KEEPING IN TOUCH: MICROBIAL LIFE ON SOIL PARTICLE SURFACES

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Microorganisms in unsaturated soil live in a world dominated by the presence of extensive surfaces, both solid and gas–liquid interfacial surfaces. Particle attachment in soils is similar to particle attachment in aquatic systems, which, because of the high abundance of suspended populations has been widely studied. Although there seems to be a general advantage to the microbes living at the interfaces in terms of enhanced nutrient concentrations and the potential to use many of the physical substrata themselves as energy or nutrient sources, the thickness of the water films in unsaturated conditions leaves the microbes little option except to adhere to the surfaces. Initial attachment to the surfaces appears to be dominated by electrostatic and hydrophobic effects that are described by Derjaguin-Landau-Verwey-Overbeek (DLVO) theory for negatively charged cells and particles. These effects result in reversible attachment and the cells are subject to rapid detachment with slight changes in solution chemistry and removal by hydraulic shear. Coatings play an important role in attachment, with metal oxide coatings

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conferring a positive charge to the particle surface resulting in a much tighter adhesion of the microbial cells to the surface. Attachment of the organisms to the particles by direct surface contact through appendages such as fimbriae or deposition of polysaccharidic slime results in irreversible attachment that can lead to buildup of colonies and biofilms.

In this chapter, considerations of theory are presented as they pertain to soil organisms, and abundant use of examples from aquatic habitats exemplifies principles and ideas not easily studied in unsaturated soil. The importance of attachment to the gas–liquid interface is also highlighted.

I. INTRODUCTION

The soil habitat represents a unique but extensive environment in which microorganisms live and carry out biogeochemical reactions critical to the maintenance of ecosystems. The uniqueness of the soil is related to the vast amount of particle surface area contained there. Other habitats also contain particles, often with a large surface area, but soils are dominated by surfaces and the matrix is generally hydrologically unsaturated. The combination of particle surface area with thin water films makes soils different in many respects from their saturated counterparts in aquatic sediments and aquifers formed from unconsolidated materials. The purpose of this chapter is to examine the relationship of soil microorganisms to soil particles in terms of their tendency to attach themselves to the particles. Much of the chapter will examine mechanisms of attachment, but this information is best understood in the context of why the bacteria attach to the particles. To accomplish this goal, it will be necessary to consider particle attachment in general, including information from other habitats such as in lakes and marine environments. Thus, while the focus of this discussion is attachment in soils, principles will be derived from other environments as needed and justified. Fletcher recently published an excellent volume (Fletcher, 1996) that examined in general the attachment of microbes to surfaces from both an ecological and physiological viewpoint. Part of this chapter formed part of a contribution to this volume, and the book also served as an important starting point for a number of topics covered here. Readers are encouraged to use the chapters contained in this reference for a more detailed coverage of the general topic of microorganisms and surfaces than can be accomplished here.

Attachment to particles could arise due to three possible reasons. Attachment might occur completely as a result of serendipity. Bacterial attachment might confer neither an advantage nor a disadvantage to the organisms, and there might be no active mechanism that pulls bacteria to the surface of particles. Experience teaches us that while events frequently arise spontaneously, their persistence in biological systems generally arises from some selective advantage conferred on the organisms involved. Furthermore, the degree to which bacteria attach to particles in soils and in aquatic ecosystems and the strength of the association once established, argue strongly against happenstance as the causative agent for bacterial attachment.

A second possible reason for the high frequency of particle association by bacteria is that there is a selective advantage to the organisms to live in close association with a particle surface. In biological systems, behaviors that are not advantageous to the populations are generally lost over evolutionary time. It is possible that attachment represents a neutral behavior (a rationale for which is discussed in the following paragraph), but we can certainly be sure that particle attachment does not represent a behavior that is generally detrimental to the cells. If this were the case, the attached bacteria would quickly be eliminated by competition with the suspended bacteria for limiting resources. Indeed, it is likely that attachment represents an advantage to the organisms in some major way; they may obtain some essential element from the particle grains, they might obtain energy from organic or inorganic molecules tightly sorbed to the mineral grain surface, or they might benefit from living in a chemically richer aqueous environment due to enhanced concentrations of soluble nutrients in the proximity of the grain surface. In some cases, attachment to particles might provide partial or complete protection from grazing by bacterivorous organisms. All these possibilities will be discussed in detail later.

A third possible reason for particle attachment in soils also exists. In unsaturated soils, microbes have little choice but to exist at or near the surface of the soil particles. Although filamentous fungi and actinomycetes have been observed to span unsaturated voids, single-celled organisms are limited to total immersion to be active (Metting, 1993); furthermore, nutritional uptake requires an aqueous phase for all phenotypes (Harris, 1981). Even under the so-called optimal soil moisture conditions in which the pore space of a silt loam soil is approximately 50% filled with water, the amount of water associated with each particle does not leave much room for the microbes to move a great distance from the surface of a particle. A simple calculation illustrates this point. Consider a soil composed entirely of uniformly spherical particles that are in the mid-silt-size range, i.e., 0.025 cm in diameter. Consider further that the soil has a bulk density of 1.2 g cm^{-3}. A final assumption is that all the water is perfectly uniformly distributed on the entire surface of all particles (note that the particles do not actually touch under this assumption). Under these oversimplified assumptions, each particle is coated with a film of water that is only about 6.4 μm thick. While no such soil exists in reality, and the actual thickness of water films under realistic soil conditions varies from a few molecules to millimeters, there is not much volume in the soil that permits the microbes to be very far from the surface of a mineral or organic soil particle. Indeed, Mills and Powelson (1996) estimated from literature data (Gardner, 1956; Green *et al.*, 1964; Holmes *et al.*, 1960; Kemper and Rollins, 1966) that at field capacity (soil moisture tension of 0.33 bar), one might expect a film thickness of only 0.2–0.3 μm assuming uniform coverage of the grains by the water film. Metting (1993) calculated that at a soil matric potential of -0.01 MPa, capillaries less than 30 μm in diameter would be saturated, but at -0.03 MPa, saturation would be only in pores of less than 4 μm diameter. When the potential is less than -0.5 MPa there is only a film of water a few molecules thick. The principal point is that the water film thins quickly as the degree of saturation decreases, forcing the microbial cells even closer to the grain surface.

The argument presented in the preceding paragraph might be quite compelling as a complete explanation if it were not for the fact that soils are not the only place where particle association is observed. In soils, attachment is so heavily dominant because the soil habitat is completely dominated by particle surface area surrounded by the thin films of water. In all systems the degree of particle association appears to be correlated to both the number of bacterial cells and the number of particles present. We cannot conclude that either selective advantage or necessity is the single reason for attachment as the way of life in soils. Obviously, the answer is a combination of the two factors. The fact that water films are generally thin and vegetative bacteria are forced to live, therefore, close to the particle surfaces is obvious. The ensuing discussion, therefore, will concentrate on factors that confer advantage to the organisms living on particle surfaces in soil.

II. NATURE OF SOIL PARTICLES RELATED TO MICROBIAL ATTACHMENT

A. PARTICLE-SIZE DISTRIBUTIONS

A number of factors influence the attachment and permanent association of bacteria with soil particles. In addition to particle composition (discussed later), particle size seems to play an important role in determining the distribution of microbial populations in soil aggregates. A study by Hattori (1973) showed the strong quantitative relationship between clay particles and bacterial cells (Fig. 1). A number of studies have shown that both the cell number and the bacterial biomass tend to be most concentrated in the smaller size silt and clay fractions (Jocteur Monrozier *et al.*, 1991; Kandeler *et al.*, 2000, 2001; vanGestel *et al.*, 1996). Obviously, therefore, the bacteria are mainly present in micropores of 5–30 μm (Amato and Ladd, 1992; Hassink *et al.*, 1993; Kirchmann and Gerzabek, 1999). Analysis of the distribution of microbial enzyme activities suggest that the bacterial activities are dominant in the silt and clay fractions, whereas enzyme activities that indicate fungi are highest in the sand fraction (Gerzabek *et al.*, 2002; Kandeler *et al.*, 1999, 2000; Stemmer *et al.*, 1998, 1999). There may be, however, even more qualitative selection for particle sizes than at a cell domain

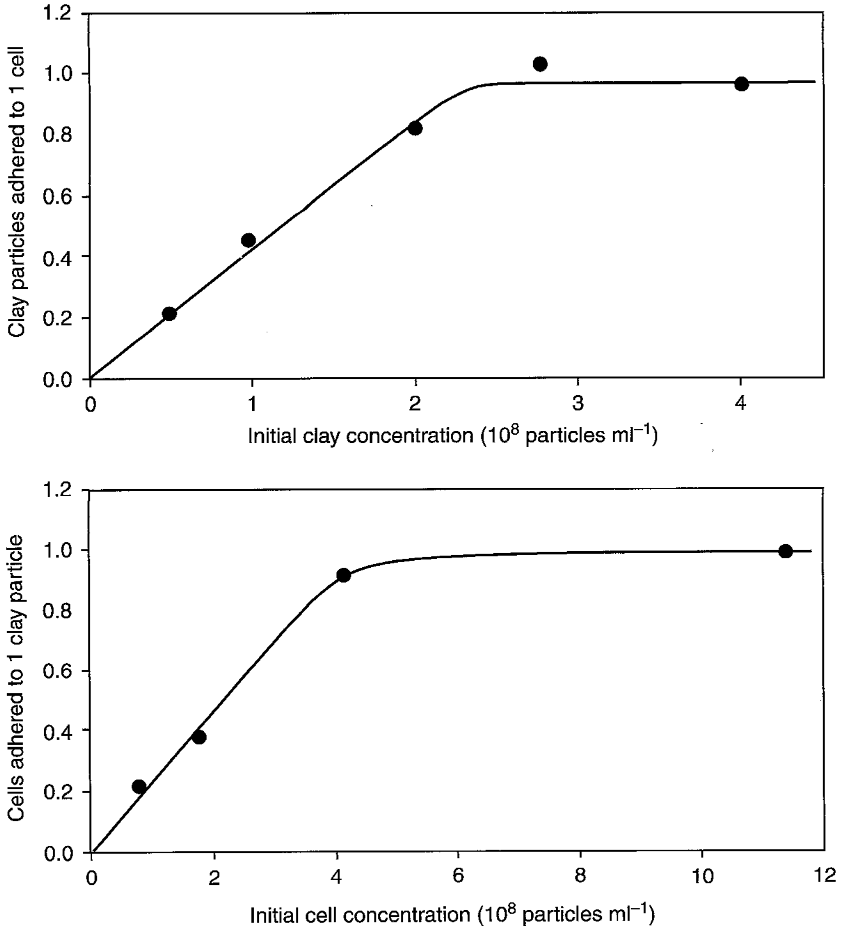

Figure 1 Adhesion of cells of *E. coli* to particles of sodium pyrophyllite as a function of particle concentration. Both cells and clay had effective mean diameters of 0.8 and 0.9 μm, respectively. Figure redrawn from Marshall (1980); original data from Hattori (1973). Reproduced with permission of John Wiley & Sons.

level, i.e., bacteria *versus* fungi. A recent article by Sessitsch *et al.* (2001) reported that not only were the numbers of attached bacterial cells greater in the finer textured fraction of Dutch soils, but also the community composition differed. Termmal Restriction Fragment Length Polymorphism (T-RFLP) analysis of the communities associated with the different size fraction indicated that different organisms were the dominant inhabitants of the coarser particles as compared with the fine materials. These authors also suggested that diversity of the amplifiable genotypes in the clay fractions was greater than that in the coarser fractions based on the number of fragments recovered in T-RFLP analysis of the particle associated DNA in each size fraction. Much of the difference noted was attributed to organic amendments in the several soils examined, and to possible competition with fungi in the coarser particle sizes.

