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Microbial colonization of polymeric materials for space applications and mechanisms of biodeterioration: A review

Ji-Dong Gu[a,b,*]

[a]*Surface Biology and Environmental Microbiology Group, South China Sea Institute of Oceanography, Chinese Academy of Sciences, 164 Xingang Road West, Guangzhou 510301, PR China*

[b]*Laboratory of Environmental Microbiology and Toxicology, Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, PR China*

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Abstract

Biodeterioration of polymeric materials affects a wide range of industries. Formation of microbial biofilms on surfaces of materials being considered for use on the International Space Station was investigated. The materials included fiber-reinforced polymeric composites, adhesive sealant, polyimide insulation foam, Teflon cable insulation, and aliphatic polyurethane coatings. In simulation experiments, bacterial biofilms formed readily on the surfaces of the materials at a wide range of temperatures and relative humidity. The biofilm population was dominated by *Pseudomonas aeruginosa, Ochrobactrum anthropi, Alcaligenes denitrificans, Xanthomonas maltophila*, and *Vibrio harveyi*. Subsequently, degradation of polymeric materials was mostly a result of both fungal and bacterial colonization in sequence, and fungi may have advantages in the early phase of surface colonization over bacteria, especially on relatively resistant polymeric materials. These microorganisms are commonly detected on spacecraft on hardware and in the air. Furthermore, degradation of polymeric materials was documented with electrochemical impedance spectroscopy (EIS). The mechanisms of deterioration of polymeric materials were due to the availability of carbon source from the polymer, such as additives, plasticizers, and other impurities, in addition to the polymeric matrices. Microbial degradation of plasticizer phthalate esters is discussed for the microorganisms involved and the biochemical pathways of degradation. Current results suggest that candidate materials for use in space missions need to be carefully evaluated for their susceptibility to microbial biofilm formation and biodegradation.

Keywords: Biofilms; Degradation; Plasmids; Polymeric materials; Resistance; Space station

1. Introduction

Synthetic polymers can be potential substrates for heterotrophic microorganisms, depending on chemical structures, composition and bonds, and the dominant microflora. Biodegradability of polymers also depends on molecular weight, crystallinity, and the physical form of the relevant materials (Gu et al., 2000; Gu, 2004), while initial adhesion of bacteria on surfaces of materials may be mediated by surface physical characteristics, e.g., hydrophobicity and smoothness. As a general rule, an increase in molecular weight of homopolymer may result in a decline of polymer degradation rate by both abiotic and biological processes. In contrast, monomers, dimers, and oligomers of the polymer repeating units can be much more easily degraded and mineralized quickly by the natural population of microflora. An increase in molecular weight of polymers tends to result in a decrease in solubility in water, making them unfavorable for microbial attack because bacteria, most of the time, require that the substrate be transported through the cellular membrane first, then degraded/metabolized by cellular enzymes, and assimilated by the cells in the synthesis of cellular components.

*Corresponding address. Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, PR China. Tel.: +852 2299 0605; fax: +852 2517 6082.
E-mail address: jdgu@hkucc.hku.hk.

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However, it should be pointed out that both abiological and biological processes may take their part in the ultimate degradation of polymers in natural and artificial environments, including manned spacecraft.

Microbial contamination of space stations is a known problem, and investigation of this topic has been carried out previously in the Soviet Union and the US (Gitel'zon et al., 1981; Pierson et al., 1994; Castro et al., 2004; Novikova, 2004; Ott et al., 2004). Because of the presence and activity of human beings, microorganisms are commonly found on surfaces of materials and hardware and in the air of the spacecraft (Castro et al., 2004; Novikova, 2004). Furthermore, the microflora on the surfaces of spacecraft do not differ greatly from those of our ambient environments on Earth because the microorganisms originate from human sources and preflight contamination (Gu, 2003a; Ott et al., 2004).

After initial attachment of microorganisms to surfaces of materials, their growth forms extensive biofilms, and damage to the materials may be observed. An example of biofilms on surfaces of a candidate polymeric material tested for use on space stations is shown in Fig. 1, illustrating the high heterogeneity of the biofilm in terms of microbial composition and also the physical form of the biofilm spatially. Biofilms are highly heterogeneous and porous, containing >95% of water in their natural state. Growth of microorganisms was observed on surfaces of materials in the space stations, including rubber seals (Gitel'zon et al., 1981), viewing windows (Castro et al., 2004) and various other types of hardware on spacecraft and space stations (Castro et al., 2004). Because of the increasing application of polymeric materials in space and aviation, deterioration and degradation of engineering materials are important considerations when selecting appropriate materials for such a program (Gu and Gu, 2005) and also for better understanding the extent of microbiological attack and problems in space.

