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Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances

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Abstract

Biodeterioration of polymeric materials affect a wide range of industries. Degradability of polymeric materials is a function of the structures of polymeric materials, the presence of degradative microbial population and the environmental conditions that encourage microbial growth. Our understanding of polymer degradation has been advanced in recent years, but the subject is still inadequately addressed. This is clearly indicated by the lack of information available on biodeterioration of polymeric materials, particularly the mechanisms involved and the microorganisms participated. In this review, polymers are treated according to their origin and biodegradability, and grouped as biopolymer, chemically modified natural polymers and recalcitrant polymers. Selective examples are used to illustrate the mechanisms and microorganisms involved in degradation of specific polymeric materials, and detection methods used for degradation and deterioration tests are discussed. In addition, new detection techniques and preventive measures are also presented.

Keywords: Biodeterioration; Biodegradation; Biodeteriogens; Fiber-reinforced composites; Fungi; Polymers; Prevention

1. Introduction

All surfaces under natural and artificial conditions except for extremely clean rooms are covered ubiquitously with microorganisms. This unique characteristic of bacterial association with surfaces was evident from the very beginning of bacterial existence and has remained part of normal living (Angles et al., 1993; Gu et al., 2000b,d; Marshall, 1976, 1992; Wächtershäuser, 1988; Woese, 1987). The process in which a complex community of microorganisms is established on a surface is known as "microfouling" or formation of biofilm. Biofilms, consisting of both microorganisms and their extracellular polysaccharides, are highly diverse and variable in both space and time. They are common on all surfaces in both terrestrial and aquatic environments (Caldwell et al., 1997; Fletcher, 1996; Ford et al., 1991; Ford, 1993; Geesey and White, 1990; Gehrke et al., 1998; Neu, 1996). Materials including metals (Gu et al., 2000a), inorganic minerals (Gu et al., 1996d, 1998c, 2000c) and organic polymers (Gu et al., 2000b) are susceptible to the formation of microbial biofilms under humid conditions, particularly those in tropical and subtropical climates (Gu et al., 2000d). Subsequent damage of materials is a result of natural processes catalyzed by microorganisms. Complete degradation of natural materials is an important part of the nutrients cycling in the ecosystem (Swift et al., 1979). Biofilm formation is a prerequisite for substantial corrosion and/or deterioration of the underlying materials to take place (Arino et al., 1997; Gu and Mitchell, 2001; Gu et al., 2000a–d; Hou, 1999; Saiz-Jimenez, 1995, 1997; Walch, 1992).

2. Biofilms and fouling on materials

Biofilm structures are highly organized and diverse on surfaces (Bitton, 1980; Bonet et al., 1993; Breznak, 1984; Caldwell et al., 1997; Costerton et al., 1978, 1994; Dalton et al., 1994; Davey and O'Toole, 2000; Freeman and Lock, 1995; Guezennec et al., 1998; Kelley-Wintenberg and

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Montie, 1994; Lappin-Scott et al., 1992; L'Hostis et al., 1997; O'Toole et al., 2000; Whitfield, 1988; Wimpenny and Colasanti, 1997; Wolfaardt et al., 1994; Zachary et al., 1980). The specific architectural structures and organization of microorganisms on a particular surface are generally materials and microorganisms specific, depending on the surface properties (Fletcher and Loeb, 1979; van Loosdrecht et al., 1987, 1990; Wiencek and Fletcher, 1995) and the ambient environmental conditions including externally supplied electrical current, cationic ions, ionic concentrations, solution chemistry, and hydrodynamic conditions (Caldwell and Lawrence, 1986; Korber et al., 1989; Lawrence et al., 1987; Lewandowski et al., 1995; Leyden and Basiulis, 1989; Little et al., 1986; Marshall et al., 1971; Martrhamuthu et al., 1995; Neu, 1996; Pendyala et al., 1996; Power and Marshall, 1988; Rijnaarts et al., 1993; Schmidt, 1997; Sneider et al., 1994; Stoodley et al., 1997). Because virtually all surfaces may act as substrate for bacterial adhesion and biofilm formation (Busscher et al., 1990; Costerton et al., 1995; Geesey and White, 1990; Geesey et al., 1996; Marshall, 1980), attack of materials by microorganisms can take place either directly or indirectly, depending on the specific microorganisms, chemical and physical properties of the materials, and their environmental conditions (Gu et al., 2000d). More specifically, important factors affecting the rate of biodeterioration include material composition (Bos et al., 1999; Busscher et al., 1990; Gu et al., 2000b; Wiencek and Fletcher, 1995), molecular weights, atomic composition and the chemical bonds in the structure, the physical and chemical characteristics of the surfaces (Becker et al., 1994; Caldwell et al., 1997; Callow and Fletcher, 1994), the indigenous microflora, and environmental conditions. Using microorganisms capable of degrading specific organic pollutants, the biofilms immobilized on material surfaces have important applications in degradation of toxic pollutants, wastewater treatment and bioleaching (Bryers, 1990, 1994; Gu, 2001; Gu et al., 2001c; Osswald et al., 1995; Sharp et al., 1998). In contrast, biofilms are undesirable in food processing, drinking-water distribution systems, petroleum transport pipeline, water-cooling systems, on submerged engineering systems and structures, medical implant materials. On molecular level, bacterial attachment on surfaces is a process controlled by chemical signaling between bacteria (Davies et al., 1998; McLean et al., 1997; Reynolds and Fink, 2001) and the specific chemical molecules involved have been elucidated as *N*-(3-oxohexanoyl)-L-acylhomoserine lactones in *Photobacterium fisheri*, *N*-(3-hydroxybutanoyl)-L-acylhomoserine in *Vibrio harveyi*, *N*-(3-oxododecanoyl)-L-acylhomoserine in *Pseudomonas aeruginosa*, *N*-(3-oxooctanoyl)-L-acylhomoserine in *Agrobacterium tumefaciens*, and γ-butyrolactone in *Streptomyces* spp. (Davies et al., 1998; Salmond et al., 1995; You et al., 1998).

Biofouling is a process defined as the undesirable accumulation of microorganisms, their products and deposits including minerals and organic materials, and macroorganisms on substratum surfaces (Gu and Mitchell, 1995; Nefedov et al., 1988; Novikova and Zaloguyev, 1985; Solomin, 1985; Sunesson et al., 1995; Viktorov, 1994; Viktorov and Novikova, 1985; Viktorov and IIyin, 1992; Viktorov et al., 1993; Zaloguyev, 1985). The thin film on fouled surfaces usually consists of microorganisms embedded in an organic matrix of biopolymers, which are produced by the microorganisms under natural conditions. In addition, microbial precipitates, minerals, and corrosion products may also coexist (Beveridge et al., 1997; Konhauser et al., 1994; Liken, 1981; Lovley, 1991; Pierson and Parenteau, 2000; Wilkinson and Stark, 1956; Zehnder and Stumm, 1988). Microfouling by microorganisms can serve as a prerequisite for the subsequent macrofouling by invertebrates such as *Balanus amphitrite*, *Janua brasiliensis*, *Ciona intestinalis* (Gu et al., 1997c; Maki et al., 1990). Industrial fouling is a complex phenomenon involving interactions between chemical, biological and physical processes resulting in enormous economic loss. To combat fouling and corrosion, large quantities of biocides have been used to control biofouling and as a result biocide resistance is an emerging problem to our society.

Both metal and non-metallic materials immersed in aqueous environments or under high humidity conditions are equally susceptible to biofouling and biodeterioration (Characklis, 1990; Gu and Mitchell, 1995; Gu et al., 1998b, 2000d; Jones-Meehan et al., 1994a, b; Knyazev et al., 1986; Little et al., 1990; Thorp et al., 1994). Specific examples include medical implants (Dobbins et al., 1989; Gu et al., 2001a–c; McLean et al., 1995; Mittelman, 1996), water pipes (Rogers et al., 1994), artificial coatings (Edwards et al., 1994; Gu et al., 1998a; Jones-Meehan et al., 1994b; Stern and Howard, 2000; Thorp et al., 1997), rubber (Berekaa et al., 2000), ultrapure systems (Flemming et al., 1994; Mittelman, 1995), porous media (Bouwer, 1992; Cunningham et al., 1990, 1991; Mills and Powelson, 1996; Rittman, 1993; Vandevivere, 1995; Vandevivere and Kirchman, 1993; Williams and Fletcher, 1996), water and wastewater treatment (Bryers and Characklis, 1990; Gillis and Gillis, 1996; Rethke, 1994; Tall et al., 1995), oilfield (Lynch and Edyvean, 1988), space station (Gazenko et al., 1990; Meshkov, 1994; Novikova et al., 1986; Pierson and Mishra, 1992; Stranger-Joannesen et al., 1993; Zaloguyev, 1985) and magnetic diskettes (McCain and Mirocha, 1995).