There is good reason why clay fractions would have the maximum interactions with bacteria. The particles' small size yields an enormous surface area per unit weight of solid, and the crystal structure of clays tends to engender a strong net negative charge on the surface that can attract nutrients, organics, and under the right circumstances, the bacterial cells themselves. Quartz and feldspars, materials with relatively inert chemical behavior usually dominate sand-size grains. As particles weather to smaller silt and clay-size particles, their composition changes to layer silicates; the smaller soil particles present not only a larger total surface area, but also a more reactive one as well. The influence of the finer textured materials is, therefore, a combination of the surface area increase and the specific mineralogy of the particles. Table I shows how cation exchange capacity (CEC) increases with increasing fineness of texture. The range of values for exchange capacity for each textural category reflects different mineralogy and different amounts of organic matter present in individual soils. Most soil particles do not present surfaces with the reactivity reflecting only the base mineralogy of the particle. Many particles have some portion of their surface coated with reactive materials, such as iron, aluminum, and manganese oxides and hydroxides, and organic matter. These coatings can alter the reactive surfaces of the particles, in some cases changing the negative surface charge to neutral or positive, and they can otherwise add reactivity to only slightly reactive surfaces. In this way, even quartz sand can become highly reactive by adsorption of a coat of negative metal oxide or organic matter. The issue of coating and soil particles' tendency to sorb bacteria will be discussed later.

Table I
Change in CEC with Change in Soil Texture

		Exchange capacity (Cmol g^{-1})	
Textural classification	No. of soils	Average	Range
Sand	2	2.8 ± 1.1	2.0–3.5
Sandy loam	6	6.8 ± 5.8	2.3–17.1
Loam	4	12.2 ± 3.6	7.5–15.9
Silt loam	8	17.8 ± 5.6	9.4–26.3
Clay and clay loam	6	25.3 ± 20.3	4.0–57.5

Note. Data for averages are expressed as the mean and standard deviation for the soils, and the range represents the minimum and maximum values reported within the textural category.

Source. Mills and Powelson (1996) based on data taken from Brady (1984). Reproduced with permission of Wiley–Liss.

Understanding the particle-size distribution in soil generally does not confer a strong predictive ability in relation to growth and interactions of the microbes, as habitats available to the organisms are defined by the structural organization of the particles into aggregates (Marshall, 1980).

B. CHEMICAL DISTRIBUTION

MINERAL

The most common minerals found in soils are listed in Table II. In general, the aluminosilicates predominate with some inclusions of carbonates, sulfates, and iron and aluminum sesquioxides. Sulfates and carbonates are much more soluble than either silicates or sesquioxides; soils tend to lose the carbonates and sulfates

Table II
Common Soil Minerals, and their Chemical Formulae

Name	Chemical formula	Importance
Quartz	SiO_2	Abundant in sand and silt
Feldspar	$(Na,K)A1O_2[SiO_2]_3$ $CaA1_2O,[SiO_2]_2$	Abundant in soil that is not leached extensively
Mica	$K,A1_2O_5[Si_2O_5]_3Al_4(OH)_4$ $K_2A1_2O_5[Si_2O_5]_3(Mg,Fe)_6(OH)_4$	Source of K in most temperate-zone soils
Amphibole	$(Ca,Na,K)_{2.3}(Mg,Fe,Al)_5(OH)_2$ $[Si,Al_4O_{11}]_2$	Easily weathered to clay minerals and oxides
Pyroxene	$(Ca,Mg,Fe,Ti,Al)(Si,Al)O_3$	Easily weathered
Olivine	$(Mg,Fe)_2SiO_4$	Easily weathered
Epidote	$Ca_2(Al,Fe)_3(OH)Si_3O_{12}$	Highly resistant to chemical weathering; used as "index mineral" in pedologic studies
Tourmaline	$NaMg_3Al_6B_3Si_6O_{27}(OH,F)_4$	
Zircon	$ZrSiO_4$	
Rutile	TiO_2	
Kaolinite	$Si_4Al_4O_{10}(OH)_8$	Abundant in clay as products of weathering; source of exchangeable cations in soils
Smectite Vermiculite Chlorite	$M_x(Si,Al)_8(Al,Fe,Mg)_4O_{20}(OH)_4$, where M = interlayer cation	
Allophane	$Si_3A1_4O_{12}\cdot nH_2O$	Abundant in soils derived from volcanic ash deposits
Imogolite	$Si_2Al_4O_{10}\cdot 5H_2O$	
Gibbsite	$Al(OH)_3$	Abundant in leached soils
Goethite	$FeO(OH)$	Most-abundant Fe oxide
Hematite	Fe_2O_3	Abundant in warm regions
Ferrihydrite	$Fe_{10}O_{15}\cdot 9H_2O$	Abundant in organic horizons
Birnessite	$(Na,Ca)Mn_7O_{14}\cdot 2.8H_2O$	Most-abundant Mn oxide
Calcite	$CaCO_3$	Most-abundant carbonate
Gypsum	$CaSO_4\cdot 2H_2O$	Abundant in arid regions

Source: Sposito (1989). Reproduced with permission of Oxford University Press.

first, while silicates are altered from primary minerals such as feldspars and micas to secondary minerals (especially the clay minerals like montmorillonite and kaolinite). Mature soils tend to be dominated by silicates, but as the soils age, the sesquioxides take on a greater importance. The ultisols of the southeastern United States are rich in iron oxide and clays (i.e., kaolinitic minerals) characteristic of old soils. The oxisols of the tropics (formerly called lateritic soils) are dominated by metal oxides and residual quartz. Charges associated with the primary minerals are generally low due to the mineralogy and the low surface area presented by the larger rock fragments from which the soil is weathered. The charge (in this case a negative charge usually expressed as the CEC) increases through a maximum for 2:1 minerals such as montmorillonite (typically 70–150 Cmol g^{-1}) through kaolinite (typically 10 Cmol g^{-1}) to the iron and aluminum oxides which often express a positive charge (i.e., anion exchange capacity).

Microorganisms interact differently with the minerals, in part because of the charge differences, but also because of mineral constituents that are in the crystal lattice or that are adsorbed to exchange sites on the mineral surface or in the interlayer spaces.

ORGANIC

The decaying mass of plant material is broken down into particles of ever decreasing size. The chemical action of microbes along with physical disintegration facilitated by invertebrate feeding, results in the pulverization of particulate organic matter into units comparable to the finest mineral particles. Many changes occur as the material is decomposed and disintegrated. The initial material is fresh or senescent plant material, but the actions of the soil biota soon generates material that has little semblance to the original plant from which it came. As the readily metabolizable components are removed, the remnants take on the character of both the plant-produced refractory compounds that remain and of the microbial cells generated during the decay process. The remnants have both dissolved and solid-phase components; the dissolved constituents often end up as coatings on all of the particles in the soil matrix.

The heterogeneous nature of starting materials for soil organic matter formation combined with the various organisms involved and conditioned by the local environmental properties operating over multiple time scales make the exact chemical composition of soil organic matter difficult to determine for any given site over long periods of time. With us, a general description of soil organic matter composition can only be discussed in the most general terms. Alexander (1977) reported that about 15% of the mass is identified as polysaccharides, polypeptides, and phenols. This value comprises about 20% carbohydrates, 20% amino acids and amino sugars, and 10–20% aliphatic fatty acids (Paul and Clark, 1989). The remainder of soil organic matter is humic materials, a dark amorphous substance derived from the degradation of organic residues. The process of conversion of plant material to soil organic matter is often referred to as *humification* and the organic matter itself is called *humus*. These references attest to the overall importance of *humic acids* in the mature organic material.

As pointed out below, the hydrophobic and electrostatic properties of soil particulates dictate, to a large extent, whether or not microbes will sorb rapidly to their surfaces. Soil organic matter has properties that include both hydrophobic and electrostatic effects. Fulvic acids are moderately size, reactive molecules with average molecular weights of 800–1500 Da. Humic acids are larger molecules with average mo lecular weights of 1500–4000 Da (Beckett *et al.*, 1987), although there are reports of weights up to 200,000 Da (Thurman, 1985). Both of these classes of compounds have many reactive sites. The sites are dominated by carboxyl- and phenolic hydroxyl groups that dissociate in water to yield a polyvalent anion (see Table III). Soil organic matter (exemplified by a model humic acid) also contains amines that can carry a positive charge at moderate to low pH (Fig. 2).