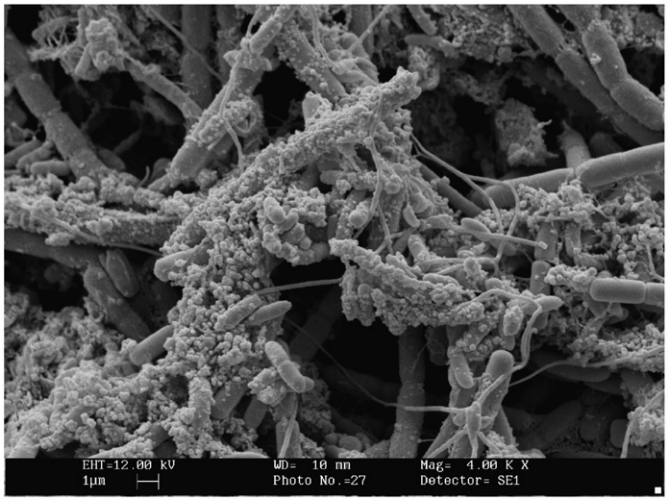

Fig. 1. A scanning electron micrograph showing a natural bacterial biofilm developed on the surface of an artificial polymeric substratum after dehydration and critical point drying.

2. Microbiology of an enclosed environment

Because of human inhabitation, the interior of space stations can be colonized by microorganisms; there are suitable environmental conditions for growth in terms of humidity and temperature. The source of the microorganisms is either human or related to the contamination of the system pre-flight. Analysis of the microflora in the air system of both Mir and the International Space Station (ISS) indicated that the two most important groups commonly found are fungi and bacteria. In monitoring programs on land, any surfaces with a microbial density $>100\,\text{cfu}/25\,\text{cm}^2$ is required to be disinfected before launch (Pierson et al., 1994). While in space, when visible growth of microorganisms is observed, on-board disinfection should be carried out using H_2O_2. For example, the crew of the Russian Salyut 6 found a 'white film' on parts of the interior surfaces, including rubber straps of the exercise machine, and the organisms were identified mainly as *Aspergillus* sp., *Penecillium* sp., and *Fusarium* sp. On Salyut 7, visible microbial growth on the hull, joints, and cables in the working module was also observed and similar microflora were identified (Pierson et al., 1994). Bacteria included *Achromobacter* sp., *Acinetobacter calcoaceticus*, *Bacillus idosus*, *Corneybacterium* sp., *Desulfovibrio desulfuricans*, *Enterobacter agglomerans*, *Enterobacter cloacae*, *Flavobacterium* sp., *Microbacteria* sp., *Micrococcus* sp., *P. aeruginosa*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Staphylococcus* sp., and *Streptococcus* sp. (Gitel'zon et al., 1981; Pierson et al., 1994). A list of bacteria isolated and identified from the Russian and US spacecraft and station is presented in Table 1. *Staphylococcus*, *Corynebacterium*, *Micrococcus*, and *Acinetobacter* were found on 55.5%, 36.0%, 27.5%, and 24.3%, respectively, of the surface samples taken from Mir surfaces (Novikova, 2004). Similarly, *Staphylococcus*, *Bacillus*, *Corynebacterium*, *Micrococcus*, and *Serratia* were detected in 53.2%, 34.0%, 16.0%, 13.8%, and 9.6% of the air samples; and *Flavobacterium*, *Xanthomonas*, *Pseudomonas*, *Corynebacterium*, *Bacillus*, *Methylobacterium*, and *Kluyvera* were in 25.0%, 21.0%, 20.8%, 16.7%, 12.5%, 12.5%, and 12.5% of the condensate samples (Novikova, 2004). The occurrence of *Corynebacterium* sp. and *Staphylococcus epidermidis* was 33.2% and 30.0%, respectively, on structural materials of Mir (Novikova, 2004). The data were derived from 1177 samples.

All of these organisms were identified based on morphological and biochemical tests at that time; no definitive molecular markers, e.g., 16S rRNA gene for bacteria, were utilized. It is apparent from the Russian and American space programs that the source of microorganisms can be minimized, but not eliminated, due to payloads, water, human inhabitation and activities; contamination of the surface is almost unavoidable due to many unexpected spills or leakage, e.g., food, feces, urine, or vomit. In Mir, bacterial contamination was maintained below $500\,\text{cfu}/\text{m}^3$ air in 95% of the air samples, while fungi

Table 1
Bacteria isolated from space programs conducted on mir and the international space station by Russia and the US

Category	Genus/Species names of microorganisms[a]	References
Surfaces in Mir	*Acinetobacter*, *Aeromonas*, *Bacillus*, *Corynebacterium*, *Enterobacter*, *Micrococcus*, *Neisseria*, *Pseudomonas*, *Serratia*, *Staphylococcus*, *Streptomyces*	Novikova (2004)
Air in Mir	*Acinetobacter*, *Aerococcus*, *Aeromonas*, *Bacillus*, *Comanonas*, *Corynebacterium*, *Flavobacterium*, *Enterococcus*, *Escherichia*, *Micrococcus*, *Pantoea*, *Pasteurella*, *Pseudomonas*, *Serratia*, *Sphingobacterium*, *Staphylococcus*, *Xanthomonas*	Novikova (2004)
Air in ISS[b]	*Bacillus megaterium*, *Pseudomonas fulva*, *Micrococcus luteus*, *Staphylococcus epidermidis*	Castro et al. (2004)
Condensate in Mir	*Bacillus, Clavibacter*, *Corynebacterium*, *Flavobacterium*, *HydrogenophagaiKingella*, *Kluyvera, Micrococcus, Pseudomonas, Psychrobacter, Staphylococcus, Xanthomonas*	Novikova (2004)
Free condensate in NASA Mir 6 and 7	*Alcaligenes faecalis, Bacillus* spp., *Citrobacter brackii, Comamonas acidovorans, Corynebacterium* sp., *Flavobacterium meningosepticum*, *Pseudomonas fluorescens*, *Ralstonia paucula, Serratia liquefaciens, Yersinia fredericksenii*	Ott et al. (2004)
Water dispensers on ISS	*Brayrhizobium japonicum*, *Methylobacterium fujisawaense*, *Ralstonia eutropha, Sphingomonas* sp.	Castro et al. (2004)
Cargo containers on ISS	*Acinetobacter radioresistens*, *Brevundimonas diminuta*, *Curtobacterium luteum*, *Micrococcus luteus*, *Staphylococcus pasteuri*	Castro et al. (2004)
ISS preflight	*Bacillus flexus*, *Staphylococcus epidermidis*, *S. diminuta*, *S. pasteuri*	Castro et al. (2004)
ISS in-flight	*Acinetobacter radioresistens*, *Bacillus pumilus*, *Coryneybacterium afermentans*, *Oerskovia xanthineolytica*, *Staphylococcus aureus*	Castro et al. (2004)