Generally, biodeterioration is the undesirable degradation of materials including both metals and polymers in the presence of and by microorganisms. Damage of materials may result in an early and unexpected consequence and the problem is often translated to system failure and economic loss. The term biodeterioration also implicitly includes both biocorrosion and biodegradation in this review. All three terms, corrosion, degradation and deterioration are used in this review. In the following sections, microbial deterioration and degradation of polymeric materials are discussed for several groups of materials.

3. Biodeterioration of polymeric materials

Microorganisms are involved in the deterioration and degradation of both synthetic and natural polymers (Gu et al., 2000b), and very little is known about the biodegradation of synthetic polymeric materials. The reason is probably due to the recent development and manufacture of this class of materials and the relatively slow rate of degradation in natural environments. Since chemically synthesized polymeric materials have become an important part of our human society and have more diversified applications than traditional metals, issues related to polymer deterioration and protection will receive increasingly attention in the time to come.

Polymeric materials are very unique in chemical composition, physical forms, mechanical properties and applications. High versatility of the carbon to carbon and carbon to non-carbon (C–C, C–R and C–H) bonds and substituent groups, the possible configurations, stereochemistry and orientation provide basis for variations of chemical structures and stereochemistry (Odian, 1991). Very small variations in the chemical structures may result in large differences in term of biodegradability. Because of this structural versatility, they are widely used in product packaging, insulation, structural components, protective coatings, medical implants, drug delivery carriers, slow-release capsules, electronic insulation, telecommunication, aviation and space industries, sporting and recreational equipment, building consolidants, etc. In service, they are constantly exposed to a range of natural and artificial conditions often involving microbial contamination, resulting in aging, disintegration, and deterioration over time (Lemaire et al., 1992; Pitt, 1992).

3.1. Microorganisms and general degradation

Polymers are potential substrates for heterotrophic microorganisms including bacteria and fungi. Polymer biodegradability depends on molecular weight, crystallinity and physical forms (Gu et al., 2000b). Generally, an increase in molecular weight results in a decline of polymer degradability by microorganisms. In contrast, monomers, dimers, and oligomers of a polymer's repeating units are much easily degraded and mineralized. High molecular weights result in a sharp decrease in solubility making them unfavorable for microbial attack because bacteria require the substrate be assimilated through the cellular membrane and then further degraded by cellular enzymes. However, it should be pointed out that concurrent abiological and biological processes may facilitate the degradation of polymers.

At least two categories of enzymes are actively involved in biological degradation of polymers: extracellular and intracellular depolymerases (Doi, 1990; Gu et al., 2000b). During degradation, exoenzymes from microorganisms break down complex polymers yielding short chains or

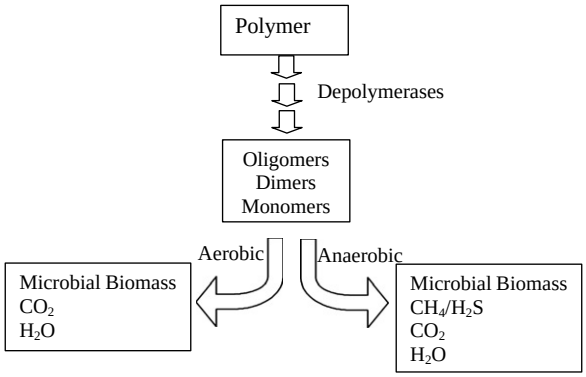

Fig. 1. Schematic diagram of polymer degradation under aerobic and anaerobic conditions.

smaller molecules, e.g., oligomers, dimers, and monomers, that are smaller enough to pass the semi-permeable outer bacterial membranes, and then to be utilized as carbon and energy sources (Fig. 1). The process is called depolymerization. When the end products are inorganic species, e.g., CO_2, H_2O, or CH_4, the degradation is called mineralization. A commonly recognized rule is that the closer the similarity of a polymeric structure to a natural molecule, the easier it is to be degraded and mineralized. Polymers like cellulose, chitin, pullusan, and PHB are all biologically synthesized and can be completely and rapidly biodegraded by heterotrophic microorganisms in a wide range of natural environment (Bérenger et al., 1985; Byrom, 1991; Chahal et al., 1992; Frazer, 1994; Gamerith et al., 1992; Gujer and Zehnder, 1983; Gunjala and Sulflita, 1993; Hamilton et al., 1995; Hass et al., 1992; Hespell and O'Bryan-Shah, 1988; Kormelink and Voragen, 1993; Lee et al., 1985, 1987a, b, 1993; Lüthi et al., 1990a, b; MacDonald et al., 1985; MacKenzie et al., 1987; Nakanishi et al., 1992; Sonne-Hansen et al., 1993; Sternberg et al., 1977; Törrönen et al., 1993; Wong et al., 1988; Yoshizako et al., 1992). In addition, natural conditions also include environments where anaerobic processes are the leading ones (Brune et al., 2000; Fenchel and Finlay, 1995). Under such conditions, the complete decomposition of a polymer will produce organic acids, CO_2, CH_4 and H_2O. It is important to note that biodeterioration and degradation of polymer substrate can rarely reach 100% and the reason is that a small portion of the polymer will be incorporated into microbial biomass, humus and other natural products (Alexander, 1977; Atlas and Bartha, 1997; Narayan, 1993).

Dominant groups of microorganisms and the degradative pathways associated with polymer degradation are often determined by the environmental conditions. When O_2 is available, aerobic microorganisms are mostly responsible for destruction of complex materials, with microbial biomass, CO_2, and H_2O as the final products (Fig. 1). In contrast, under anoxic conditions, anaerobic consortia of microorganisms are responsible for polymer deterioration. The primary products will be microbial biomass, CO_2, CH_4 and H_2O under methanogenic conditions (Barlaz et al.,

1989a, b; Gu et al., 2000e, 2001; Gu and Mitchell, 2001) or H_2S, CO_2 and H_2O under sulfidogenic conditions (Fig. 1). It is known that aerobic processes yield much more energy and are capable of supporting a greater population of microorganisms than anaerobic processes because thermodynamically O_2 is a more efficient electron acceptor than SO_4^{2-} and CO_2. These conditions are widely found in natural environments and can be simulated in the laboratory with appropriate inocula. Both aerobic and strictly anaerobic microorganisms are involved in the degradation of polymers.

In this review, synthetic polymers are divided into three groups: (1) degradable, (2) slowly degradable, and (3) resistant. Natural polymers, e.g., cellulose, chitin, chitosan, lignin, and polysaccharides, etc. are excluded.

3.2. Biodeterioration of polymers

3.2.1. Microbiologically synthesized polymers

Microorganisms are capable of manufacturing a range of complex polymers under conditions when excessive carbon source is available, e.g., C/N 10. The polymers include a diverse class of polyesters (Doi, 1990; Stenbüchel, 1991), polysaccharides (Linton et al., 1991), silk (Kaplan et al., 1991). Microbial degradation of polymers depends on their molecular compositions, molecular weights and the presence of specific microorganisms on surfaces of materials. Some can be almost completely utilized as a source of carbon and energy while others are only partially degraded. Examples of the former include the poly(hydroxyalkanoate)s (PHAs) (Anderson and Dowes, 1990; Brandl et al., 1988; Choi and Yoon, 1994; Doi, 1990; Nakayama et al., 1985; Stenbüchel, 1991; Stuart et al., 1995; Tanio et al., 1982); γ-poly(glutamic acid) (Cromwick and Gross, 1995), cellulose acetates with degree of substitution values lower than 2.5 (Buchanan et al., 1993; Gross et al., 1993, 1995; Gu et al., 1992b, c, 1993a–c, 1994b), polyethers (Kawai, 1987; Kawai and Moriya, 1991; Kawai and Yamanaka, 1986), polylactide (Gu et al., 1992b,c), polyurethanes (Blake et al., 1998; Crabbe et al., 1994; El-Sayed et al., 1996; Filip, 1978; Gillatt, 1990; Gu et al., 1998b; Mitchell et al., 1996; Nakajima-Kambe et al., 1995; Szycher, 1989), and natural rubbers (Berekaa et al., 2000; Heisey and Papadatos, 1995).

