of maintenance medium and incubation continued. Plates were observed daily for evidence of cytopathic effect for a total of seven days (6).

RESULTS

Results of the fecal streptococci analysis indicated that the wastewater treatment system significantly reduced numbers of this group (Table 3). Influent samples over the 10 week period averaged 53 CFUs (colony forming units)/100 ml. None of the effluent samples exhibited any growth. Consequently, the system is 100% effective in the reduction of fecal streptococci/enterococci.

To date, no viruses have been isolated from any portion of the wastewater system. 27 samples were screened for the presence of enterics with no evidence of cytopathic effect.

DISCUSSION

It is encouraging to find that fecal streptococci are virtually removed from the wastewater. This group, also known as the Group D streptococci, has been linked to high incidences of urinary tract infections as well as abdominal lesions and are resistant to numerous antibiotics (7). Similarly, the absence of enteric

viruses is a promising finding. There are several factors that may account for the low levels and/or absence of these groups. First, the septicity of the tank preceding the artificial wetland may be such that conditions are unfavorable for both groups. Factors such as high NH_3 content may limit survival, particularly with respect to enteric viruses. It is also possible that the relative numbers of both groups are comparatively low in the raw wastewater. The majority of sewage that is used for the BioHome studies is derived from that which is generated on site at SSC. Consequently, the presence or absence of a particular group of microorganisms is a reflection of resident microbial population associated with the raw wastewater.

The analysis of these data along with previous studies support the finding that artificial wetlands may provide a suitable means of reducing the number of pathogens in wastewater (8, 9). Several studies have documented the advantages of aquatic and wetland plants for the treatment of wastewater (10, 11). It has been theorized that plants perform two functions in an artificial wetland system. The first is that they provide increased surface area for microbial attachment, an important consideration since the treatment process relies on microbial activity. The second function relates to the transport of oxygen to the root zone, or rhizosphere, thereby producing an aerobic environment (12). The resultant aerobic zone supports a microbial consortium that effects modification of nutrients, ions, and other compounds while the aerobic/anaerobic interface serves to enhance the processes of nitrification and denitrification (13).

It is interesting to note that plants have additional mechanisms to dictate the types of microorganisms found within the rhizosphere. Studies by Bowen and Rovira (14) revealed that several regions of the root produce compounds that leak from the root or may be pumped out as a result of metabolic activity. Such compounds were identified as inhibitory to certain microorganisms. Broadbent et al (15) theorized that such antibiotic activity may be involved in significant coliform reductions associated with artificial wetlands. Similarly, Palmateer et al (16) found that coliform reduction was enhanced substantially during the summer. This reduction coincided with an anoxic period, suggesting the ability of plants to translocate oxygen to the rhizosphere, thereby providing an explanation for improved coliform removal in vegetated systems.

These findings are also supported by Seidel (17) whose studies included Juncus effusus, Scirpus lacustris and Phragmites communis. Seidel maintains that excretions from the plants either partially or completely kill pathogenic bacteria while heterotrophs are left unharmed. Unfortunately, the author neglected to include relevant reference material. Consequently, the validity of these finds must be carefully considered.

Pathogens are known to be removed by physical/chemical processes (filtration and adsorption) and by biological inactivation and predation (18). However, biofilm development also plays an important role in their presence or absence. In a study utilizing granular activated carbon (GAC) , it was determined that the autochthonous microbial community influenced pathogen survival (2). When pathogens were introduced to sterile GAC in the presence of heterotrophs, they attached at levels similar to those found in pure culture, then decreased. However, when the two were added to GAC with a mature biofilm, the pathogens attached at lower levels and decreased at a more rapid rate.

Future research will address the enumeration of bacterial pathogens as it relates to biofilm development on activated carbon. similarly, efforts will continue in the characterization of fecal streptococci and enteric viruses associated with the wastewater treatment system.

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TABLE 1
BIOHOME MEAN MONTHLY BOD VALUES

DATE	INFLUENT (mg/L)	EFFLUENT (mg/L)
6/89	368	1.2
7/89	264	2.0
8/89	217	20.6
9/89	388	3.8
10/89	293	7.2
11/89	304	2.0
12/89	245	2.2
1/90	114	1.8
2/90	234	7.2
3/90	236	1.5
4/90	224	11.6
5/90	357	3.1

TABLE 2
BIOHOME MEAN MONTHLY FECAL COLIFORM COUNTS

DATE	INFLUENT	EFFLUENT
6/89	4.8×10^5	1
7/89	1.5×10^5	1
8/89	8.0×10^6	800
9/89	4.4×10^6	6000
10/89	8.5×10^5	8000
11/89	4.0×10^4	1
12/89	8.0×10^5	6000
1/90	4.2×10^4	530
2/90	8.0×10^4	10
3/90	8.0×10^4	1
4/90	2.5×10^4	150
5/90	8.0×10^5	1