[a]Microorganisms present at <1% of the 1177 Mir samples collected are not included.
[b]ISS, International Space Station.

were more variable, between 2 and 1.0×10^4 cfu/m^3 air (Novikova, 2004). It was therefore argued that selection of structural materials for the spacecraft is a crucial step in the overall program (Pierson et al., 1994). An effective strategy in dealing with this issue is to select materials with known resistance to microbial attack and possibly to modify easily degradable components. Commonly detected fungi in free condensate during NASA, Mir 6 and 7 flights were *Acremonium* sp., *Candida guilliermondii*, *C. lipolytica*, *Cladosporium* sp., *Fusarium* sp., *Penicillium* sp., *Rhodotorula glutinis*, and *R. rubra* (Ott et al., 2004). *Penicillium* spp., *Aspergillus* spp., and *Cladosporium* spp. were detected in 76.8%, 39.4%, and 27.2% of surfaces on Mir, respectively, while each of these genera was found in 75.8%, 76.6%, and 24.2%, respectively, of the air samples (Novikova, 2004).

3. Microbial colonization and deterioration of material surfaces

Dominant groups of microorganisms in space are very similar to those commonly observed to be associated with the deterioration of engineering materials on land (Gu, 2004; Gu et al., 1998a; Mitchell et al., 1996; Ott et al., 2004; Novikova, 2004); the biochemical pathways associated with polymer degradation are often determined by the environmental conditions and the microorganisms involved. Considering the availability of O_2 as an electron acceptor, aerobic and anaerobic conditions are the two broad categories to be considered. When O_2 is available, e.g., with thin microbial biofilms or oxygenated conditions, aerobic microorganisms are mostly responsible for destruction of complex materials. In contrast, under strictly anaerobic conditions, e.g., in a thick biofilm or niche where oxygen is limited due to diffusion transport,

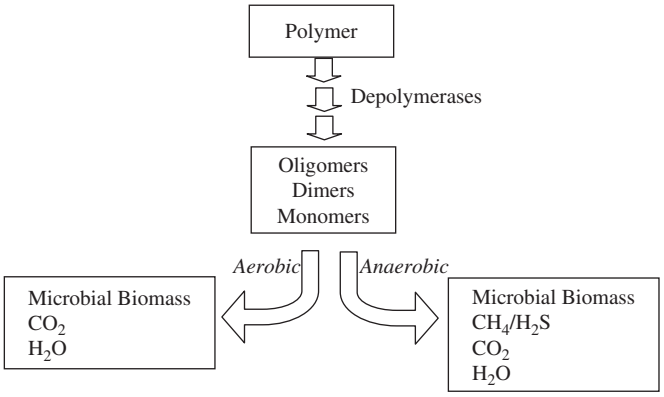

Fig. 2. A schematic diagram of the processes involved in degradation of polymeric materials under natural conditions, including both aerobic and anaerobic, and the relevant degradation products (redrawn from Gu, 2003).

anaerobic microorganisms, including fermentative and sulfate-reducing bacteria, may become active and are responsible for polymer deterioration (Gu et al., 2000; Gu, 2003b). Because of the difference in environmental conditions and hence the presence of active microorganisms, the possible degradation processes will lead to different end-products, even though the biochemical processes of hydrolysis are identical (Fig. 2). These environmental conditions are widely found in natural environments and can also be simulated in the laboratory with appropriate chemical media and inocula from the relevant environments (Gu, 2003a; Gu et al., 1992a, b, 1993a, b, c, 1994; Gu and Gu, 2005). In addition, both aerobic and strictly anaerobic microorganisms can coexist in natural environments in close association; both members can benefit from such associations, being spatially separated but biochemically connected.