The magnitude of the charge generated in soil organic matter can be quite large. Depending on the existing soil conditions, soil organic matter can have a high exchange capacity for either cations or anions. Negative charges are generated by the dissociation of a proton from a carboxyl or a phenolic hydroxyl group. Protonation of amine groups ($R{-}NH_2 + H^+ \rightarrow R{-}NH_3^+$) results in a positively charged site. The involvement of protons and protonation–deprotonation reactions gives rise to a substantial pH-dependent charge. Mineral particles demonstrate a combination of permanent and pH-dependent charge, but the most highly charged particles are dominated by permanent charges generated by substitution within the crystal lattice. The CEC of organic particles may

Table III
Elemental and Functional Group Analysis of Humic and Fulvic Acids

Sample	Elemental analysis (%)					
	C	H	N	S	O	Ash
Fulvic acids	49.5	4.5	0.8	0.3	44.9	2.4
Humic acids	56.4	5.5	4.1	1.1	32.9	0.9

Sample	Functional group analysis (meq g^{-1})			
	OCH_3	COOH	Phenolic OH	Total acidity
Fulvic acids	0.5	9.1	3.3	12.4
Humic acids	1.0	4.5	2.1	6.6

Source: Smith (1993), from data in Tate (1987). Reproduced with permission of Marcel Dekker.

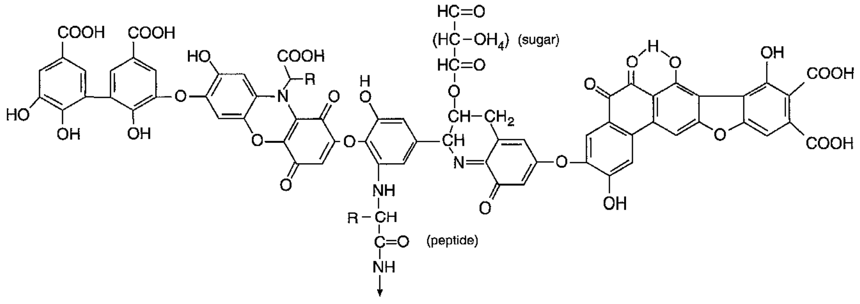

Figure 2 Structure of a sample humic molecule. Note the high proportion of aromatic groups and the opportunities for both positive and negative charges on the molecule as a result of the carboxyl and amine groups. Redrawn from Stevenson (1982). Reproduced with permission of John Wiley & Sons.

double over the range of pH 4.0–8.0 (Smith *et al.*, 1993); the exchange capacity of organics is also much higher (2–30 times) than that of mineral colloids (Smith *et al.*, 1993).

Soil organic matter does not comprise only humic materials. Depending on the nature of the starting plant material, it may contain substantial amounts of readily available carbon compounds and nitrogen and other essential elements may also be abundant. The amount of such nutrients is highly variable as is the overall degradability of the material. The most degradable materials, sugars, free amino acids, etc., are soluble and leach quickly from the particulate fraction. Little of the material is lost, however, because microorganisms colonizing the detritus surface or in close proximity to the decaying particles rapidly utilize the dissolved material as soon as it is lost from a particle. Indeed, rapid microbial colonization of organic particles probably occurs because of the readily available carbon and nitrogen compounds leaching from them. Once the pool of readily available (i.e., soluble) compounds has been depleted, the microbial community turns its attention to the solid phase material that remains, mineralizing carbon and assimilating other nutrients during the humification process.

ORGANIC-COATED MINERAL

As described later, sorption due to surface charge or hydrophobicity or some combination of the two would seem to be a likely phenomenon to be affected by the presence of a coating over the substrate. Ferric coatings, for example, were found, to greatly enhance bacterial sorption, by Mozes *et al.* (1987), Scholl *et al.* (1990), Mills *et al.* (1994), Knapp *et al.* (1998), and Bolster *et al.* (2001), among others. The importance of metal oxide coatings will be described in detail later. One of the most ubiquitous of all coatings is that of organic matter. Hunter (1981) found that, without exception, the suspended particles in river and gesturing waters were negatively and quite uniformly charged. Since the particles themselves varied widely as to composition, Hunter concluded that this was likely due to a coating of organic matter or metal oxide. Immersing positively charged hydrous iron oxide particles in natural water containing organic matter results in a negative charge being imparted to the particles, presumably due to the sorption of organic molecules (Loder and Liss, 1985; Tipping, 1981; Tipping and Cooke, 1982). The effect of organic coatings on microbial sorption is variable, as will be described later. In some cases, sorption is enhanced, but in many cases, the addition of organics to the surface of minerals decreases the sorptive capacity of the grains. Other effects such as hydrophobicity and active adhesion as to organic particles may have an overall stimulatory effect on microbial attachment to the coated grains.

III. BENEFITS OF LIVING ON PARTICLES

A. Particle Surfaces as a physical Substrate for Collection of Nutrients

A common observation is that, given access to surfaces and interfaces, microbes quickly colonize those habitats. Basic ecological principles dictate that if organisms inhabit a site, there must be some advantage accruing to the organisms. The high frequency of microbial association with surfaces, therefore, must be interpreted as advantageous to the surface-associated cells. Although the exact nature of that advantage is not completely understood, it is commonly accepted that the adhesion of microorganisms to surfaces allows the organisms to utilize higher concentrations of nutrients, especially energy sources, that are also found to be associated with the interface.

Since the mid-1930s, microbiologists have recognized the overwhelming tendency of bacteria (especially heterotrophs) to associate with particles or surfaces. Waksman and Carey (1935) noted that bacteria grew rapidly when water samples were placed in bottles and stored for even short periods of time, and ZoBell and Anderson (1936) demonstrated that the increases in growth in the bottles was proportional to the surface-to-volume ratio of the storage containers. The so-called "bottle effect" was thought to be related to the tendency of nutrients, especially organics, to collect on the walls of the container. This speculation was confirmed by Stark *et al.* (1938), who reported that clean glass slides accumulated organic matter when immersed in sterile lake water. Later studies observed a stimulation of bacterial growth in the presence of increased surface area provided by the additional particular materials to liquid cultures with low nutrient concentrations when compared with similar cultures that lacked particles (Heukelekian and Heller, 1940; Jannasch, 1973; ZoBell, 1943). In the presence of high nutrient concentrations, the effect was not observed. The nutrient enhancement principle applied to the effect of surfaces has persisted, even though it may not completely explain the rapid growth of suspended organisms in sampling containers. Kaper *et al.* (1978) observed a doubling of the number of suspended cells in polyethylene sampling bags within 20 min of sample collection from the Chesapeake Bay. Wall-associated organisms were not enumerated, so neither attachment nor growth on the bag's surfaces was determined. Given that some fraction of the suspended cells probably became attached during the brief incubation, the results represented an underestimate of the actual growth of bacteria in the bag. Although the container effect as described by the early workers does not account for prolific growth of suspended organisms, that growth does not contradict the potential effects of the surfaces on enclosed samples.

Nutrient enhancement associated with particle surfaces is of great importance in soil. Metting (1985, 1993) has described the soil outside of the rhizosphere as "in essence a nutritional desert." He further describes the microbial lifestyle as one in which activity is ephemeral and sensitive to fluctuations in substrate and nutrient availability, along with microscale variations in physical and chemical conditions. Microbial life exists in microhabitats that exist on or near particle surfaces on the exterior and interior of soil aggregates.

In some cases, association of organic compounds with surfaces can actually cause a decrease in biodegradation. Otherwise metabolizable compounds are sometimes rendered non-degradable when associated with particle surfaces. Mills and Eaton (1984) noted nearly complete inhibition of degradation of bromobenzene when sand was added to the reaction mixture. Guerin and Boyd (1997) observed reduced degradation of naphthalene in some of the soils tested to determine the effect of particle sorption on bioavailability of the compound, but in no case was degradation enhanced when the soil particles were present. Other work examining different contaminant compounds showed reduced or completely inhibited biodegradation activity in the presence of sorptive particles (Gordon and Millero, 1984; Ogram *et al.*, 1985).

Difference in degradability is generally considered to be related to the availability of a given compound. For example, bovine serum albumin, a rapidly sorbing protein complex, was degraded by attached bacteria but not by unattached bacteria, whereas suspended bacteria metabolized a readily desorbable dipeptide that was less available to the attached bacteria (Griffith and Fletcher, 1991). Based on observations from the literature, Mills and Powelson (1996) speculated that for situations in which availability to organisms is decreased by sorption to particles, there appears a competition between the microbes in surface for the compound. If the microbes can extract the compound from the surface, there may be no observable change in, or even a possible enhancement of, degradation. But, if the surface attraction for the compound is stronger than the ability of the microbes to extract it from the surface, then the compound will be less available. Similarly, sorption to the interlayer spaces of expanding lattice clays may further affect the situation. For example, Weber and Coble (1968) observed that Diquat could be degraded when it was sorbed to the external exchange sites of kaolinite (a non-expanding clay), but it was not available when bound in the interlayer spaces of montmorillonite (an expanding lattice clay mineral).

Evidence exists for the strong attachment of nitrifying bacteria, in particular the ammonium oxidizers, to soil particles. Ammonium (NH_4^+) is strongly sorbed to negatively charged soil particles (it is equivalent to K^+ in charge and radius), and bacterial residence on the particle surface could facilitate uptake of NH_4^+ directly from the surface of the colloids. Indeed, Aakra *et al.* (2000) observed that indigenous ammonia-oxidizing bacteria in a clay loam soil were extremely difficult to release from soil particles compared to most of the heterotrophic bacteria. Less than 1% of indigenous NH_4^+-oxidizers were extractable by the dispersion-density-gradient centrifugation technique, at least 10-fold less than the extractability of heterotrophic bacteria. When urea was applied to the soil, the authors observed a 5-fold increase in the potential ammonia oxidation rate, with the concomitant result of in a much higher percentage (8%) extractability of NH_4^+-oxidizers. The newly grown oxidizers in the urea-treated soil seemed less strongly attached to the soil particles, suggesting that the strong attachment of indigenous oxidizers is either a gradual process taking place due to a long residence time (infrequent/slow cell division) compared to heterotrophic organisms, or that there were differences in species composition of the original community compared with that growing in response to urea inputs.

Although sorption of bacteria and chemicals to surfaces can represent an increased availability to the bacteria, at the same time, the soil minerals may also serve to immobilize some toxic materials from the soil. Clay minerals have been shown to provide protection to NH_4^+-oxidizers from the effects of the organic nitrification inhibitor nitrapyrin (Powell and Prosser, 1991). Similarly, if toxic metal ions are sorbed so strongly to the minerals as to make them unavailable to the microbes, the presence of the surfaces can have a secondary beneficial effect for the microbes (Stotzky, 1979). Incorporation of the clay minerals, kaolinite or montmorillonite, into synthetic media (Babich and Stotzky, 1977) or soil (Babich and Stotzky, 1977) reduced the toxicity of cadmium to a variety of organisms, including *Bacillus megaterium*, *Agrobacterium tumifaciens*, *Nocardia corallina*, *Fomes amnosus*, and several other fungi and bacteria tested. The reduction in the toxicity of Cd was correlated with the CEC of the clays. Although particle association by the cells themselves is not an element in this phenomenon, the fact that they are also present on the surface suggests that attraction of toxic levels of (at least heavy metal) contaminants is not likely to inhibit microbial activity and may enhance it when the toxic materials might otherwise have a damaging effect.