Chemical structure of a polymer determines the extent of biodegradation. A general rule is that biologically synthesized polymers are readily biodegradable in natural environments and synthetic polymers are either less biodegradable or degraded very slowly. This widely accepted rule suggests that the degradation processes have evolved through time and complexity of biochemical pathways may increase with the structure diversification of polymeric materials. However, the rate of degradation is largely affected by the chemical structure, e.g., the C–C and other types of bonds, molecular weights, structures and configuration as well as the participating microorganisms and the environmental conditions. High molecular weight polymers are less biodegradable or degraded at a slower rate than those with low molecular weights. For example, the rate of hydrolytic chain cleavage of ester bonds in the following polymers is dependent on the co-polymer composition: poly(3-hydroxybutyrate-co-27% 4-hydroxybutyrate) [P(3HB-co-27% 4HB)] > [P(3HB-co-17% 4HB)] > [P(3HB-co-10% 4HB)] > poly(3-hydroxybutyrate-co-45% 3-hydroxybutyrate [P(3HB-co-45% 3HV)] > [P(3HB-co-71% 3HV)] (Doi, 1990). Similarly, the sequence of enzymatic hydrolysis is [P(HB-co-16% HV)] > [P(HB-co-32% HV)] > PHB (Parikh et al., 1993). In addition, crystallinity and stereochemistry of polymers also affect the rate of degradation significantly, but is rarely taken into account (Budwill et al., 1992; Gu et al., 2000b,e). This characteristic of molecules and its effects on degradation has received attention recently (Kohler et al., 2000).

3.2.2. Poly(β-hydroxyalkanoates)

Bacterial poly(β-hydroxyalkanoates) are formed during nutrient limited growth when the carbon source is in excess, e.g., high C/N ratio, as energy storage materials (Anderson and Dowes, 1990; Brandl et al., 1988; Doi, 1990; Holmes et al., 1985; Kim et al., 1995; Lemoigne, 1926; Stenbüchel, 1991). Under condition of nutrient limitation, these materials can be depolymerized and utilized by microorganisms. They consist of homo or co-polymers of [R]-β-hydroxyalkanoic acids. This polymer is a microbial intracellular inclusion in the cytoplasmic fluid in the form of granules with diameters between 0.3 and 1.0 μm (Stenbüchel, 1991). Biopolymers may comprise as much as 30–80% of the total cellular biomass. The polymer has been isolated from *Bacillus megaterium* by extraction in chloroform and has a molecular weight of approximately 10^5–10^6 with more than 50% in crystalline form (Gu et al., unpublished data). Unlike other biopolymers, such as polysaccharides, proteins and DNAs, PHB is thermoplastic with a melting temperature around 180°C, making it a good candidate for thermoprocessing. Furthermore, PHB and co-polymers have also been produced in genetic engineered plants (John and Keller, 1996) and through chemical synthesis (Kemnitzer et al., 1992, 1993), which provide potential for commercial production in the future.

Both homopolymers and co-polymers can be degraded under biologically active environments, e.g., soil (Albertsson et al., 1987; Mas-Castellà et al., 1995; Tsao et al., 1993), sludge, compost (Gilmore et al., 1992, 1993; Gross et al., 1993, 1995; Gu et al., 1993b), river water (Andrady et al., 1993; Imam et al., 1992) and seawater (Andrady et al., 1993; Imam et al., 1999; Sullivan et al., 1993; Wirsen and Jannasch, 1993). Extracellular PHB depolymerases have been isolated from *Pseudomonas lemoignei* (Lusty and Doudoroff, 1966) and *A. faecalis* (Saito et al., 1989; Tanio et al., 1982). Other bacteria capable of degrading these polymers include *Acidovorax facilis*, *Variovorax paradoxus*,

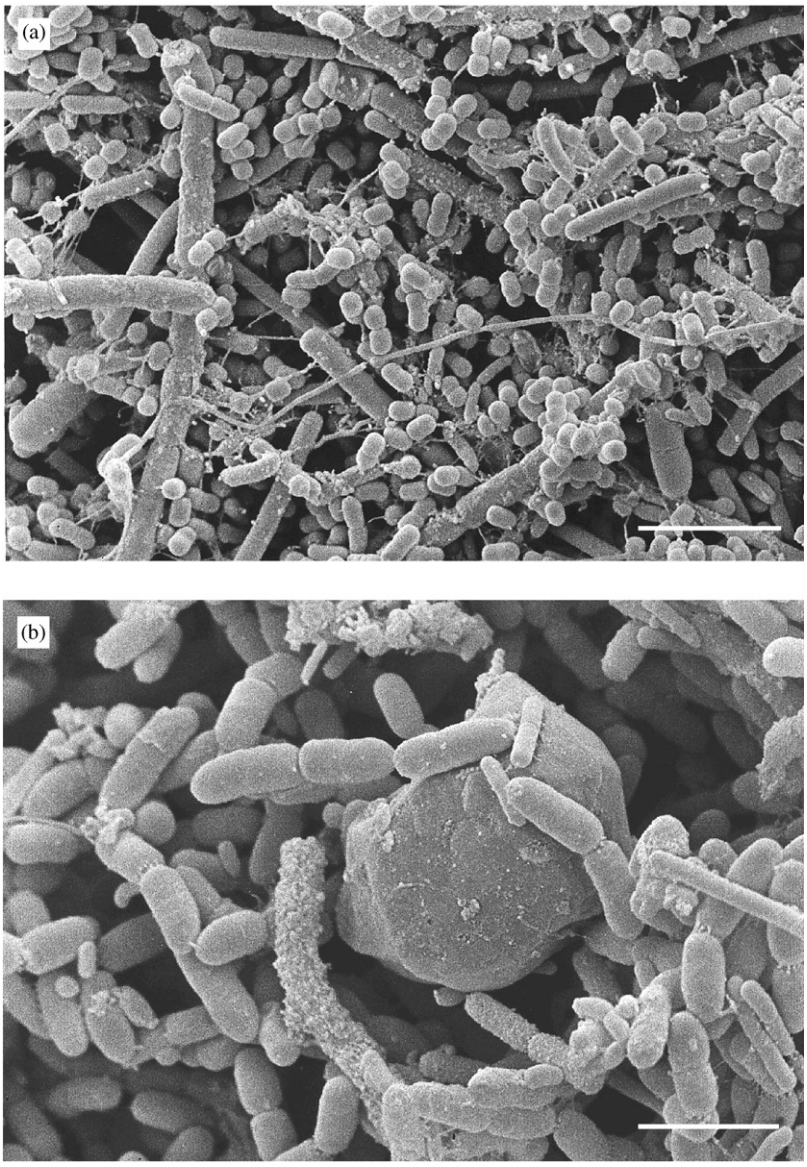

Fig. 2. Scanning electron micrographs of (a) aerobic soil bacteria growing on surface of poly-β-hydroxybutyrate (PHB) (scale bar, 10 μm) and (b) bacteria surrounding a PHB granule after incubation under mesophilic conditions (35°C) (scale bar, 5 μm).

Pseudomonas syringae subsp. *savastanoi*, *Comamonas testosteroni*, *Cytophaga johnsonae*, *Bacillus megaterium*, *B. polymyxa*, and *Streptomyces* spp. (Mergaert et al., 1993). The enzymatic degradation occurs initially at the surfaces of the polyester film after microbial colonization (Fig. 2), and the rate of surface erosion is highly dependent on both the molecular weight (degree of polymerization), composition of the polyester, crystallinity and the dominant species of bacteria.