Table 2
Polymeric materials tested for their susceptibility to degradation and deterioration by environmental microorganisms

Name	Description	References
Adhesive	RTV142 silicone rubber with methyl alcohol	Gu et al., 1998(a, b)
Thin film	Cellulose acetates with degree of substitution values 0.8–2.5	Gu et al. (1992a, b, 1993b, c, 1994)
Insulation foam	Benzophenonetetracarboxylic imide polymer foam	Gu et al., 1998(a, b)
Cable insulation	Polytetrafluoroethylene, fluorinated ethylene propolene coated polyimides and Perfluocarboxyl	Gu et al., 1998(a, b)
Composites	Fluorinated polyimide/glass fibers, bismaleimide/carbon fibers, epoxy/ carbon fibers unidirectional, epoxy/carbopn fibers [0, 45, 90, −45]2S, poly(ether-ether-ketone)	Gu et al. (1996a, b)
	Epoxy/graphite fiber unidirectional	Gu et al., 1998(a, b)
	Epoxy/carbon fibers, epoxy/ glass fibers, bismaleimide/ aluminum	Thorp et al. (1994)
Protective coating	Aliphatic polyurethane coating	Gu et al., 1998(a, b)
Kapton polyimides	Pyromellitic dianhydride and 4,4′-diaminodiphenyl ether	Gu et al. (1996a, b)
Polyimides	BTDA-ODA/MPDA (benzophenone tetracarboxylic acid dianhydride oxidianiline/*m*-phenyldianiline	Mitton et al. (1998)

Both bacteria and fungi have been observed to colonize on a very wide range of engineering and high-strength polymeric materials indiscriminately (Gu, 2003b; Gu et al., 1996a, b, 1998a, b, 2000; Mitchell et al., 1996) (Table 2). High-strength engineering materials are structurally important for the spacecraft, while electronic insulation polymers are essential in the operation systems commanding the flight of the vehicle; polymers selected and used in electronic industries are chemically synthesized with exceptionally high strength and resistance against degradation, both chemical and biological processes. For example, the thermosetting polyimides are a major class of high-performance polymer used in electronic insulation and fiber-reinforced composites (Brown, 1982). Wide acceptance of polyimides in the electronics industry (Lai, 1989; Verbicky, 1988) has drawn serious attention to the stability of these materials over long periods of use. The National Research Council (NRC (National Research Council), 1987) pointed out the need to document the potential deterioration of these polymers in the electronics industries because data acquisition, information processing, and communication are critically dependent on their performance. The inter-layering of polyimides and electronics in integrated circuits prompted several studies on the interactions between materials and microorganisms (Gu, 2003a; Gu et al., 1998b, 2000; Mitton et al., 1996, 1998). Such information is discussed in detail below.

3.1. Electronic insulation materials

Polyimides are widely used in load-bearing applications, e.g., struts, chassis, and brackets in automotive and aircraft structures, due to their flexibility and compressive strength, as well as their chemical resistance to oils, grease, and fats, microwave transparency, and thermal resistance. Their dielectrical properties are ideally suited for use in electronics, especially as high-temperature insulation materials and passivation layers in the fabrication of integrated circuits and flexible circuitry. The high flame resistance of this class of polymers provides a halogen-free flame-retardant material for aircraft interiors, furnishings, and wire insulation, and they are also commonly chosen for space applications. Other possible uses include fibers for protective clothing, advanced composite structures, adhesives, insulation tapes, foam, and optics operating at high temperatures (Verbicky, 1988).

Electronic packaging polyimides are particularly useful in the aviation and space industries because of their outstanding performance and engineering properties, as indicated above. It is only recently that the biodeterioration problem of these polymers has been investigated, using pyromellitic dianhydride and 4,4′-diaminodiphenyl ether with molecular weight of 2.5×10^5 (Mitton et al., 1996; Gu et al., 1998b; Mitton et al., 1998). Using fungi isolated from previously deteriorated circuit parts, both polyimide films and sputter-coated silicon wafers were tested for degradation, after inoculation with the fungal culture, using electrochemical impedance spectroscopy (EIS) (Mitton et al., 1996; Gu et al., 1998b; Mitton et al., 1998). Both forms of polyimides were found to be susceptible to deterioration by a mixed culture of fungi (Gu et al., 1996b; Mitton et al., 1998). The fungi formed a biofilm on the surface of the material and its degradation was confirmed by monitoring the dielectrical properties of the polymer film using EIS (Gu et al., 1996b). The dielectric properties of polyimides could be altered drastically following growth of a microbial biofilm (Mitton et al., 1996; Gu et al., 1996b; Mitton et al., 1998). At the end of the incubation experiments, physically damaged but intact polyimide film showed a severe decrease of capacitance and increase of conductance, further supporting the suggestion that degradation of polymer dielectric properties were solely due to damage of polymer by fungal growth. It should be pointed out that this form of deterioration may be slow under ambient conditions due to limited availability of organic carbon and other nutrients for the colonizing microorganisms. However, the deterioration processes can be accelerated in humid conditions or in enclosed environments, e.g., submarines, space vehicles, and aircraft, because the humidity of these environments promotes the development of bacterial biofilms on surfaces, and different

microorganisms may form a succession, according to the conditions. Under such conditions, very small amounts of impurities or polymerized by-products will be able to support the growth of fungi preceding bacteria, as observed in our simulation study (Gu et al., 1996a). Changes of material properties, if not prevented, will affect communications and control systems, resulting in catastrophic consequences.