B. UTILIZATION OF THE PARTICLE AS A CHEMICAL SUBSTRATE

For many materials, both mineral and organic, the soil particles serve as more than a physical habitat for the microorganisms. In some cases, the particles also serve as the source of nutritional components for cell growth, including essential nutrients and even carbon, electron, and energy sources. In these cases, the organisms must possess enzymes capable of accelerating the dissolution of the particle, in effect, extracting the desired materials from the solid phase, or they must possess the ability to rapidly incorporate dissolved products of weathering reactions as rapidly as they are formed.

MINERALS

The most common soil minerals are listed in Table II. The substances there do not represent sources of macronutrients to microbes, although some elements such as base cations might be derived by acid leaching of the minerals or by contact exchange of sorbed ions. It is well documented that bacteria can generally obtain adequate PO_4^{3-} by dissolution of PO_4^{3-}-containing minerals through acid excretion (Alexander, 1977). Some elements, however, can serve as energy sources that can be extracted by some bacteria directly from crystalline material. The oxidation of metal sulfides such as pyrite by chemoautolithotrophs is an excellent example of microbes attaching to inorganic particles for the purpose of extracting and exploiting the elements held therein as a source of energy.

Some bacteria, such as *Thiobacillus ferrooxidans*, attach to pyrite or sulfur by means of extracellular lipopolysaccharides. The primary attachment to pyrite at pH 2 is mediated by exopolymer-complexed iron(III) ions in an electrochemical interaction with the negatively charged pyrite surface. The extracellular polymers from sulfur-grown cells possess increased hydrophobic properties compared with that of cells grown on pyrite and the polymers do not attach to pyrite, indicating that organisms can adapt their attachment ability to match the substratum (Gehrke *et al.*, 1998).

Further evidence for adaptation of attached organisms is given by Knickerbocker *et al.* (2000) who observed "blebbing" (sloughing of outer membrane vesicles) in *Thiobacillus thiooxidans* attached to sulfur grains, but not when grown with sulfite as an energy source. Because the sulfite is dissolved, the authors concluded that the cells formed the blebs to assist in the dissolution of the solid phase substrate. Edwards *et al.* (2000) examined the growth of *Thiobacillus caldus* on pyrite and marcasite and observed that more cells attach to marcasite than pyrite and the authors suggested that was because of the greater solubility of the former. Edwards *et al.* (2000) also concluded that preferential colonization of surfaces relative to solution and oriented cell attachment on the sulfide surfaces suggest that *T. caldus* may chemotactically select the optimal site for chemoautotrophic growth on sulfur (i.e., the mineral surface).

Using an enzyme-linked immunofiltration assay, Dziurla *et al.* (1998) were able to estimate directly and specifically *T. ferrooxidans* attachment on sulfide minerals. The mean value of bacterial attachment was about 10^5 bacteria mg^{-1} of pyrite at a particle size of 56–65 μm. The geometric coverage ratio of pyrite by *T. ferrooxidans* ranged from 0.25 to 2.25%. From their results, Dziurla *et al.* (1998) inferred attachment of *T. ferrooxidans* on the pyrite surface to well-defined limited sites with specific electrochemical or surface properties. This conclusion was supported in a laboratory study (Sanhueza *et al.*, 1999) of the attachment of a pure strain of *T. ferrooxidans* on films of synthetic pyrite. Pyrite films representing a wide range of structural and electronic properties were produced by sulfuration of pure iron films at different annealing temperatures, viz., 250, 300, 350, 400, 450, and 500°C. The patterns and degree of attachment of *T. ferrooxidans* to the synthetic pyrite depends strongly on the degree of crystallization of the sulfide films, which varies with the sulfuration temperature. In the low range of sulfuration temperatures (250, 300, 350°C), where there is a major presence of amorphous pyrite, elongated clusters of densely packed bacteria attach to the films. In the range of sulfuration temperatures (400, 450°C) where formation of highly crystallized pyrite predominates, bacteria attachment occurs as isolated bacteria or short bead-like chains. The percentage of pyrite surface coverage by *T. ferrooxidans* is lower at high sulfuration temperatures, where pyrite is fully crystallized. The microorganisms seem to attach preferentially to the less crystallized or amorphous zones of the pyrite films which provide a better availability of sulfide ions for bacterial oxidation. No examination of attachment to sulfur is presented in this work.

From the above, and other evidence, it is clear that many metal sulfide oxidizing bacteria colonize the mineral grain surfaces, often selecting a specific site on the crystal that provides the maximum access to the elements required by the organism (Andrews, 1988), and some work suggests that treatment of the mineral surfaces with surfactant compounds (Jiang *et al.*, 2000; Nyavor *et al.*, 1996) can inhibit the formation of acid mine drainage, at least in part by reducing attachment and thereby the colonization of the particle surfaces.

ORGANICS

The colonization of organic particles by microorganisms is a well-documented phenomenon in both aquatic and terrestrial systems. Both aquatic detritus and soil organic matter are made up of particles of various sizes that comprise decaying plant material along with the microorganisms that are the mediators of decay. As the particles decay their chemistry becomes less and less plant-like and more and more microbial in nature as the organic material in the particles is assimilated and converted to microbial biomass (both living and dead). It is usually observed that organic matter decomposition includes a shift in elemental ratios from those of the native plant material to the equilibrium value associated with well decomposed soil organic matter, i.e., about 10–1 (roughly 5% by weight). This value is also a typical one observed for a large number of soil microbes (Alexander, 1977). Thus, the close association of microbial cells with soil organic matter particles is well accepted. Microscopic observations of organic particles from aquatic habitats (i.e., detritus) generally yield bacterial abundance values of about 10^8 cells g^{-1}, and those organisms are counted only after rigorous extractions to separate them from the detrital particles. For routine microscopic techniques such as epifluorescence counting, it is difficult to separate the organic from the inorganic particles in soil for the purpose of counting the attached microorganisms. It is reasonable to assume, however, that the number of bacteria associated with organic particles in soil are not dissimilar from those in detritus systems. Given the chemical nature of the organic compounds composing the particles, attachment of the microbes through electrostatic mechanisms is not direct. Electrostatic bonding occurs between charges created on the surface of the bacterial cell and the organic particle by functional groups associated with the molecules, make up the structures associated with each entity. Surfaces of bacteria are dominated by teichoic acid (gram positive strains) or polysaccharides (gram negative strains) (Brock and Madigan, 1991). The most common functional groups on the bacterial surface are carboxyl and some amine groups (although a variety of other, less common groups may also be involved), whereas charge-generating groups on humified particles tend to be dominated by carboxyl groups and phenolic hydroxyls combined with a lower percentage of amine groups than found on the bacterial cells (Stevenson, 1982). Given that acid carboxyl groups on humic materials are largely dissociated at about pH 5.0 and above, and that phenolic hydroxyls are undissociated at pH values below about 10, the overall charge of both the organic particles and the bacterial cells is negative (Plette *et al.*, 1995; Posner, 1964; Stevenson, 1982). Amines can be protonated at a variety of pH values (depending on the specific amino acids with which the groups are associated), but they are rarely so heavily protonated at typical soil pH values that the net charge becomes positive (see Figs. 2 and 3).

Given the net negative charge of both particles, it is clear that some intermediate bridge must be used to span and join the two negatives together. Polyvalent cations generally provide such a bridge, and complexation of ions such as Ca^{2+}, Mg^{2+}, etc., with the negative charges on either the cell or particle surface can leave exposed positive charges to complex with negative charges on the corresponding particle. While the presence of a large proportion of polyvalent ions tends to cause electrostatic attraction and flocculation of particles in suspensions, the organometallic complexes are much stronger than simple

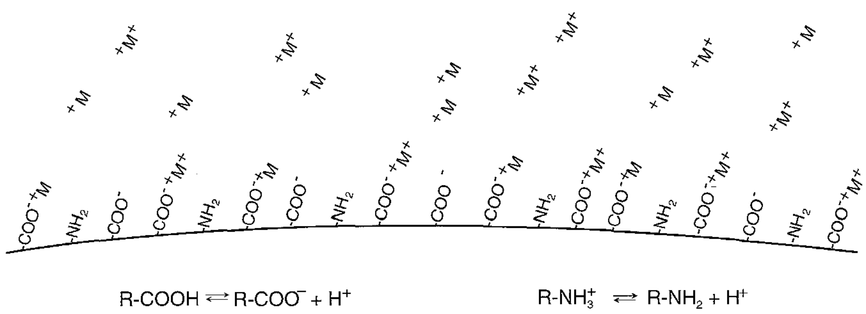

Figure 3 Generation of charges on the surface of bacterial cells and on organic particle surfaces. The degree of positive and negative charge on any cell will reflect the relative proportion of acids and bases exposed to the solution, the pK_a for any proton donating–accepting group and the pH of the surrounding solution. Other groups may be involved with this type of reaction; carboxyl groups and amines are common groups and are shown for simplicity. The presence of polyvalent cations can generate a net positive charge that can lead to a strong ionic bonding of the particles together.

electrostatic attractions and attachment is irreversible unless some major change in ionic strength or composition occurs.

IV. IMPORTANCE OF ATTACHED MICROBES IN SOIL

A. NUMBERS OF ATTACHED *VERSUS* FREE-LIVING MICROBES

The total abundance of bacteria in soil is typically $10^8\,g^{-1}$ or greater, depending on conditions of soil moisture, organic concentration, pH, etc. The bacterial biomass is generally on the order of 500 kg ha^{-1}. It is difficult to determine what fraction of these organisms reside attached to the soil particle surfaces as opposed to being suspended in the soil solution. As pointed out above, the liquid volume in unsaturated soils is small; in a soil with a porosity of 0.4 at 50% saturation, the amount of water in a cm^3 of the soil would only be 0.2 ml. If the solution contains about 10^6 cells ml^{-1} (a value often associated with surface waters), the ratio of attached to unattached cells would be determined as:

$$\frac{\text{total cells} - \text{suspended cells}}{\text{total cells}} = \frac{10^8 - 2 \times 10^5}{2 \times 10^5} = 5000 \qquad (1)$$

Data to support this calculation are not readily available. Classical counting methods are grossly inaccurate for determining such a ratio; extraction of cells for direct microscopic enumeration fails to differentiate between truly suspended cells and those that are loosely associated with the particle surface via electrostatic or hydrophobic interactions and are easily removed. It is not clear if use of newer techniques can overcome preparation artifacts to help determine the fraction of cells in intimate contact with particle surfaces. However, calculations such as the one presented above suggest that the role of attached organisms must be dominant in soils.