3.3. Modified natural polymers

3.3.1. Cellulose acetates (CAs)

Cellulose acetates (CAs) are a class of natural polymers with chemical modification to improve their processibility and mechanical properties for different applications (Bogan and Brewer, 1985). Because the backbone is natural cellulose, theoretically they can carry substitution values from as low as near zero to as high as 3.0. Current knowledge is that CAs with a degree of substitution values less than 2.5 can be degraded in thermophilic compost (Gross et al., 1993, 1995; Gu et al., 1992b, c, 1993a–c, 1994b) or transformed to solvents through biological catalyzed reactions (Downing et al., 1987). Apparently, increasing the DS value makes the polymers less degradable. As discussed above, it is clear that slightly deviation from the natural structures will lead to increasing resistance to deterioration and degradation.

CA degradation occurs more rapidly under oxic conditions than anoxic conditions. The mechanisms of initial degradation reaction are de-acetylation, which releases the

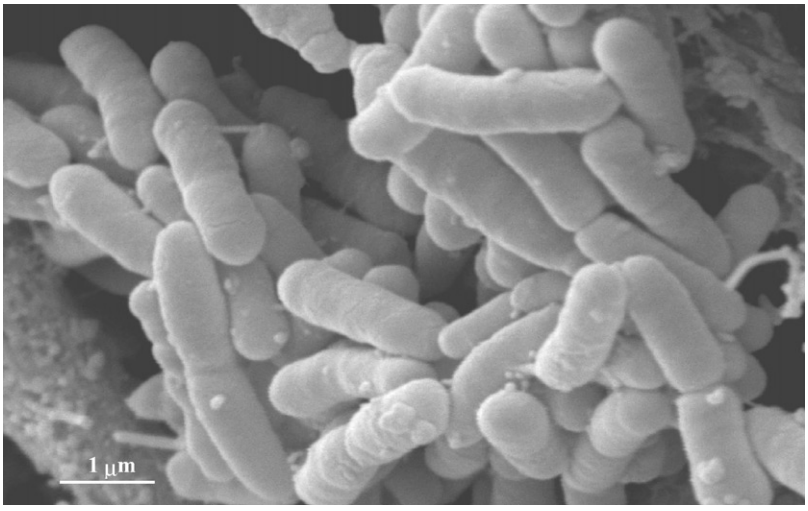

Fig. 3. Scanning electron micrograph showing bacteria growing on surfaces of cellulose acetate.

substituted groups, followed by cleavage of the C–C backbone. In this case, substituting group has a strong influence on the degradability of polymer. It is also demonstrated recently that the decrease of molecular weight by cleavage of C–C chain and de-acetylation proceed simultaneously during degradation after CA reaches to a critical value of substitution of approximately 1.0. Structural substitution groups, and their numbers per repeating unit, affects the degradation kinetics remarkably. For example, cellulose acetate (CA) with a lower degree of substitution (DS) value is more quickly degraded than those with higher substitution values under both oxic and anoxic conditions (Buchanan et al., 1993; Gross et al., 1993, 1995; Gu et al., 1992b, c, 1993a–c, 1994b) (Fig. 3). CAs with lower substitution values ($\cong$ 0.82) also show relatively higher solubility which is favored by microbial metabolism. During degradation of CA, both molecular weight and degree of substitution decreased, suggesting that de-acetylation and decomposition of the polymer backbone proceed simultaneously (Gu et al., 1993c). Earlier data also suggested that CA with DS values greater than 0.82 are recalcitrant to biodegradation and that the limiting step is de-acetylation, followed by breaking of the polymer carbon–carbon bonds (Reese, 1957). Current results showing degradation of CA indicated that CA degradation has been observed with DS values as high as 2.5.

Microorganisms capable of CA degradation are mostly actinomyces, fungi and selective bacteria (Gross et al., 1993, 1995; Gu et al., 1992b, c, 1993a–c). One bacterium *Pseudomonas paucimobilis* was isolated for ability to degrade CA with DS value 1.7 from a composting bioreactor containing CA films (Gross et al., 1993).

3.4. Synthetic polymers

3.4.1. Polyethers

One of the most commonly used synthetic polymers with wide application and usage is polyethers. The polymers include polyethylene glycols (PEGs), polypropylene glycols (PPGs) and polytetramethylene glycol (PTMGs). They are used in pharmaceuticals, cosmetics, lubricants, inks, and surfactants. Contamination of natural waters, including coastal waters and streams where wastewater is discharged have been reported (Kawai, 1987, 2002).

Degradability of this class of polymers has been studied under both oxic (Kawai, 1987, 2002; Kawai and Moriya, 1991; Kawai and Yamanaka, 1986) and anoxic conditions (Dwyer and Tiedje, 1983; Frings et al., 1992; Schink and Stieb, 1983). Their degradability is highly dependent on molecular weight. Molecules with molecular weights higher than 1000 have been considered resistant to biodegradation (Kawai, 1987). However, degradation of PEGs with molecular weights up to 20,000 has been reported (Kawai and Yamanaka, 1989). The ability of a microflora to degrade PEG molecules with high molecular weights is dependent primarily on the ability of a syntrophic association of different bacteria to metabolize the chemicals (Fig. 4). For example, *Flavobacterium* sp. and *Pseudomonas* sp. can form an effective association and mineralize PEG completely. During degradation, PEG molecules are reduced by one glycol unit after each oxidation cycle.

The central pathway of PEG degradation is cleavage of an aliphatic ether linkage. In a co-culture of aerobic *Flavobacterium* and *Pseudomonas* species, PEG degradation proceeds through dehydrogenation to form an aldehyde and a further dehydrogenation to a carboxylic acid derivative (Kawai, 1987; Kawai and Yamanaka, 1986). It is important to note that either of the two bacteria in pure culture cannot degrade PEG alone. Cellular contact between them seems to be essential for effective activity (Kawai, 1987).

In the investigated *Flavobaterium* sp. and *Pseudomonas* sp. system, three enzymes are involved in the complete degradation of PEG (Kawai, 1987). PEG dehydrogenase, PEG-aldehyde dehydrogenase, and PEG-carboxylate dehydrogenase (ether-cleaving) are all required. All of them

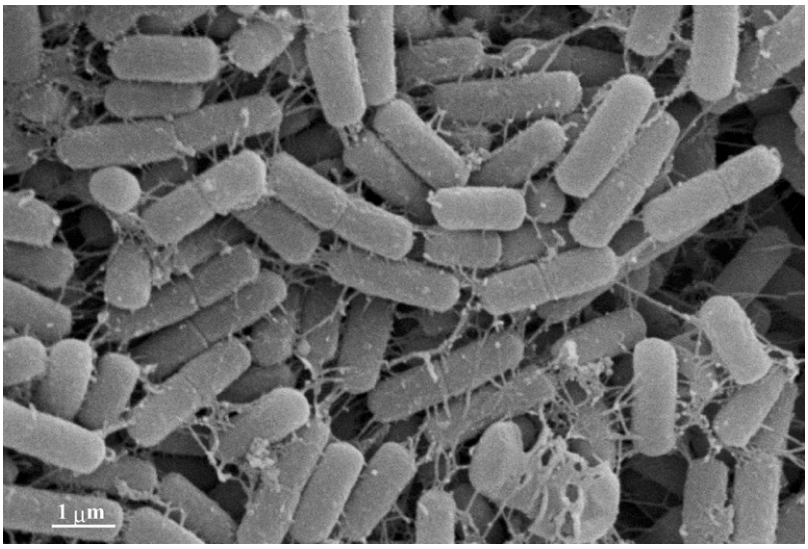

Fig. 4. Scanning electron micrograph showing a pure culture of bacteria capable of utilizing polyethylene glycol as a source of carbon and energy.

are found in *Flavobaterium* sp., while only PEG-carboxylate dehydrogenase is present in *Pseudomonas* sp. Using PEG 6000 as a sole substrate no degradation can be observed with either of the two bacteria alone. In addition, the ether cleavage is extremely sensitive to the presence of glycoxylic acid. However, *Pseudomonas* sp. though not directly involved in the degradation, is capable of utilizing the toxic metabolite that inhibits the activity of the *Flavobacterium* sp. This connection appears to be the essential link for their closely syntrophic association in achieving completely degradation of PEG.

Under anaerobic condition, EG, PEG can also be degraded (Dwyer and Tiedje, 1983) but only one bacterium *Pelobacter venetianus* was reported (Schink and Stieb, 1983).