Polyimide deterioration occurs through biofilm formation and subsequent dielectrical changes in the polymer. Using EIS, a very sensitive technique for monitoring the dielectric constant of polymers (Mansfeld, 1995), fungal growth on polyimides was shown to yield distinctive EIS spectra, indicative of increased conductivity and failing resistivity of the colonied polyimides over a year of monitoring (Gu et al., 1996b, 1998b). Two steps were involved during degradation: An initial decline in coating resistance was related to the partial ingress of water and ionic species into the polymer matrices. This was followed by further deterioration of the polymer by fungal activity, resulting in a large decrease in resistance. Microorganisms involved were identified as *Aspergillus versicolor*, *Cladosporium cladosporioides*, and *Chaetomium* sp. (Gu et al., 1996b, 1998b); these are commonly found in our atmosphere. The data suggest that polyimides are susceptible to microbial deterioration and also confirm the versatility of EIS as a method for detecting biosusceptibility of polymers at an early stage. EIS is a very sensitive electrochemical technique widely used by corrosion engineers to assess corrosion at the oxidized surface layer. The new application in biodeterioration detection offers another application for this technique.

3.2. Structural and engineering composites

Fiber-reinforced polymeric composite materials (FRPCMs) are new materials widely used in aerospace and aviation because of their easy molding and light weight. The increasing usage of FRPCMs as structural components in aerospace applications has generated an urgent need to evaluate the biodegradability of this class of materials. FRPCMs are also susceptible to attack by microorganisms, but the mechanisms involved are more complicated than with the previously discussed polyimides, due to the multi-components within any FRPCM (Gu et al., 1996a, 1997). Impurities and additives, which can promote microbial growth as potential sources of carbon and energy for environmental microorganisms, are widely incorporated into the materials during manufacturing processes. In addition, no attention is given to minimizing the contamination of microorganisms during fabrication of each component or each directional layer of fibers in resins such as ply. There is no environmental control during storage of these plys before overlaying them to the designed orientation of FRPCMs.

Natural populations of microorganisms are capable of growth on surfaces of FRCPM coupons at both relatively high (65–70%) and lower humidity (55–65%) (Gu et al., 1996a, 1997, 1998a; Mitchell et al., 1996). The accumulation of bacteria on surfaces of composites develops into a thick biofilm layer and decreases the sensitivity of microorganisms to further environmental changes. The FRCPMs can provide enough organic carbon for detectable growth of microorganisms to be observed using microbiological techniques (Gu et al., 1996a, 1998a; Mitchell et al., 1996). More changes within material matrices can be detected by using highly sensitive techniques such as EIS. Monitoring using EIS showed that the resistance of FRPCMs, as reflected by capacitance, declined significantly after the initial three months of a one-year monitoring program (Gu et al., 1997). Clear differences resulting from biofilm development were detected on FRCPMs used in aerospace applications (Gu et al., 1998a; Mitchell et al., 1996). Further study indicated that many fungi are capable of utilizing chemicals, e.g., plasticizers, surface treatment chemicals, and impurities, introduced during composite manufacture (Gu et al., 1998a, 2000). Fungi are the most aggressive microorganisms attacking FRCPMs over longer periods, while bacteria cause limited damage. These observations clearly establish that materials can provide nutrients for the growth of environmental microorganisms and, because of this, systematic selection of candidate materials, including fiber type, surface coating chemicals, and resins, is crucial in the long-term success of space and aviation.

Currently, a critical question remains about the effect of FRPCM deterioration of the mechanical properties of the composite materials though separation—technically called delimination by engineers—of the fibers from resins; this has been observed in laboratory experiments. Thorp et al. (1994) attempted to determine mechanical changes in composite coupons after exposure to a mixed fungal culture in a simulation study. Mechanical tests showed that no significant changes could be detected after 120 days of exposure. It was suggested that methodologies were not sufficiently sensitive to detect surface changes during incubation and the whole coupon-based technique currently used would need to be modified for higher sensitivity. Wagner et al. (1996) suggested an acoustic technique as a means of detecting changes in the physical properties of the FRPCMs.

A mixed culture of microorganisms, including a sulfate-reducing bacterium, was used to show FRPCM deterioration (Wagner et al., 1996). In contrast, we used a fungal consortium, originally isolated from a degraded polymeric composite, on a range of materials, including fluorinated polyimide/glass fibers, bismaleimide/graphite fibers, poly (ether–ether–ketone) (PEEK)/graphite fibers, and epoxy/graphite fibers (Gu et al., 1998a). This consortium of microorganisms consisted of *Aspergillus versicolor*, *Cladosporium cladosporioides*, and a *Chaetomium* sp. When analyzing bacteria and fungi capable of growing on the graphite and glass fibers of FRPCMs, only fungi were

shown to cause deterioration detectable over more than 350 days (Gu et al., 1997).

Physical and mechanical tests were not sufficiently sensitive to detect any significant physical changes in the materials (Gu et al., 1997; Thorp et al., 1994). However, the resins were actively degraded, indicating that the materials were at risk of failure. It is clear that both fiber surface treatment and resin processing supply enough carbon for microbial growth (Gu et al., 1998a). It became clear that FRPCMs are not immune to adhesion of environmental microflora, and attack of materials may be observed (Gu et al., 1998a; Mitchell et al., 1996). Unfortunately, the mechanical evidence has not been established quantitatively.