Such a conclusion would be consistent with the findings related to particle attachment in aquatic environments. While typical counts of suspended bacteria are on the order of $10^6\,\mathrm{ml}^{-1}$, counts associated with organic particles are generally two orders of magnitude larger (i.e., in the order of $10^8\,\mathrm{g}^{-1}$).

B. PHYLOGENY OF ATTACHED *VERSUS* FREE-LIVING MICROBES

Given the lack of a real suspended phase for microorganisms as suggested above, one might anticipate that there would be no phylogenetic differences in the attached and suspended soil microbial community. However, whenever there is an opportunity for the two communities to develop there does seem to be a different set of microbes found attached to particles as opposed to remaining in suspension. In aquatic environments where detrital particles are dominant in the water column, molecular analysis has shown important differences in the attached and suspended communities. Examination of ribosomal RNA (both amplified and cloned) (DeLong *et al.*, 1993) and low-molecular-weight (transfer and 5S ribosomal) RNA (Bidle and Fletcher, 1995) has shown that particle-associated communities differ from those in suspension. More recent work by the latter group, however, suggested that there was little difference in the communities during the summer, perhaps due to a rapid exchange of organisms between the attached and suspended phases (Noble *et al.*, 1997). At this point, similar analyses have not been reported for soil; it may be that the lack of a clear suspended phase makes the question uninteresting. It may also be that concern over the removal of particle-associated microbes (which are most certainly the predominant in soil) during the extraction process may make the results of such an exercise suspect. One would logically expect, however, that differences in attached and suspended organisms in soil would be much less than found in aquatic environments.

C. QUANTITATIVE CONSIDERATIONS OF ACTIVITY OF ATTACHED *VERSUS* NON-ATTACHED

There is little information for soils on the relative contribution to microbial activity of attached *versus* unattached cells. In most cases, it is simply assumed that all the organisms in a soil sample are associated with particle surfaces. Indeed, all other things being equal, the earlier calculation which suggested that 5000 times more microbial cells are associated with the mineral grain surfaces and in suspension would suggest that metabolic activity should be divided on a similar ratio. Although it is not examination of soil, a study by Hopkinson *et al.* (1989) in Georgia coastal waters, supports the concept that in the presence of large particulate surface areas, metabolic activity associated with particle surfaces is greater than comparative activity in the suspended phase. In the estuary, 80% of heterotrophic activity was associated with particles $>3.0\ \mu m$ and 20% of the activity was associated with particles less than $3.0\ \mu m$. These results suggest that the organisms (and their activities) are primarily associated with particles. In the open ocean, however, the circumstances were reversed. Eighty percent of the metabolic activity passed a $3.0\ \mu m$ filter, indicating non-particle association. This seems perfectly reasonable, given that the particulate load in estuarine waters can be orders of magnitude higher than in the open ocean.

V. DIVERSITY OF MODES OF ATTACHMENT

A. REVERSIBLE *VERSUS* NON-REVERSIBLE

Reversible attachment describes the situation in which microbes are easily removed from particle surfaces by shear forces from tangential flow across the surface, or by small changes in the aqueous phase chemistry that causes a desorption of cells attached by electrochemical forces. Non-reversible attachment, or permanent adhesion, of microbes to surfaces occurs due to the formation of polymer bridges between the cells and the surfaces to which they attach (Marshall, 1980; Marshall *et al.*, 1971). Non-reversible sorption also includes the formation of metal–organic complexes that do not dissociate except under conditions of significant chemical alteration, i.e., major change in ionic strength or composition such that the binding polyvalent metals would be displaced by monovalent forms through mass action, or loss of ionic strength through dilution. Hydraulic shear is not considered sufficient to break the complexes holding the bacteria to the surface.

B. ELECTROSTATICS

ELECTROSTATICS AND HYDROPHOBIC EFFECTS: DLVO THEORY

Reversible sorption to particles is often explained by a combination of electrostatics combined with hydrophobic effects to overcome the natural repulsion of bacteria and particles that arises from the similar charges expressed at their surfaces. The surface charges of both mineral particles and bacterial cells are slightly to strongly negative at common soil pH values (4–8). For an excellent review of surface charge interactions in soil, see Bolan *et al.* (1999).

The pH at which positive and negative electrical charges on a particle balance is the isoelectric pH ($\mathrm{pH}_{\mathrm{iep}}$), also referred to frequently as the point of zero charge or ZPC. Harden and Harris (1953) found that bacterial $\mathrm{pH}_{\mathrm{iep}}$ varied from 1.75 to 4.15 for 18 gram-positive species and from 2.07 to 3.65 for 13 gram-negative species. Typical soil solid $\mathrm{pH}_{\mathrm{iep}}$ values are 2.0 for quartz and 4.6 for kaolinite (Bolan *et al.*, 1999; Stumm and Morgan, 1996). Both bacteria and common soil components have $\mathrm{pH}_{\mathrm{iep}}$ values lower than the typical pH of soil solution, pH 5–8 (Table IV). Consequently, bacteria and solids generally have net negative charges and will repel each other electrostatically. It is important to keep in mind that some soil components may be positively charged in near-neutral pH conditions, e.g., amorphous $Fe(OH)_3$, which has a $\mathrm{pH}_{\mathrm{iep}}$ of 8.5 (Stumm and Morgan, 1996). Scholl *et al.* (1990) found that attachment of negatively charged bacteria was much greater to positively charged surfaces of limestone, $Fe(OH)_3$-coated quartz, and $Fe(OH)_3$-coated muscovite than to uncoated quartz and muscovite.

The negative charge on bacteria and solids is neutralized by a swarm of cations that becomes progressively less dense away from the surface, resulting in a diffuse double layer of charge (see Fig. 4). The approximate thickness of the diffuse layer is κ^{-1}. At 20°C,

$$\kappa \approx \frac{I^{0.5}}{0.28\ \mathrm{nm}} \tag{2}$$

where I is the ionic strength expressed in units of molality (m; note that many authors use molarity, M, to quantify ionic strength; although not strictly correct,

Table IV
Surface Charges at Typical Soil pH Values

Soil mineral	Surface charge and pH at which charge was measured (Cmol kg^{-1})	Reference
Kaolinite	−13 (pH 7.0)	Hendershot and Lavkulich (1983)
Illite	−21 (pH 7.0)	Hendershot and Lavkulich (1983)
Montmorillonite	−90.4 to −127.6 (pH 7.0)	Cowan *et al.* (1992)
Vermiculite	−195.3 (pH 7.0)	Bouabid *et al.* (1991)
Muscovite	−22 (pH 7.0)	Hendershot and Lavkulich (1983)
Quartz	−2 (pH 7.0)	Hendershot and Lavkulich (1983)
Al hydroxide	+24 (+16.0) to +10.0 (pH 6.0)	Hendershot and Lavkulich (1983)
Gibbsite	+7.2 (pH 6.0) to −0.88 (pH 9.0)	Hingston *et al.* (1974)
Fe hydroxide	+34 (pH 5.8)	Bolan *et al.* (1999)
Humic acid	−330 to −340	Posner (1964)

Source: Bolan *et al.* (1999).

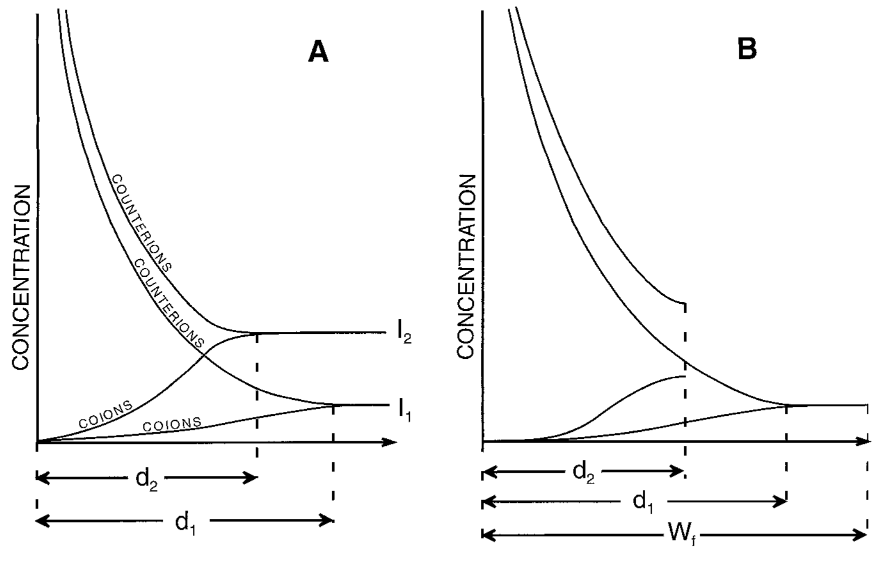

Figure 4 Distribution of charges (ions) near a charged surface. (A) Represents the distribution at two different ionic strengths (*I*) representing two equilibrium concentrations of ions in the soil solution. Co-ions are those with the same charge as the surface and counterions are those with a charge opposite to that of the surface. *d* represents the thickness of the diffuse double layer near the particle surface (i.e., the distance from the surface at which the concentration of counter and co-ions equals that of the equilibrium solution). Note that as the ionic strength increases, the thickness of the double layer decreases. (B) Represents the situation in which part of the water layer has been removed due to drying. The initial thickness of the water film is greater than the thickness of the double layer. Note that the integral of the ion concentrations from 0 to d_1 and d_2 remains the same as no salts are removed upon evaporation of the water. Concept from Bolt and Bruggenwert (1976). Reproduced with permission of Elsevier Science Publishers.

molarity is a good approximation for dilute solutions) (Stumm and Morgan, 1996). For example, when $I = 0.001$ m, $\kappa^{-1} = 8.9$ nm.