3.5. Recalcitrant polymers

3.5.1. Electronic insulation polyimides

Polymers used in electronic industries are chemically synthesized with the objective of high strength and resistance to degradation. Thermosetting polyimides are major class in this application (Brown, 1982). Wide acceptance of polyimides in the electronics industry (Brown, 1982; Jensen, 1987; Lai, 1989; Verbicky, 1988; Verbiest et al., 1995) has drawn attention to the stability of these materials. The National Research Council (NRC, 1987) emphasized the need to apply these polymers in the electronic industries because data acquisition, information processing and communication are critically dependent on materials performance. The interlayering of polyimides and electronics in integrated circuits prompted several studies on the interactions between these two materials (Hahn et al., 1985; Kelley et al., 1987).

Polyimides are also widely used in load bearing applications, e.g., struts, chasses, and brackets in automotive and aircraft structures, due to their flexibility and compressive strength. They are also used in appliance construction, cookingware, and food packaging because of their chemical resistance to oils, greases, and fats, microwave transparency, and thermal resistance. Their electrical insulation properties are ideally suited for use in the electrical and electronics markets, especially as high temperature insulation materials and passivation layers in the fabrication of integrated circuits and flexible circuitry. In addition, the flammability resistance of this class of polymers may provide a halogen-free flame-retardant material for aircraft interiors, furnishings, and wire insulation. Other possible uses may include fibers for protective clothing, advanced composite structures, adhesives, insulation tapes, foam, and optics operating at high temperatures (Verbiest et al., 1995).

Electronic packaging polyimides are particularly useful because of their outstanding performance and engineering properties. It is only recently that biodeterioration of these polymers was investigated using pyromellitic dianhydride and 4, 4′-diaminodiphenyl ether with molecular weight (M_w of 2.5×10^5) (Ford et al., 1995; Gu et al., 1994a, 1995a, 1996b, c, 1998a, b; Mitton et al., 1993, 1996, 1998). They are susceptible to deterioration by fungi (Fig. 5) (Ford et al., 1995; Gu et al., 1994a, 1995a, 1996b; Mitton et al., 1993, 1998). Though bacteria were isolated from culture containing the deteriorated polyimides, further tests did not show comparable degradation by bacteria.

Our studies showed that the dielectric properties of polyimides could be altered drastically following growth of a microbial biofilm (Ford et al., 1995; Gu et al., 1995a, 1996b; Mitton et al., 1993, 1998). This form of deterioration may be slow under ambient conditions. However, the deterioration processes can be accelerated in humid conditions or in enclosed environments, e.g., submarines, space vehicles, aircraft, and other closed facilities. Very small changes of

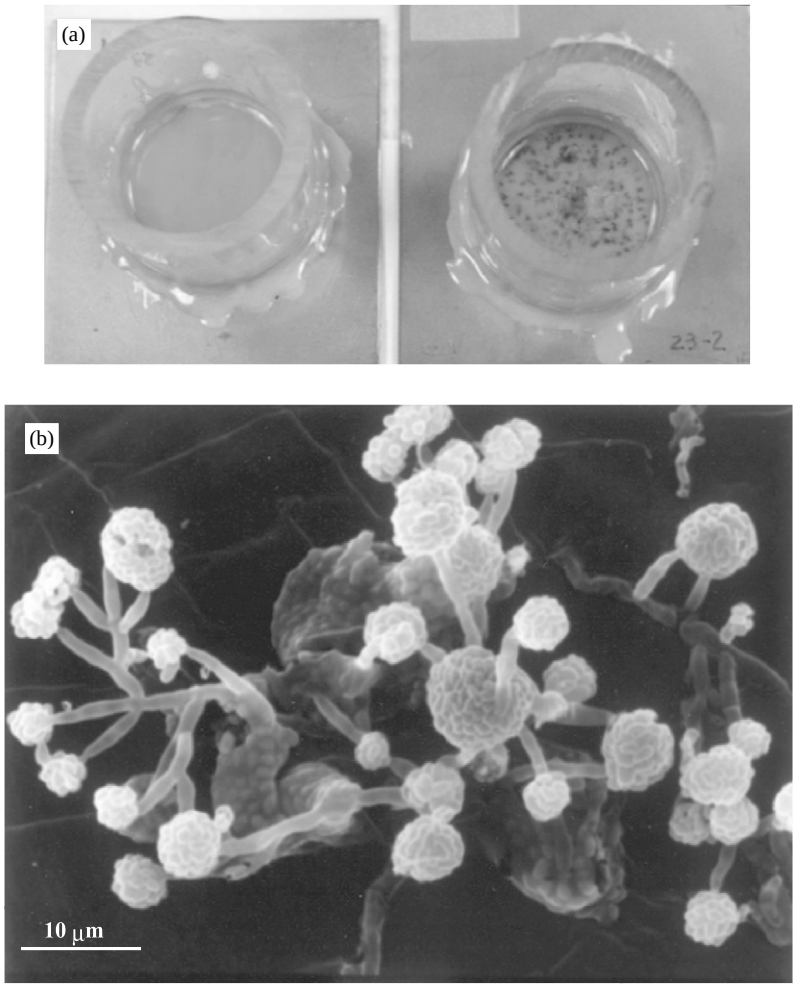

Fig. 5. Photograph and scanning electron micrograph showing: (a) the visible colonization of microorganisms in the inoculated cell containing polyimides and (b) the microorganisms colonizing and growing on the surface of polyimides.

material insulation properties may result in serious and catastrophic consequences of communications and control systems.

Polyimide deterioration occurs through biofilm formation and subsequent physical changes in the polymer. Using electrochemical impedance spectroscopy (EIS) (Mansfeld, 1995; van Westing et al., 1994), a very sensitive technique for monitoring dielectric constant of polymers, fungal growth on polyimides have been shown to yield distinctive EIS spectra, indicative of failing resistivity. In the degradation processes, two steps are involved during degradation: an initial decline in coating resistance is related to the partial ingress of water and ionic species into the polymer matrices. This is followed by further deterioration of the polymer by activity of the fungi, resulting in a large decrease in resistivity. Fungi involved include *Aspergillus versicolor*, *Cladosporium cladosporioides*, and *Chaetomium* sp. (Gu et al., 1995a, 1996a, e, 1997a, b, 1998a). The data support the hypothesis that polyimides are susceptible to microbial deterioration and also confirm the versatility of EIS as a method in evaluation of the biosusceptibility of polymers.

Initial isolation of microorganisms associated with deterioration of polyimides indicated the presence of both fungi and bacteria. Bacteria include *Acinetobacter johnsonii*, *Agrobacterium radiobacter*, *Alcaligenes denitrificans*, *Comamonas acidovorans*, *Pseudomonas* spp, and *Vibrio anguillarum*. These bacteria were not capable of degrading the polymer after inoculation while fungi were more effective in degrading the polyimides.

3.5.2. *Fiber-reinforced polymeric composite materials*

Fiber-reinforced polymeric composite materials (FRPCMs) are newly developed materials important to aerospace and aviation industries (Gu et al., 1994a, 1995a–d, 1996a, 1997a, b; Wagner, 1995; Wagner et al., 1996). The increasing usage of FRPCMs as structural components of public structures and particularly in aerospace application has generated an urgent need to evaluate the biodegradability of this class of new materials. FRPCMs are also susceptible to attack by microorganisms (Gu et al., 1997b). It was suggested that impurities and additives that can promote microbial growth are implicated as potential sources