TABLE 3
BIOHOME FECAL STREPTOCOCCI DENSITIES (CFUs/100 ml)

WEEK #	INFLUENT	EFFLUENT
1	58	0
2	57	0
3	59	0
4	45	0
5	56	0
6	50	0
7	57	0
8	49	0
9	50	0
10	52	0

BIOREGENERATING CLOSED HABITAT

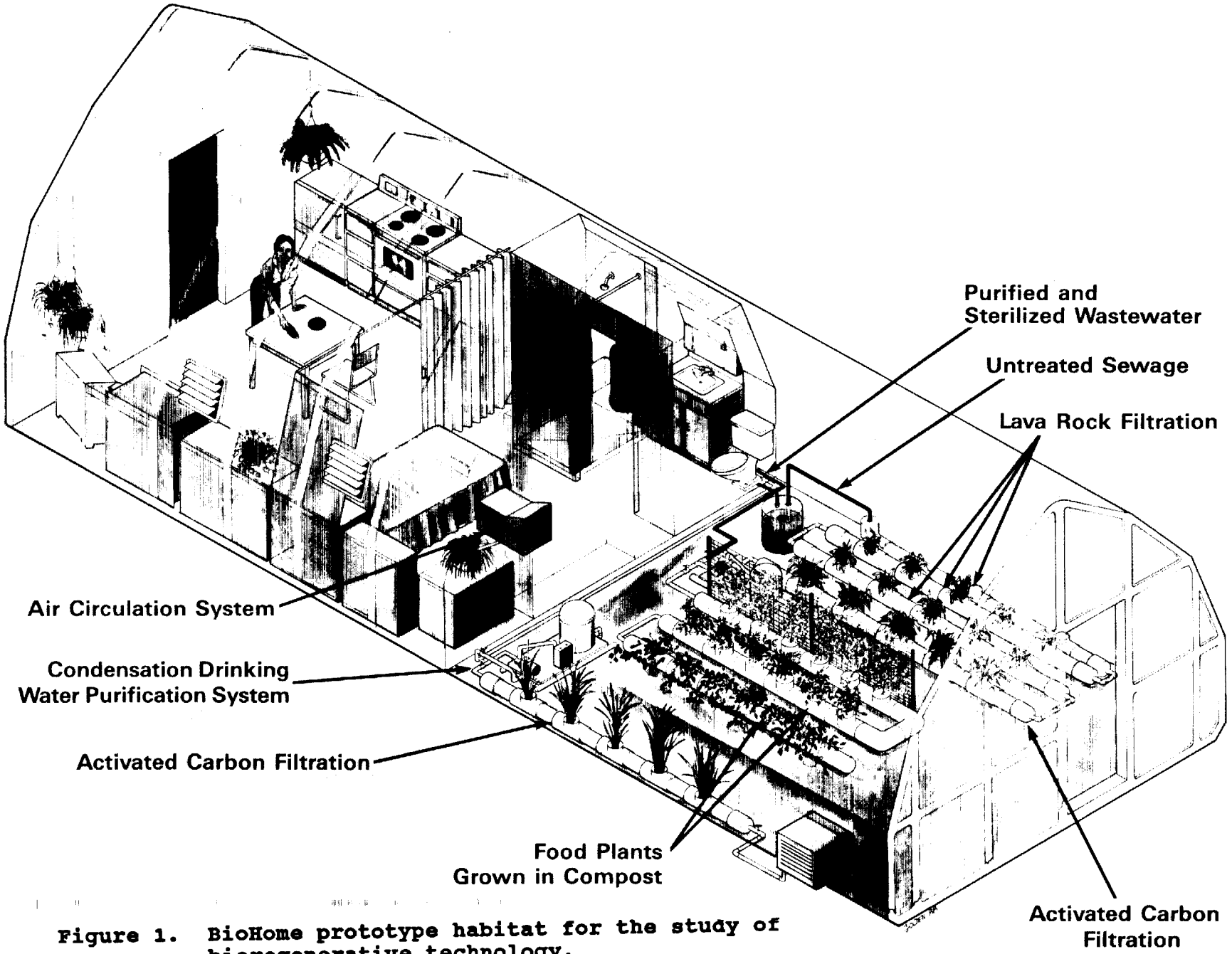

Figure 1. BioHome prototype habitat for the study of bioregenerative technology.

ENVIRONMENTAL AND FACILITIES MANAGEMENT SYSTEM

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