3.3. Polymer plasticizers and additives

Polymeric materials commonly contain a wide array of chemicals to improve their processibility and product quality; these chemicals include phthalate esters, namely dimethyl phthalate esters (*ortho*-dimethyl phthalate ester, dimethyl isophthalate ester, and dimethyl terephthalate ester), di-*n*-butyl phthalate ester, and dibutylbenzyl phthalate ester (Gu, 2003b; Gu et al., 2004). These chemicals are not covalently bound to the polymer resins and are therefore susceptible to release into the environment. This class of chemicals has been investigated for their biodegradability by pure cultures of bacteria isolated from activated sludge (Wang et al., 2003a, b; Fan et al., 2004; Wang, 2004), mangrove sediments (Gu et al., 2005; Li et al., 2005a, b; Xu et al., 2005a, b; Li and Gu, 2006), and deep-ocean sediment (Wang, 2005; Wang and Gu, 2006) (Table 3). These microorganisms are well characterized by morphological, biochemical, and 16S rRNA gene analyses. The biochemical degradation mechanisms of plasticizers commonly used in polymer formulation and processing are discussed below.

3.3.1. Isomers of dimethyl phthalate ester

Degradation of phthalate esters has been reported for selective isomers, but the best-characterized biochemical pathway is that of phthalic acid. Information on degradation of phthalate esters is comparatively very limited in the literature (see review by Gu, 2003b). Our earlier research has been focused on the degradation of dimethyl phthalate ester (DMP) and its isomers, and the biochemical pathways involved by pure cultures of bacteria (Gu et al., 2004). One important fact derived from the research was that biochemical cooperation by different bacteria was needed for the utilization of *ortho*-dimethyl phthalate ester (Fig. 3) and dimethyl terephthalate ester (Fig. 4) (Gu et al., 2004; Li et al., 2005a, b; Wang, 2005). *Sphingomonas paucimobilis* has been demonstrated as a very important participant in the bacterial consortium degrading phthalic acid (Wang et al., 2003a; Fan et al., 2004; Wang et al., 2004). This strain lacks the ability to hydrolyze ester bonds, required for transforming DMP to monomethyl phthalate (MMP) (Fig. 3), but can degrade down-stream degradation intermediates. Mineralization of *ortho*-dimethyl phthalate ester was carried out by a biochemical cooperation between *Arthrobacter* sp., which can transform DMP to MMP, and *Sphingomonas yanoikuyae* DOS01, which is distinct from the *S. paucimobilis* in the previous study, consumes the substrates DMP and MMP. However, *S. yanoikuyae* DOS01 was incapable of degrading PA. In addition, *Arthrobacter keyseri* degraded several *ortho*-dimethyl phthalate esters, including dimethyl phthalate, diethyl phthalate, and di-*n*-butyl phthalate.

Variovorax paradoxus T4, isolated from deep-ocean sediment of the South China Sea, was capable of utilizing dimethyl terephthalate ester as sole carbon and energy source (Fig. 4); the intermediates monomethyl terephthalate (MMT) and terephthalic acid (TA) were also degraded. However, *Sphingomoas* sp. DOS01, also involved in the complete degradation of dimethyl terephthalate ester, was only capable of transforming dimethyl terephthalate ester to MMT without further degradation (Fig. 4) (Wang, 2005;

Table 3
Degradability of plasticizers by bacteria isolated from various environments

Substrate	Microorganism(s) involved	Source of Inoculum	Degradation intermediates	References
Dimethyl phthalate	*Comamonas acidovorans* fy-1,*Xanthomonas maltophila*, and *Sphingomonas paucimobilis*	Activated sludge	monomethyl phthalate, phthalic acid	Wang et al. (2003a, b)
Dimethyl phthalate	*Rhodococcus rubber* Sa	Mangrove sediment	monomethylphthalate, phthalic acid	Li et al. (2005a, b)
Dimethyl isophthalate	*Klebsiella oxytoca* Sc, and *Methylobacterium mesophilicum* Sr	Mangrove sediment	monoisophthalate,phthalic acid	Gu et al. (2004)
Dimethyl isophthalate	*Rhodococcus rubber* Sa	Mangrove sediment	monoisophthalate,phthalic acid	Li et al. (2005a)
Dimethyl terephthalate	*Rhodococcus rubber* Sa	Mangrove sediment,	monoterephthalate,phthalic acid	Li et al. (2005a)
Dimethyl terephthalate	*Pasteurella multocida* Sa	Mangrove sediment	monoterephthalate,phthalaic acid	Li et al. (2005b)
Di-*n*-butyl phthalate	*Pseudomonas fluorescens* B-1	Mangrove sediment	monobutyl phthalate,phthalic acid	Xu et al. (2005a, b)
Butylbenzyl phthalate	*Pseudomonas fluorescens* B-1	Mangrove sediment	monobutyl phthalate, Monobenzyl phthalate phthalic acid	Xu et al. (unpublished data)

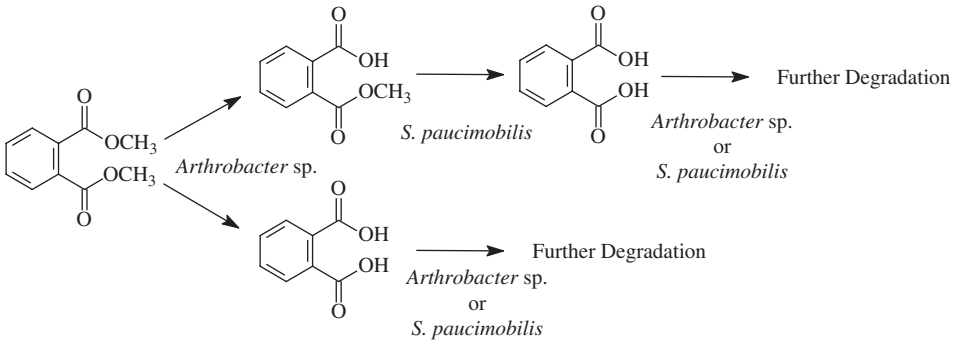

Fig. 3. Proposed biochemical pathways for degradation of *ortho*-dimethyl phthalate ester by *Arthrobacter* species and *Sphingomonas paucimobilis* isolated from deep-ocean sediment of the South China Sea (Wang, 2005).