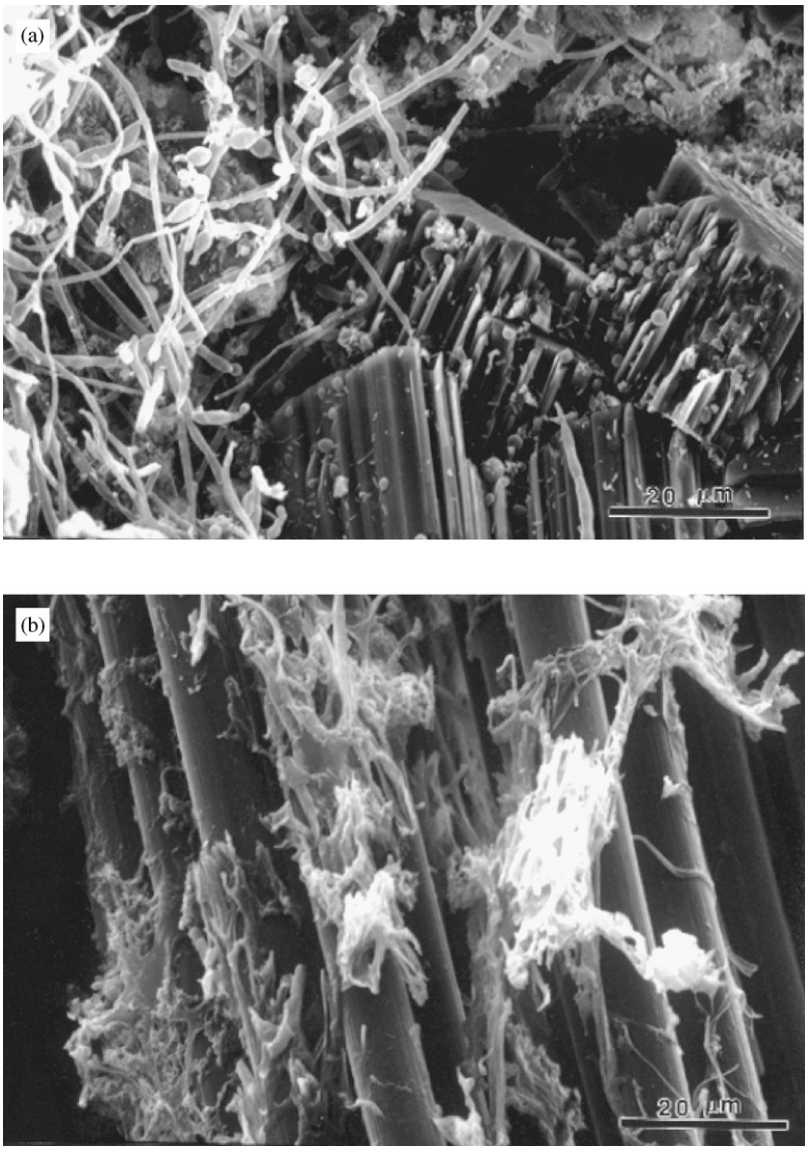

Fig. 6. Scanning electron micrographs showing colonization of surfaces of: (a) fiber-reinforced polymeric composite by both bacteria and fungi and (b) graphite carbon fibers by mostly fungi.

of carbon and energy for the environmental microorganisms (Fig. 6).

In this area of research, two groups reported microbial degradation of FRPCMs (Gu and Mitchell, 1995; Gu et al., 1995b–d, 1996a, 1997a, b; Wagner et al., 1996). A mixed culture of bacteria including a sulfate-reducing bacterium was used to show the material deterioration (Wagner et al., 1996). In contrast, Gu et al. (1994a, 1996a–c, 1997a, b) used a fungal consortium originally isolated from degraded polymers and a range of material composition including fluorinated polyimide/glass fibers, bismaleimide/graphite fibers, poly(ether-ether-ketone) (PEEK)/graphite fibers, and epoxy/graphite fibers (Gu et al., 1995b). The fungal consortium consisted of *Aspergillus versicolor*, *Cladosporium cladosorioides*, and a *Chaetomium* sp. Both bacteria and fungi are capable of growing on the graphite fibers of FRPCMs, but only fungi have been shown to cause deterioration detectable over more than 350 days (Gu et al., 1995b, 1997a, b). It was also found that plasticizers are biodegradable and utilized by natural microorganisms as source of carbon and energy (Gu et al., 1994a, 1996a). Phthalate and phthalate esters are the largely groups of chemicals used as plasticizers in plastics manufacturing, they are also detected at high concentrations in landfill leachate (Mersiosky, 2002) and degraded by aerobic microorganisms quickly (Fan et al., 2001; Wang et al., 2003). Physical and mechanical tests were not sufficiently sensitive to detect any significant physical changes in the materials after the duration of exposure (Gu et al., 1997b; Thorp et al., 1994). However, the resins were actively degraded,

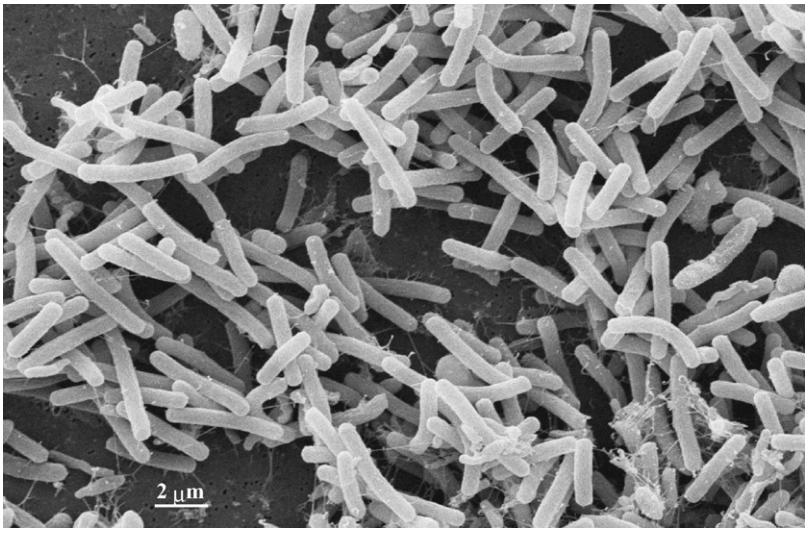

Fig. 7. Scanning electron micrograph of a pure culture of bacteria capable of degrading water-soluble polyurethane.

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., 1995d). It has become clear that FRPCMs are not immune to adhesion and attack by microorganisms (Ezeonu et al., 1994a,b; Gu et al., 1998b; Mitchell et al., 1996).

Natural populations of microorganisms are capable of growth on surfaces of FRCPM coupons at both relatively high (65–70%) and lower humidity conditions (55–65%) (Gu et al., 1998b). The accumulation of fungi on surfaces of composites develops into a thick biofilm layer and decreases the resistance to further environmental changes. However, the resistivity of FRPCMs was found to decline significantly after the initial 3 months during a year of monitoring using EIS (Gu et al., 1996c, 1997b). Clear differences resulting from biofilm development were detected on FRCPMs used in aerospace applications (Gu et al., 1997b). Further study indicated that many fungi are capable of utilizing chemicals, e.g., plasticizers, surface treatment chemicals and impurities, introduced during composite manufacture as carbon and energy sources (Gu et al., 1996a). Similarly, lignopolystyrene graft copolymers were also susceptible to attack by fungi (Milstein et al., 1992).

A critical question remains about the effect of FRPCM deterioration on mechanical properties of the composite materials. Thorp et al. (1994) attempted to determine mechanical changes in composite coupons after exposure to a fungal culture. No significant mechanical changes could be measured after 120 days exposure. They suggested that methodologies sufficiently sensitive to detect surface changes need to be utilized. Acoustic techniques have also been proposed as a means of detecting changes in the physical properties of the FRPCMs (Wagner et al., 1996).

Many bacteria were capable of growth on surfaces of FRPCMs and resins (Gu et al., 1996a). The bacteria are believed to be introduced onto the polymers during production. Similar to the microorganisms isolated from polyimides, bacteria are less effective in degrading the composites than fungi (Gu et al., 1995b). Degradation of composites were detected using electrochemical impedance spectroscopy.

3.5.3. *Corrosion protective polymers*

Corrosion protective coatings also have wide application because the development of metallic materials and susceptibility to corrosion both environmentally and microbiologically (Mitchell et al., 1996). Polymeric coatings are designed to prevent contact of the underlying materials with corrosive media and microorganisms. However, microbial degradation of coatings may accelerate and severely damage the underlying metals. Typical example includes the corrosion of underground storage tanks. Natural bacterial populations were found to readily form microbial biofilms on surfaces of coating materials, including epoxy and polyamide primers and aliphatic polyurethanes (Blake et al., 1998; Filip, 1978; Gu et al., 1998b; Stern and Howard, 2000; Thorp et al., 1997) (Fig. 7). Surprisingly, the addition of biocide diiodomethyl-*p*-tolylsulfone into polyurethane coatings did not inhibit bacterial attachment or growth of bacteria effectively due to development of biofilm and bacterial resistance (Gu et al., 1998b; Mitchell et al., 1996).