If a bacterial suspension with pH > pH_{iep} is placed in an electric field, the cells will be drawn towards the positive pole, and the cations farthest away from the surface of a cell will be sheared off. The resulting potential at the outside of the diffuse layer, determined from the bacterial velocity, is called the *zeta potential.* This potential is dependent on the ionic strength and pH of the suspension as well as density of charge on the bacteria. Gannon *et al.* (1991) measured zeta potentials for 19 bacterial strains suspended in deionized water that ranged from -8 to -36 mV.

Due to the diffuse layer, the electrostatic potential that repels ions of like charge (negatively charged particles, for the case considered here) increases as the particle approaches the solid. For the case of a negatively charged, 1 μm bacterium approaching a soil particle, the two surfaces may be approximated as spheres. The repulsive electrostatic potential (V_R) is

$$V_R = \frac{cr^2\,e^{-\kappa x}}{(2r + x)\kappa^2} \tag{3}$$

where c is a constant for fixed temperature and decreases the separation distance for a given level of repulsion.

If electrostatic repulsion is not strong enough to keep bacteria away from a solid, close approach may result in strong adsorption due to van der Waals force. This force is sometimes referred to as *electrodynamic* or *fluctuating dipole–induced dipole*, since it results from the motion of electrons in their orbitals (Weber *et al.*, 1991). This potential (V_A) is always attractive; for spheres,

$$V_A = \frac{Ar}{12x} \tag{4}$$

where A is the Hamaker constant (about 100 g nm^2 s^{-2}) (Stumm and Morgan, 1996).

The total interaction potential is the sum of the attractive and the repulsive forces, $V_R + V_A$. This is the basis of what is called the DLVO theory (for the researchers Derjaguin and Landau (1941), and Verwey and Overbeek (1948)). According to the theory, for most groundwater ($I < 0.01$ m), suspended bacteria are electrostatically repelled from solids with increasing force as they approach the solid, until a separation distance of about 1 nm is reached. Brownian motion, convection, or motility may push the bacteria over the repulsive barrier. If the separation distance becomes less than 1 nm, the potential rapidly decreases to a strongly adsorptive van der Waals minimum. At very close range, other mechanisms such as hydrogen bonding may also contribute to adsorption. Increasing ionic strength decreases the magnitude of the barrier (equation 2). Mills *et al.* (1994) found that increasing the ionic strength from 0.001 to 0.01 reduced recovery of bacteria from sand columns by as much as an order of magnitude. Similarly, Gross *et al.* (1995) found that increasing ionic strength from 0.01 to 1 M increased adsorption of bacteria to borosilicate beads by a factor of 9. For high ionic strengths (generally $I > 0.1$ M) the diffuse-layer potential is so weak that the total potential is always negative (attractive), and there will be no net energy barrier to contact. Murray and Parks (1980) discuss details of the DLVO equations for the interaction of a virus particle and an oxide surface. They emphasize the importance of differences in van der Waals potentials for different materials, with metals having strong potential and organics weak potential.

In addition to the primary van der Waals minimum described above, a "secondary minimum" may be important for reversible adsorption of bacteria (see Fig. 5). The total interaction potential of a sphere and a plate (using representative values for the Hamaker constant and electrostatic potential and for ionic strengths around 0.01 M) is negative at about 5–20 nm separation, as well

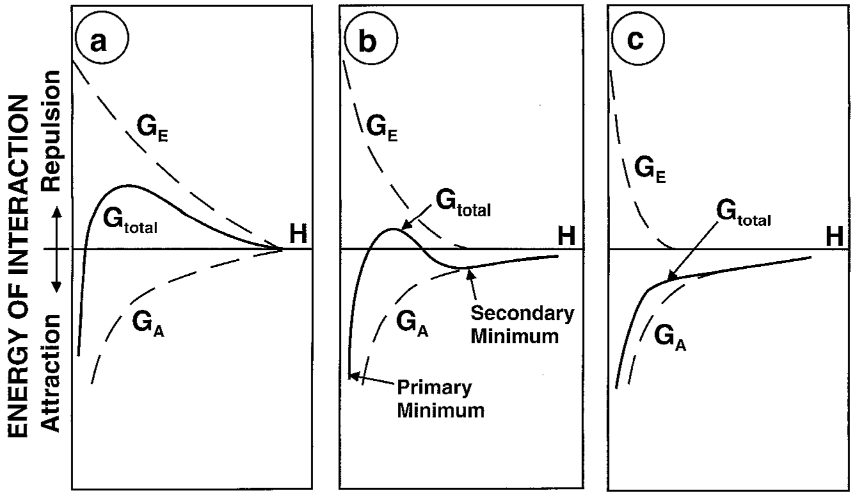

Figure 5 Repulsive and attractive relationships near a charged surface. The three diagrams represent conditions of: (a) very low ionic strength; (b) intermediate ionic strength; (c) high ionic strength. Repulsive forces that keep same charged particles far from the surface, i.e., unadsorbed, dominate surfaces covered with very dilute solutions. Surfaces associated with solutions of intermediate ionic strength have a "secondary minimum" in the repulsive forces in which like-charged particles can rest in a "reversibly sorbed" state, subject to shear stresses. Under high ionic strength conditions, the diffuse double layer has collapsed to allow a deep primary minimum to form at the surface. Particles that reside in that region are considered irreversibly sorbed, although changes in pH, ionic strength, etc., can change the distribution of repulsive and attractive forces that can cause desorption of the particles. Concept from Van Loosdrecht *et al.* (1990). Reproduced with permission of The American Society of Microbiology.

as at <1 nm (McDowell-Boyer *et al.*, 1986; Van Loosdrecht *et al.*, 1989). Support for this hypothesis is found in the attachment energy calculated from adsorption isotherms. In many cases, bacteria reversibly adsorb to solids such that there is a linear relationship between suspension concentration and adsorbed cells per gram of solid (the adsorption isotherm). The slope of this relationship (K_d) and the number of adsorption sites per gram (M) may be used to calculate the energy of bacterial adhesion with the van't Hoff equation:

$$\ln\left(\frac{K_d}{M}\right) = -\frac{\Delta H^0}{RT} + C \tag{5}$$

where ΔH^0 is the standard state enthalpy, R the gas constant, T the absolute temperature, and C a constant (Hendricks *et al.*, 1979). van Loosdrecht *et al.* (1989) found that the adhesion energy calculated from adsorption isotherms is about the same as the predicted potential at the secondary minimum using the DLVO equations and suggested that initial bacterial adhesion takes place at the secondary minimum.

Electrostatic repulsion may result in enhanced transport of microbes relative to the average mass of water by keeping them exposed to greater velocities in the central "fast lane" of flowing soil pore water. Water adheres to pore walls and slows adjacent flow according to Newton's law of shear. Consequently, water and entrained substances in the center of a pore move the microbes most rapidly. At the molecular level this entire process is called *anion exclusion*.

Microbes have hydrophobic regions on their surfaces where there are high densities of hydrocarbon (C–H) groups. Water molecules "prefer" (have lower free energy) to associate with other water molecules or hydrophilic surfaces. Water molecules in contact with hydrophobic surfaces exhibit a surface tension that tends to minimize water contact with hydrophobic regions. Surface tension may squeeze hydrophobic particles out of the water environment and into other hydrophobic regions. Murray and Parks (1980) defined hydrophobic bonding as "the aggregation of non-polar surfaces resulting from the minimization of reoriented and thus higher (than bulk) free energy water structure adjacent to the non-polar surfaces." For a bacterium attaching to a solid surface, this change in free energy is

$$\Delta G_{\mathrm{adh}} = \gamma_{\mathrm{BS}} - \gamma_{\mathrm{BL}} - \gamma_{\mathrm{SL}} \tag{6}$$

where γ_{BS} is interfacial tension of the bacterium–solid surface, γ_{BL} is that of the bacterium–liquid, and γ_{SL} is that of the solid–liquid surface. Values of the interfacial tensions are calculated from Young's equation by the relationship

$$\gamma_{\mathrm{SV}} - \gamma_{\mathrm{SL}} = \gamma_{\mathrm{LV}} \cos \theta \tag{7}$$

where γ_{SV}, γ_{SL}, and γ_{LV} are the solid–air, solid–water, and air–water interfacial tensions and θ the contact angle of the water on the solid (Marshall, 1990; Neumann *et al.*, 1980).

Bacterial hydrophobicity is, therefore, most appropriately evaluated by contact angle measurement, but indexes of cell surface hydrophobicity have also been obtained by measuring bacterial adherence to hydrocarbons (BATH) and by hydrophobic interaction chromatography (HIC) (Rosenberg and Doyle, 1990). The bacterial contact angle is the angle formed when a drop of water contacts a surface such as a lawn of bacterial cells. More hydrophobic bacterial surfaces cause the water drop to "ball up," resulting in larger contact angles. Wan *et al.* (1994) concentrated three strains of bacteria on filters and used a goniometer eyepiece to observe the angle formed by a drop of 1 mM $NaNO_3$ on the bacteria. The contact angles ranged from 77.1° (relatively hydrophobic bacteria) to 24.7° (relatively hydrophilic).

Huysman and Verstraete (1993) used the BATH method with octane to measure hydrophobicity of *Escherichia coli*, *Streptococcus faecalis*, and seven strains of *Lactobacillus*. Hydrophobicity, measured as percent removal by octane, ranged from 2% for *E. coli* to 94% for *Lactobacillus* strain Lc4. They then tested bacterial adsorption to sand and transport through sand columns and found significant correlations between hydrophobicity and adhesion to sand. The hydrophobic characteristics of poliovirus (which has a protein surface) were evaluated by Murray and Parks (1980) by testing its sorption to $C_2C1_3F_3$. There was no significant difference in virus concentration between a viral suspension mixed with this hydrophobic liquid and a control suspension similarly mixed without it. The authors concluded that the protein surface was very hydrophilic. They neglected to account for sorption of virus to the air–water interface, however, which could explain an equal loss in virus concentration for the control and $C_2C1_3F_3$ treatments.

HIC measures the amount of bacteria retained by a hydrophobic gel. Gannon *et al.* (1991) used this method as well as the BATH method to estimate hydrophobicity of 19 bacterial strains. The HIC assay retained from 7 to 91% of the cells, and the BATH assay retained from 5 to 85% of the cells, although the correlation between the HIC and BATH assays was poor. Furthermore, there was no significant relationship between the HIC or BATH results and transport of cells through loam columns.