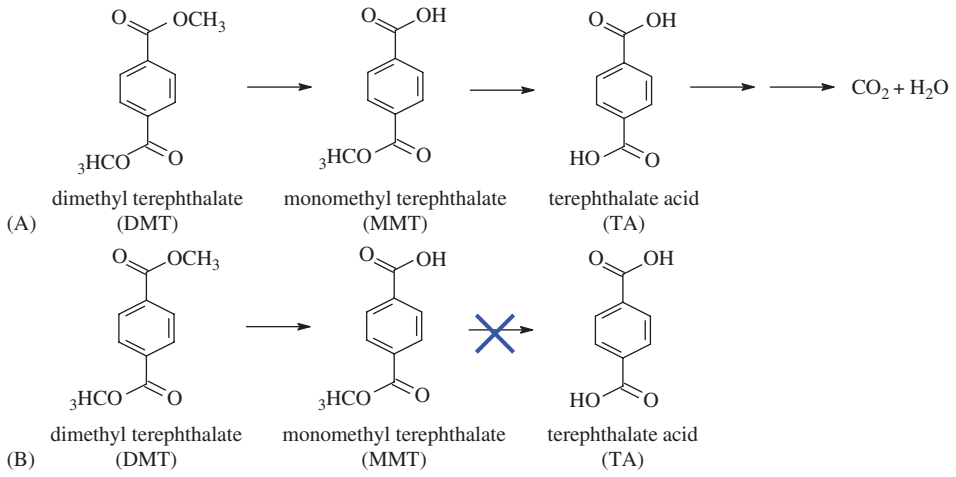

Fig. 4. Proposed biochemical pathways for degradation of dimethyl terephthalate ester by *Variovorax paradoxus* T4 (A) and *Sphingomonas yanoikuyae* DOS01 (B) isolated from deep-ocean sediment of the South China Sea (Wang, 2005).

Wang and Gu, 2006), indicating the complexity of biochemical cooperation between different microorganisms in the natural environment.

3.3.2. *Di-n-butyl phthalate ester and dibutylbenzyl phthalate ester*

Degradation of di-*n*-butyl phthalate ester was accomplished by *Pseudomonas fluorescence* B-1 isolated from mangrove sediment (Xu et al., 2005a, b). Monobutyl phthalate was the major intermediate produced during the initial phase of degradation, and a small amount of phthalic acid was also produced. Detection of both intermediates in this study suggests that similar biochemical mechanisms to the isomers of dimethyl phthalate esters are involved in degradation of di-*n*-butyl phthalate. A major difference was also apparent in that *P. fluorescens* alone was capable of completely degradating di-*n*-phthalate ester. Similarly, dibutylbenzylphthalate ester was first transformed to monobutylphthalate and monobenzylphthalate and then phthalic acid by the same *P. fluorescens* B-1 (Xu et al., 2005b). Results of this part of the study indicated that enzymes involved in the initial cleavage of the ester bonds were not selective, which is very different from the similar enzymes catalyzing transformation of isomers of dimethyl phthalate ester. Because of the differences in selectivity of the hydrolytic esterases for different substrates, further proteomics work will be needed to elucidate the specific nature of the esterases involved in degradation of this collection of environmental pollutants.

4. Susceptibility testing methodologies

Currently available methods are not applicable for assessing deterioration of high-strength and engineering materials due to their slow degradation rate. At the same time, traditional methods rely heavily on observation and rating for characterization of the extent of degradation. Most of the time, the qualitative results reflect the development of biofilms on surfaces of materials (review by Gu, 2003a) and no scientifically sound inference of material integrity or damage can be made. Quantitative and reliable methods are needed for screening and accurate determination of biodeterioration, especially early detection methods for engineering applications (Gu and Gu, 2005). A series of testing methods has been developed for testing the biodeterioration and biodegradation of organic materials, particularly polymers of various chemical

compositions and a range degradability (Gross et al., 1995; Gu, 2003a). Cellulose acetate with degree of substitution (d.s.) values from 0.8–2.5 was used for the validation of the test methods conducted under thermophilic composting and landfill simulations (Gu et al., 1992a, b, 1993a, b, c, 1994). These methods can also accommodate polymers in various physical forms, e.g., thick films and powders.

The highly sensitive and quantitative method of EIS has been introduced in evaluation of polymer integrity of polyimide thin films, fiber-reinforced polymeric composites, and aliphatic polyurethane top-coatings (Gu et al., 1996b, 1998b; Gu, 2004). With proper controls, without inoculation of microorganisms, and inoculated ones, the effects of microorganisms and their growth on polymer deterioration can be evaluated. The mechanisms may also be delineated by further study, after gaining information about the early changes. With the latest advances, new techniques can be adopted in tests specific to the materials and their use of environments, so that data generated on different materials will provide a quantitative basis for the description of the biological deterioration potential.