Using EIS, both primers and aliphatic polyurethane top-coatings were monitored for their response to biodegradation by bacteria and fungi. Results indicated that primers are more susceptible to degradation than polyurethane (Gu et al., 1998b). The degradation process has similar mechanisms as polyimides and FRPCMs as mentioned above. Aliphatic polyurethane-degrading bacteria have been isolated and one of them is *Rhodococcus globerulus* P1 base on 16S rRNA sequence (Gu, unpublished data).

Polyurethane-degrading microorganisms including *Fusarium solani*, *Curvularia senegalensis*, *Aureobasidium pullulans* and *Cladosporidium* sp were isolated (Crabbe

et al., 1994) and esterase activity was detected with *C. senegalensis*. A number of bacteria were also claimed to be capable of degrading polyurethane and they are four stains of *Acinetobacter calcoaceticus, Arthrobacter globiformis*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Pseudomonas putida*, and two other *Pseudomonas*-like species (El-Sayed et al., 1996). A *Comamonas acidovoran* TB-35 was also reported (Akutsu et al., 1998; Nakajima-Kambe et al., 1995, 1997). In addition, *Pseudomonas chlororaphis* was isolated and encoded a lipase responsible for the degradation (Stern and Howard, 2000).

3.6. Resistance polymers

3.6.1. Polyethylene

Polyethylenes (PEs) of high and low density are primarily used in product packaging as sheets and thin films. Their degradability in natural environments poses serious environmental concerns due to their slow degradation rates under natural conditions, and the hazard they present to freshwater and marine animals. Prior exposure of PEs to UV promotes polymer degradation. It is believed that polymer additives, such as starch, antioxidants, coloring agents, sensitizers, and plasticizers may significantly alter the biodegradability of the parent polymers (Karlsson et al., 1988). Degradation rates may be increased by 2–4% following photosensitizer addition. However, degradation is very slow, estimated in decades. Crystallinity, surface treatment, additives, molecular weight, and surfactants are all factors affecting the fate and rate of PE degradation, and may accelerate the process.

Biodegradation of PEs has been studied extensively earlier (Albertsson, 1980; Breslin, 1993; Breslin and Swanson, 1993; Imam and Gould, 1990), but the results were based on PE blent with starch. For example, extracellular concentrates of three *Streptomyces* species cultures were inoculated to starch containing PE films (Pometto et al., 1992, 1993). Subsequently, PE was claimed to be degraded. Realizing that degradation may occur and the extent could be extremely small, conclusion on PE degradation should be treated with caution. Other data describing degradation of PE containing starch is questionable, and microbial metabolites may contaminate the PE surfaces and could be interpreted as degradation products of the parent PE. Abiotic degradation of PE is evident by the appearance of carbonyl functional groups in abiotic environments. In contrast, an increase of double bonds was observed when polymers showed weight loss resulting from biodegradation (Albertsson et al., 1994). It was proposed that microbial PE degradation is a two-step process involving an initial abiotic photooxidation, followed by a cleavage of the polymer carbon backbone. However, the mechanism of the second step needs extensive analysis before plausible conclusions can be drawn confidently. Lower molecular weight PEs including paraffin can be biodegraded and paraffin undergoes hydroxylation oxidatively to form an alcohol group, followed by formation of carboxylic acid.

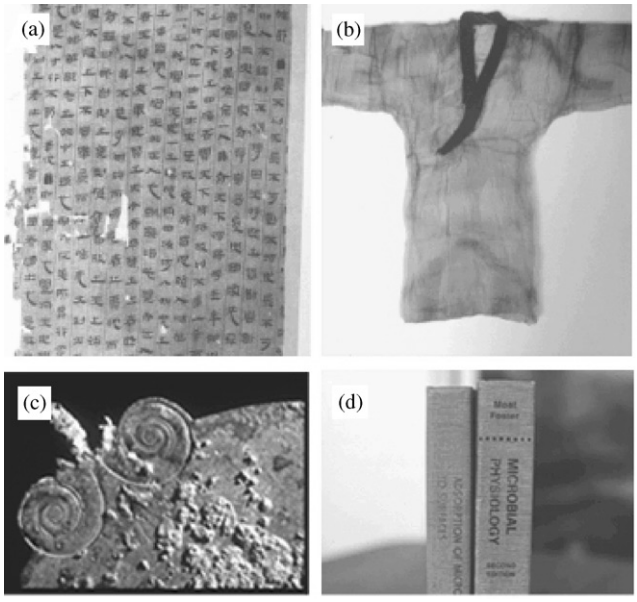

Fig. 8. Photographs showing the: (a) ancient writing script, (b) textile, (c) bronze, and (d) books with molding development from a library in the tropical region.

At higher temperatures, ketones, alcohols, aldehydes, lactones, and carboxylic acids are formed abiotically in 6 weeks (Albertsson et al., 1994). PE pipes used in gas distribution systems may fail due to cracking. It is unlikely that biological processes are involved (Zhou and Brown, 1995).

3.6.2. Polypropylenes

Polypropylenes (PPs) are also widely utilized as engineering pipes and containers. Degradation of PPs results in a decrease of their tensile strength and molecular weight. The mechanism may involve the formation of hydroperoxides which destabalize the polymeric carbon chain to form a carbonyl group (Cacciari et al., 1993; Severini et al., 1988). Degradability of pure and high molecular weight PPs is still an open question.

4. Biodeterioration of cultural heritage materials

Other materials of interest and importance to society for protection from biodeterioration are cultural objects with historical and cultural value. Examples of these materials are bronze (Wang et al., 1991, 1993; Wu et al., 1992; Zou et al., 1994), jade, ceramic and glass (Fuchs et al., 1991; Lauwers and Heinen, 1974), lacquer, silk, papers (Adamo et al., 1998; Arai, 2000; Fabbri et al., 1997; Florian, 1996; Zyska, 1996), paintings (Fabbri et al., 1997; Piñar et al., 2001; Rölleke et al., 1998), animal bones and shells, wood (Blanchette, 1995; King and Eggins, 1972), and mummified bodies. Fig. 8 shows ancient script on paper and textile, which have been held in museum condition, and modern books from library in tropic region. These materials are either in need

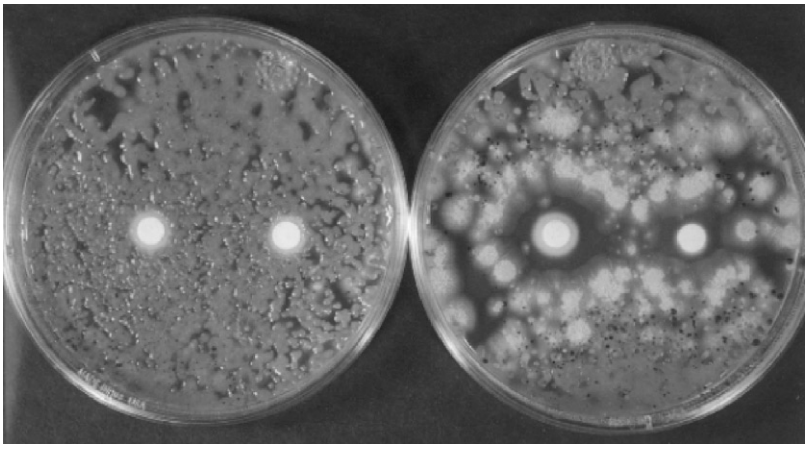

Fig. 9. Photograph showing inhibition of microorganisms on surface of agar plates by a biocide in the discs placed on the agar plates.

for protection or suffer from potential biodeterioration due to the growth of microorganisms which have been established their population on surfaces of materials.

Significant importance for protection and preservation of them is on the social, culture, and archaeological and historical value and scholarly meaning for future study. Conservation and preservation of them are a major task in all museums worldwide and integrated research effort deserves more attention in understanding the processes contributing to the problem and proposing preventive measure or solution. Apparently, such understanding and the preventive measure can only be achieved by collective research effort from biologists, chemists and conservators.