Hydrophobic interactions are not simple relationships since humic material (generally the most important hydrophobic component of soil solids) occurs in solution as well as associated with solids. Dissolved organic molecules may compete with microbes for adsorption sites or modify interfacial tensions and thereby interfere with hydrophobic adsorption (Powelson *et al.*, 1991).

Although there is little controversy over the fact that both electrostatic effects and hydrophobic effects influence bacterial sorption to surfaces, there are different opinions about which is more important. Both Stenström (1989) and Van Loosdrecht *et al.* (1987a) found that increased hydrophobicity resulted in increased sorption and that hydrophobicity was more important than surface charge in determining the extent of sorption. Van Loosdrecht *et al.* (1987a,b) used negatively charged polystyrene with a contact angle of 70°, making it relatively hydrophobic. Stenström (1989), however, found that sorption increased with cell hydrophobicity even with a hydrophilic substratum such as quartz. On the other hand, Fletcher and Loeb (1979) found sorption of a *Pseudomonas* sp. was lessened considerably on hydrophilic surfaces relative to hydrophobic surfaces. The sorption of a hydrophobic cell would be less with a hydrophilic surface would seem to be borne out by the findings of Absolom *et al.* (1983).

One notable difference between studies which have found an electrostatic effect and those which have found a hydrophobic effect is the ionic strength at which they have been run. Studies which have found an electrostatic effect, whether it is due to ionic strength of the suspending liquid (Fontes *et al.*, 1991; Marshall *et al.*, 1971; Scholl *et al.*, 1990) or the surface charge of the bacteria (Sharma *et al.*, 1985), have used ionic strengths below 0.1 M. Studies which have observed a hydrophobic effect (Absolom *et al.*, 1983; Fletcher and Loeb, 1979; Stenström, 1989; Van Loosdrecht *et al.*, 1987a,b) have been carried out at ionic strengths greater than 0.1 M, except for that performed by Mozes *et al.* (1987), who used distilled water. Gordon and Millero (1984) found that the nature of electrostatic interactions changed at 0.1 M. Below this level, bacterial sorption increased with ionic strength, as predicted by DLVO theory. Above 0.1 M, DLVO theory was no longer applied; bacterial sorption was unaffected by ionic strength, but decreased with increasing cation concentration. This may help to explain why electrostatic interactions have been found to be, at best, of secondary importance at higher ionic strengths. It may also serve to explain why Scholl *et al.* (1990) and Mozes *et al.* (1987) observed changes in sorption with pH, while Stenström (1989) did not.

PRIMARY MINERAL DIFFERENCES

Soil minerals comprise a variety of chemical classes (Sposito, 1989), but the dominant ones are silicates, carbonates, sulfates, and oxides of Fe and Al (Table II). In addition, PO_4^{3-} and a few sulfides can also be found in some environments. There has not been a great deal of work comparing bacterial attachment rates on a variety of pure minerals, but some literature reports indicated differences among the minerals that have been tested. In a field study, Mills and Maubrey (1981) examined the effect of mineralogy on the initial colonization of chips of freshly exposed rock submerged in lakes or ponds. The chips were made by sawing parallel faces on pieces of rock or of museum-grade specimens of single mineral crystals, then carefully cleaning the surfaces to remove any organic contaminants left as a result of the processing. The chips were left in place for only 24 h because after that time, the film was invariably more than one cell thick in some places, precluding obtaining an accurate count of the attached bacteria. Mills and Maubrey (1981) observed that chips immersed in flowing water always accumulated more bacteria than chips of the same material placed in still water, a fact that probably has little relevance to the unsaturated soil situation in which shear and boundary layer considerations are not the important factors. Quartz chips were colonized more rapidly than calcite. When limestone and hematitic sandstone were compared, either no difference was observed, or the quartz-based rock was colonized more slowly than the carbonate. Environmental factors notwithstanding, this set of seemingly contradictory observations were deemed by the authors to be consistent with the findings of Marszalek *et al.* (1979) that surfaces which are biologically inert (such as glass) tend to be colonized more rapidly than those which are biologically active (i.e., that leach ions toxic to the colonizing community). Mills and Maubrey (1981) suggested that the presence of potentially leachable Fe in hematitic sandstone may increase the activity of the rock surface and thereby inhibit microbial colonization.

In a similar study, the same research group exposed chips of quartz, muscovite, and limestone to a suspension of an organisms isolated from groundwater and measured the rate of attachment over a 16 h period (Scholl *et al.*, 1990). In doing so they obtained results in direct opposition to the earlier work of Mills and Maubrey (1981). Attachment to the silicates was lower than to the limestone (Fig. 6), which compared favorably with quartz and muscovite after coating with iron hydroxide. Clearly the speculation of Mills and Maubrey (1981) about the leachable iron was incorrect. Scholl *et al.* (1990) observed that the attachment to the various materials corresponded with the surface charge of the mineral at the experimental pH. The latter finding is consistent with expectations based on DLVO theory and is supported by nearly all of the work on the importance of metal oxides that followed.

EFFECT OF MINERAL COATINGS (e.g., IRON OXIDES)

Most temperate soils have silicate minerals making up the bulk of their composition (Table II). Other minerals occur in soil with high frequency, notably oxides of iron or aluminum. As phyllosilicates weather, the silicon-containing layers are stripped away leaving behind abundant aluminum mixed with residual oxides of iron. Most highly weathered soils are characterized by accumulation of

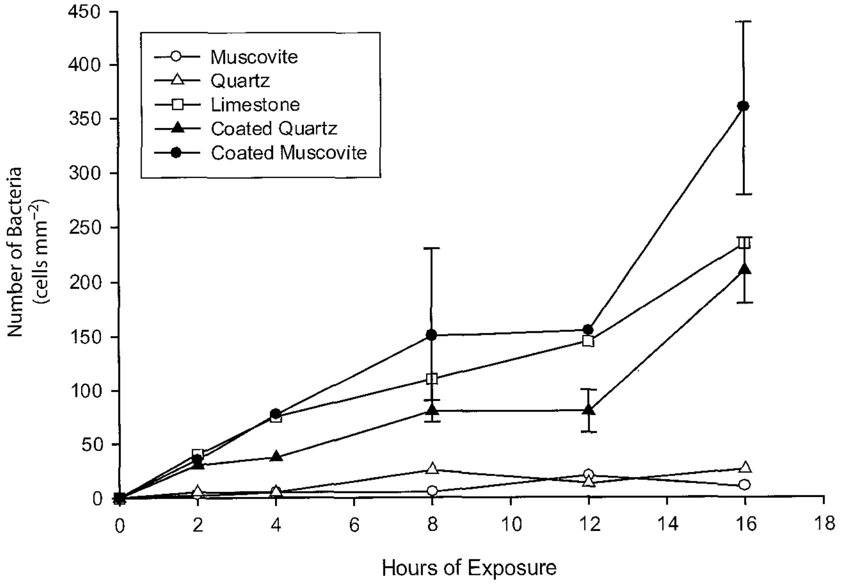

Figure 6 Attachment of a gram negative bacterium isolated from ground water to uncoated chips of quartz, muscovite and limestone and to quartz and muscovite chips coated with Fe-hydroxide. Values are mean ± 1SEM; some fell within the size of the point as drawn. Figure redrawn from Scholl *et al.* (1990). Reproduced with permission of Elsevier Science Publishers.

metal sesquioxides as part of the normal pedogenic process. In mature soils, often even in young soils, the oxides and hydroxides exist as coatings on the surfaces of silicate mineral grains. Podsolization of soils is the downward translocation of Fe, Al, and organic matter. The iron and aluminum are dissolved in the surface horizons (often with the assistance of complexation by soil organic matter) and are moved downward in the profile where they are deposited as coatings on sand-, silt-, and clay-sized particles.

There is a substantial opinion that nearly all mineral grains have coatings that differ somewhat from the bulk mineralogy of the grains. Given that the surfaces of pure quartz and silicate minerals express a net negative charge, it is reasonable to assume that cations such as $Al(OH)^{2+}$ or $Fe(OH)_2^+$ (products of the hydrolysis reactions of Al^{3+} and Fe^{3+}) would be readily attracted to the silicate surface. Indeed, coatings of amorphous iron and aluminum sesquioxides are common on silicate grains. At typical soil pH values, these layers of amorphous material, as well as the more crystalline coatings such as $Al(OH)_3$ (gibbsite) or $Fe(OH)_3$ (goethite), can block negative charges from the silicate and can often act as bases accepting protons and imparting a positive charge to the mineral surface (Fig. 7). Coatings do not completely cover the entire grain surface; a soil may retain its net negative charge even though there are abundant sites of less negative or even positive charge distributed over the surface.

The presence of metal oxide coatings on mineral grains has a profound effect on the interaction of those surfaces with bacterial cells. Scholl *et al.* (1990)

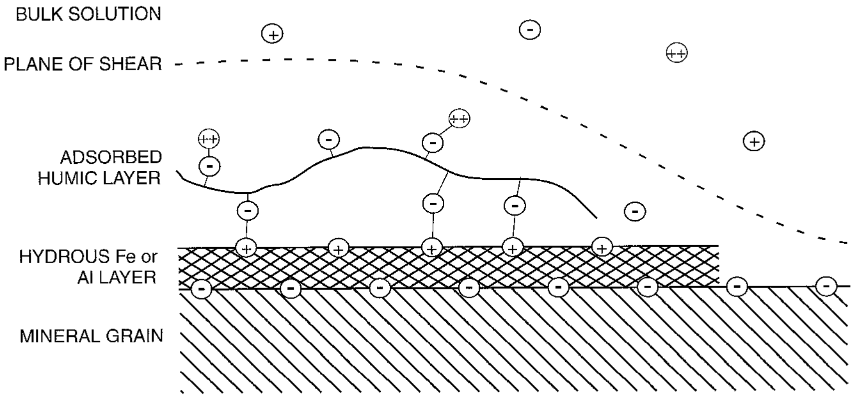

Figure 7 Changes in charge distribution upon coating of a silicate mineral with metal oxides and subsequent over-coating with organic materials. While soil particles normally have a net negative charge at most soil pH values, coating with metal sesquioxides or organics may generate regions of net positive charge at those same pH values. This figure illustrates the type of surface exposure expected given a discontinuous coat of sesquioxide or humic material over the surface of the mineral grain. Note the importance of (especially) polyvalent cations in conferring a positive charge over the organic layer. Figure reproduced from Mills and Powelson (1996) with permission of Wiley–Liss.

demonstrated that the deposition of iron sesquioxide on quartz and muscovite greatly increased the sorption of bacteria from suspension. The retention of bacteria in columns and clean quartz sand coated with iron sesquioxide was much greater than for the uncoated analog (Scholl *et al.*, 1990). Mills *et al.* (1994) demonstrated that bacterial sorption to clean sand followed a linear isotherm for the range of concentration of bacterial cells used but that sorption to iron-coated sand was nearly complete for all concentrations up to the sorption limit of 10^8 cells g^{-1}. Using that limit, Mills *et al.* (1994) constructed a simple model for sorption of bacteria to partially coated sand. Initial sorption was complete and irreversible up to the sorption limit imposed by the amount of sesquioxide coating available (i.e., 10^8 cells g^{-1} of coating included) in the porous medium. Once the coating was saturated with cells, sorption was controlled by the linear isotherm developed for the clean quartz sand. The intercept for the linear isotherm was the maximum number of cells that could be sorbed by the coated sand in the mixture used for the experiment. The model differs significantly from standard saturation models (e.g., Langmuir isotherm) which is concentration independent up to the saturation level of the coated sand. The theory that underlies the simple model can describe any mixture of reversible and irreversible sorption processes.