5. Protective measures against microorganisms

Microbiological contamination of space travel and enclosed systems is a challenge for the successful application of any long-term program because damage of materials by microorganisms will increase over time. At the same time, health-related problems may also appear due to the increasing load of microorganisms in the environments. Control of microbial growth and propagation on material surfaces can be achieved by using the proper criteria in selecting materials that are not easily susceptible to microbial growth or utilization (Gu, 2004). Physical and chemical manipulations of the materials and the artificial environments can also delay the initial establishment of microorganisms on surfaces and the subsequent development of biofilms; environmental control by lowering humidity can slow the growth of microorganisms (Gu et al., 1998a). Obviously, because of the presence of astronauts, there is a limit to the extent to which the humidity can be manipulated. Experience from Russian space programs on materials formulation by incorporation of biocides and physical cleaning clearly shows both the practicality of application and also the problems involved (Pierson et al., 1994). Similarly, temperature can be controlled to reduce the growth of microorganisms (Gu et al., 1998a; Mitchell et al., 1996), to prevent potential contamination and to extend the lifetime of the hardware. It should also be pointed out that very little is known about slow-growing bacteria on the surface of polymers (Gu and Pan, 2006). This group of bacteria has been largely ignored, but their ability to utilize polymeric materials may be higher than that of fast-growing bacteria. Such information will be very useful in the fundamental understanding of material damage and half-life prediction.

Biocides are commonly applied in polymeric materials. Silver ion has been used as a microbial inhibitor against microorganisms in water purification systems of Russian spacecraft, while iodine was chosen on the ISS by the US (Pierson et al., 1994). These chemicals have been shown to be of variable efficacy at the initial stage in killing planktonic bacterial cells. After adhesion to surfaces, biofilm bacteria show great resistance to a whole range of biocides (Mitchell et al., 1996; Gu, 2003a, b). Organic biocides, used to prevent bacterial growth in industrial systems, may selectively enrich the resistant population of microorganisms on long-term of exposure (Gu et al., 1998a). Microorganisms are known to uptake genes from the environment through horizontal gene transfer. No effective solution to these problems is currently available and alternative biocides have been screened from natural products. Resistant microorganisms have often been isolated because of the high mobility of resistant genes in the environment; microorganisms are capable of incorporating such genes into the cell to increase their survival. The high frequency and diversity of plasmids found in environmental bacteria are an example illustrating the plasticity of bacteria (Wang et al., 2006; Zhang et al., 2006). These bacteria show a wide range of resistance to antibiotics and chemicals, including Hg^{2+}. It should also be pointed out that very little information is available about the functional genes in the plasmids isolated from environmental bacteria, e.g., *Vibrio* spp. (Wang et al., 2006; Zhang et al., 2006). In addition to their environmental unacceptability, biocides also serve as a selection pressure for development of a resistant population through mutation or up-taking resistance genetic elements from the environment. Current research by biologists and materials scientists is focused on the prevention of adhesion of microorganisms to surfaces, using surface treatments and modifications. A less opportunistic pathogenic strain of *Pseudomonas aeruginosa* was detected on surfaces incorporating Ag^+ or Ag^+ plus lectin, because both biocidal and initial adhesion inhibition were activated in such treatments (Gu et al., 2001).

Since bacteria are capable of forming biofilms on surfaces of materials, future tests should focus on the quantification of biofilm rather than descriptively showing biofilms on surfaces using scanning electron microscopy. In assaying the efficacy of biocides against bacteria, tests should be conducted based on simulated biofilm conditions rather than the traditional liquid culture efficacy tests (Gu et al., 1998a, 2000). Because bacteria are more resistant to antibiotics and biocides after biofilms are formed and developed, the planktonic cells of liquid cultures are not truly representative of their actual condition on the surfaces of materials (Gu, 2003a). And as mentioned above, horizontal gene transfer allows bacteria to gain resistance to higher concentrations of biocides, metals, and antibiotics (Zhang et al., 2006).

Prevention of biofilm formation and biodeterioration on materials used in space should emphasize surface engineering

of materials, so that initial attachment and susceptibility to microorganisms can be minimized. Biofilm microorganisms can be reduced greatly on surfaces. Early detection of material integrity is an important component in diagnosis and prevention of deterioration (Mansfeld, 1995). New detection technologies, including optical fiber, DNA probes and microarrays (Gu, 2003a), will find valuable applications in this field, and advance our understanding of the mechanisms involved in bacterial adhesion and its prevention in the near future.

In conclusion, microbial contamination and colonization of surfaces are problems facing the long-term application of space programs, particularly in terms of polymeric materials used. Since both bacteria and fungi are common microflora in spacecraft, the population size should be maintained at a lower level to provide microbial safety to the materials and also to the astronauts. Almost all materials are susceptible to colonization by environmental microorganisms, including both fungi and bacteria; candidate materials for space applications should be screened using quantitative methods for their susceptibility and should be tested for the possible extent of deterioration and degradation by microorganisms. Further coupling of information derived from applied microbiology and materials science will allow the purpose-oriented design of materials for such specialized applications and extension of service life.

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