4.1. Consolidant polymers

In addition to the polymers and applications described above, organic polymers are widely used in consolidation of monuments and repairing of art works (Selwitz, 1992). Utilization of these materials by common flora of microorganisms transported in the atmosphere has been documented (Gu and Mitchell, unpublished data) and guidelines are needed for systematic evaluation of candidate polymers and their suitability in specific applications. Since these polymers are mostly commercial products, polymer additives and other constituents are more likely to serve as a source of carbon and energy for microbial growth when temperature and moisture (humidity) are favorable for the proliferation of microorganisms (Gu et al., 1998b, 2000b; Tilstra and Johnsonbaugh, 1993). Even organic pollutants can be degraded by natural microorganisms (Gu and Berry, 1991, 1992; Gu et al., 1992a). These physical conditions are generally available particularly in developing countries where resource is limited for preservation and conservation.

Among several consolidants including acryloids, polyurethane, and epoxies, none of them is resistance to microbial colonization (Gu, unpublished data). Though application of biocides becomes a routine practice in the conservation of art works, the effectiveness of the addition is questionable from our past experience (Fig. 9). This problem will be more serious than expected when eradication of microorganisms becomes harder using these chemicals due to resistant development in microorganisms after exposure (Bingaman and Willingham, 1994).

5. Prevention and detection of biodeterioration

5.1. Preventive measures

Microbial growth and propagation on material surfaces can be controlled by physical and chemical manipulations of the material and the artificial environments. Prevention against biodeterioration include surface engineering so that attachment by and susceptibility to microorganisms and then the fouling organisms can be reduced greatly (Gu and Cheung, 2001; Mansfeld, 1994; Matamala et al., 1994; Scamans et al., 1989; Williamson, 1994; Young, 1948). Basic information on microorganisms are widely available from textbook (e.g., Madigan et al., 2000) and microbiological manuals (Balow et al., 1992; Krieg and Holt, 1984; Sneath et al., 1986; Staley et al., 1989; Williams et al., 1989). As a control measure, lowering humidity is a very effective means to slow down the growth of microorganisms on surfaces in an enclosed environment (Gu et al., 1998b) and prevention against potential contamination will prolong the life time of the objects. Under museum conditions, sensitive art pieces should be carefully protected environmentally and the numbers of visitors should also be controlled to maintain a relatively constant temperature and humidity, and to decrease chance of contamination.

Basic measures in control of biodeterioration should be focused on the surface especially the initial population of organisms. Without a better understanding of what is on the surface, subsequent protection measure cannot be tar-

get specific. In this area, recent development in molecular technique involving DNA based information allows a better examination of any surface due to the shortcomings with traditional microbiological techniques (Amann et al., 1995). Coupling the understanding of surface microbial ecology using molecular techniques and then controlling measure, better results can be achieved. By modification of the microbial community, Sand et al. (1991) proposed oxygenation as a means of alleviating the propagation of SRBs under anoxic conditions. At the same time, biocides can be effective in controlling biofilms and subsequent deterioration of materials to some extent (Bell and Chadwick, 1994; Bell et al., 1992; Wakefield, 1997). Other attempts at community modification include precipitation of microbially produced H_2S by ferrous chloride ($FeCl_2$) (Morton et al., 1991), and displacement of *Thiobacillus* sp. by heterotrophic bacteria (Padival et al., 1995). All of these efforts have met with limited success.

5.2. Use of biocides

Biocides are commonly applied in repairing, cleaning and maintenance of artworks. Chlorine, iodine and other organic biocidal compounds are used widely and routinely in controlling biofilms which cause corrosion and deterioration of a wide range of materials in industries (Bloomfield and Megid, 1994; Cargill et al., 1992; Chen and Stewart, 1996; Stewart et al., 1996) and conservation of art (Bianchi et al., 1980; Bingaman and Willingham, 1994). These chemicals have been shown to be ineffective in killing biofilm bacteria (Gu et al., 1998b; Huang et al., 1996; Keevil and Mackerness, 1990; Koenig et al., 1995; Liu et al., 1998; Lü et al., 1984, 1989; McFeters, 1991; McFeters et al., 1995; Moore and Postle, 1994; Myers, 1988; Pyle et al., 1992; Reinsel et al., 1996; Rossmoore and Rossmoore, 1993; Srinivasan et al., 1995; Stewart, 1996; Stewart et al., 1996; Suci et al., 1998; Wakefield, 1997; Xu et al., 1996; Yu and McFeters, 1994). In addition to their environmental unacceptability most of the time because of toxicity, biocides induce the development of biofilms that are highly resistant to the levels of chlorine normally utilized to prevent biocorrosion. Organic biocides, used to prevent bacterial growth in industrial systems, may selectively enrich population of microorganisms capable of biocide resistance (Fig. 9). No solution to these problems is currently available and alternative biocides have been screened from natural products (Abdel-Hafez and El-Said, 1997; Bell and Chadwick, 1994; Bell et al., 1992; Brözel and Cloete, 1993). Current research by materials scientists is focused on the prevention of adhesion of corrosive microorganisms to surfaces through surface treatments and modification (Costerton et al., 1988).

Since bacteria are capable of forming biofilms on surfaces of materials, future tests should be focused on the dynamics of biofilm and quantification than descriptively showing biofilm of scanning electron micrographs. In particular, test of assaying efficacy of biocide should be conducted based on biofilm condition than liquid culture efficacy (Gu et al., 1998b, 2000d). This major discrepancy has not fully been resolved. Because biofilm bacteria are more resistance to antibiotics and biocides, tests based on planktonic cells are not truly representative of their actual conditions on surfaces of materials. New initiative is needed for innovative methodology to assess biocidal effects using surface oriented assays.

5.3. Testing methodologies

Another critical issue in this area is the standardization of test methodologies. Current available methods are certainly not representative of the actual conditions for each individual case, but very little flexibility is offered in the methods. Simulation testing of microbial growth on materials includes only a small selection of fungal species (ASTM, 1993a–e) while deterioration under natural environment is hardly carried out by those species. Furthermore, biodeterioration assessment has hardly been quantitative because presence of bacteria or fungi on surface of materials has generally been assumed as biodeterioration (Zachary et al., 1980). Actually, the interpretation is about the potential for biodeterioration not actually biodeterioration and biodegradation. More methods are now becoming available for test the biodeterioration and biodegradation of organic materials, particularly polymers in various chemical composition and degradability (Gross et al., 1993, 1995; Gu et al., 1992b, c, 1993a–c, 1994b, 2000b, d). Both gravimetric method and respirometry have been tested and used successfully with CAs and PHB as testing polymers. Highly sensitive and quantitative method has also been introduced in evaluation of polymer integrity using EIS (Gu et al., 1998a, 1995a–d, 2000b). With the latest advances, new techniques should be adopted in tests according to the characteristics of materials and their application environments, so that data generated on the materials will be a quantitative description of the biological deterioration potential.

Prevention against biofilm formation and biodeterioration include surface engineering so that attachment and susceptibility to microorganisms and the fouling organisms can be reduced greatly (Mansfeld, 1994; Matamala et al., 1994; Scamans et al., 1989; Williamson, 1994; Young, 1948). Early detection is an important component in diagnosis and prevention of severe deterioration of materials (Li et al., 1997). It should also be pointed out that new detection technologies including optical fiber (Bacci, 1995), DNA probes and microarray (Raychaudhuri et al., 2001; Salama et al., 2000) will find valuable applications in this exiciting field of research and development in the near future.

6. Conclusions

Microorganisms are involved in the degradation and deterioration of polymers under both aerobic and anaerobic

conditions. We have only recently begun to understand the complex nature of interactions between the microflora and deterioration of polymeric materials.

Degradation mechanisms are specifically related to the chemical structures, molecular weights, presence of the microorganisms and environmental conditions. Protection of materials can be achieved to some extent through surface engineering and control of the physical, chemical and biological environments, so that the material surfaces can be as inert as possible. Application of biocides has been widely used but the development of resistant bacteria is a more serious problem than even anticipated before. Utilization of molecular techniques to detect specific groups of microorganisms involved in the degradation process will allow a better understanding of the organization of the microbial community involved in the attack of materials. Control methods should be developed based on the combined information o the material characteristics and microbial specie composition.

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