EFFECT OF ORGANIC COATINGS ON ATTACHMENT

The effect of organic matter on bacterial sorption is not well studied, although there is substantial evidence to indicate that the presence of a "conditioning film" of organic matter enhances and may even be a prerequisite for bacterial colonization (see Marshall (1996) for a thorough discussion of conditioning films). Pringle and Fletcher (1986) found that a variety of macromolecules inhibited attachment of bacteria to polystyrene when the macromolecules were present in the suspension during the attachment, and a number of the macromolecules also inhibited attachment when the surfaces were preconditioned with them. Scholl and Harvey (1992) observed that retention of bacteria in artificial groundwater was greatest in sand which had been leached of organic matter but still retained an iron oxyhydroxide coating (and was therefore positively charged), as opposed to sand which had both an organic and iron coating or sand which had neither. They also obtained less sorption to sand that was coated with iron oxyhydroxides and organic matter when organic matter was present in the suspension (but not with iron-coated sand with the organic matter coating removed, as might be expected for competition mechanism). Scholl and Harvey (1992) concluded that experiments with better defined components were necessary. Both Lance and Gerba (1984) and Powelson *et al.* (1991) found that organic matter in solution (derived from sewage sludge in both studies, and from natural humic material as well in the latter study) decreased sorption of virus, and they concluded that the effect was due to competition between the viruses and the organic matter for sorption sites.

Richardson *et al.* (1998) used two strains of bacteria of differing hydrophobicity and surface charge in experiments designed specifically to look at the effect of humic acid on attachment of bacteria to sand. The presence of dissolved humate added to the bacterial suspension did not affect sorption of cells to clean quartz sand at the concentrations studied (up to $1.0\ \mathrm{mg\ l^{-1}}$) in batch experiments using 50 ml of artificial ground water (AGW), 25 g sand, and an incubation period of 3 h. A strain effect was observed, with 50% of the added cells of the relatively more hydrophilic, less negatively charged strain (S138) retained across the treatments, but only 2.3% of the added cells of the more hydrophobic, more negatively charged strain (S139) retained (Table V). The authors concluded that the humic acid did not sorb strongly to the clean sand. However, when humate was sorbed to iron-coated quartz sand at two concentrations, but was not present in the AGW, bacterial sorption to iron-coated sand decreased, and the sorption decreased with increasing humate concentration in the range examined. An effect of strain was not seen. The presence of a humate "overcoat" over a metal oxide coating modifies the high sorptive properties of the oxide with respect to bacterial cells. The results suggested that the organic matter blocks otherwise available sorption sites, and sorption of the bacterial cells to the organic coating does not make up for the loss of the sites associated with the metal oxide. Figure 7 illustrates how organic coatings can block the electric positive effect of metal oxide coatings.

Table V
The Effect of Na-humate on Bacterial Sorption to Sand Percentage of Cells Sorbed to Sand. Humate was Either Added to the Aqueous Phase in the Case of Clean Quartz Sand, or "Overcoated" on Iron-oxyhydroxide-coated Quartz Sand

Strain	Dissolved Na-humate (mg l^{-1})		
	0	0.1	1
		Clean quartz sand	
S138	53 ± 2	61 ± 2	37 ± 8
S139	2.0 ± 5.9	−2.0 ± 2.8	68 ± 3.9
	Concentration of Na-humate (mg g^{-1} sand)		
	0	9.4×10^{-3}	2.1×10^{-2}
		Iron-coated sand	
S138	94 ± 0	80 ± 3	64 ± 4
S139	98 ± 0	87 ± 1	60 ± 8

Note. All values are mean of three replicate treatments ± 1 standard error of the mean.
Source: Richardson *et al.* (2000).

This concept is consistent with reports in the literature that coatings of mineral grains remove sorptive effects due to the properties of the bulk mineral and replace them with those of the coating material. Different colloids revert to a similar electronegative surface charge after exposure to dilute solutions of humic substances (Beckett *et al.*, 1987).

C. APPENDAGES AND CEMENTS

A variety of microfibrillar structures, usually called fimbriae or pili may be found on the surfaces of various bacteria. While they are composed of proteins similar to those of flagella, these hair-like structures tend to be straighter, thinner (0.004–0.008 μm diameter), and shorter than flagella (Joklik *et al.*, 1992). These structures are similar to flagella in that they are composed of self-aggregating monomers that originate from the membrane. The fimbriae are known to be associated with cellular attachment to surfaces, although they have other purposes as well. Because different fimbriae act in different ways to confer survival advantage, they are often categorized by their functional role. The best-studied roles of fimbriae are in host–pathogen relationships, and fimbriae have been grouped in categories relative to their function. The classes include adhesins, lectins, evasins, agressins, and sex pili; the latter three categories have little to do with bacterial attachment in nature. Evasins are structures that assist pathogens (such as *Neisseria gonorrhoeae*) to evade the host with immune system by covering large portions of the cell with non-antigenic materials. Fimbriae serve multiple purposes. In some strains of *Streptococcus pyogenes*, a surface virulence factor, the M protein, serves as an adhesin allowing the organism to colonize the pharynx, and this microfibril can also prevent phagocytosis (evasin) and is leukocidal (agressin). Many adhesins are directly associated with pathogenicity; the greatest volume of literature concerning adhesins is related to the role these molecules play in conferring virulence on (especially) opportunistic pathogens such as *Pseudomonas aeruginosa* (Prince, 1996), *E. coli* (Sokurenko *et al.*, 2001), *Streptococcus pneumoniae* (Jado *et al.*, 2001), *Helicobacter pylori* (Ikehara *et al.*, 2001), etc.

Microfibrils and their importance are not well studied in soils, but the fact that adhesins help cells bind strongly to hydrophobic surfaces suggests these structures may play a role in bacterial attachment to soil particles. Lectins, on the other hand, are known to be important in the soil environment. Lectins are adhesins which bind to specific sugars on cell surfaces. In soil, the lectins are best studied in rhizosphere situations, particularly those of the *Rhizobium*–legume association. In rhizosphere habitats, many of the organisms actively attach to the root surface. Many of the organisms adhere to specific plants at specific locations. Dazzo *et al.* (1976) found that *Rhizobium leguminosarum* biovar *trifolii* which nodulates the roots of clover, adhered specifically to those roots. However, binding to pea roots was observed in *R. leguminosarum* biovar *vicae* which nodulates pea, and by *R. leguminosarum* biovar *trifolii* and *A. tumifaciens* neither of which nodulates the plant (Smit *et al.*, 1986, 1989). Smit *et al.* (1987) attributed the attachment to microfibrils. Matthysse (1996) asserts, however, that no fimbrial adhesions have been found in *R. leguminosarum.* She indicated that there are occasionally cellulose fibrils that are involved in adhesion that have been mistaken for the proteinaceous fimbriae (Smit *et al.*, 1987). On the other hand, *Bradyrhizobium japonicum* does produce fimbriae at times that coincide with the cells' ability to bind to soybean root hairs (Vesper and Bauer, 1986). In an attempt to exclude specific symbionts by adding large number of competing but non-nodulating cells, Wall and Favelukes (1991) found that only about 10% of the inoculated alfalfa nodulator *Rhizobium meliloti* sorbed to the root hairs, but addition of a 1000-fold excess of *R. leguminosarum* did not reduce the sorption of the proper symbiont.

The role of lectins in defining the specificity of the diazotroph-plant symbiosis is well accepted (Matthysse, 1996), although detailed mechanisms are not yet worked out for all bacteria–plant pairs. In some cases, the lectin is produced by the bacterium (e.g., *B. japonicum*; Loh *et al.*, 1993) to allow it to recognize the plant, and in other cases the lectin is produced by the plant, presumably to allow identification of the bacterium (Lodeiro and Favelukes, 1995; Wall and Favelukes, 1991). In either case, the protein serves as an attachment point for the bacterium to bind to the hairs of the plant root.

The decay of organic matter, much of which is cellulose, in soil requires the use by the cells of extracellular enzymes to initiate the depolymerization of the large molecules that make up the fibers. Many cellulolytic organisms attach directly to cellulose by means of an organelle called the cellulosome (Bayer *et al.*, 1996). This discrete multifunctional multicomponent surface protein complex is not only responsible for the adhesion of cells to cellulosic material, but it also provides the catalytic power for conversion of cellulosic substrates to cellobiose which can be readily taken up and assimilated by the bacterial cell (Bayer *et al.*, 1983, 1996; Lamed and Bayer, 1988a,b, 1991; Lamed *et al.*, 1987; Strobel *et al.*, 1995).

After initial adhesion, bacteria may become more permanently moored with polymers that extend beyond the bacterial and solid surfaces. Because of their small diameter, these fibers may be able to overcome electrostatic repulsion to link the bacterium with the solid by a process called bridging (Van Loosdrecht *et al.*, 1989). Many bacterial cells secrete a polysaccharide layer that may effectively cement them to solids and to each other. Gannon *et al.* (1991), however, found that the presence of polysaccharide capsules or the presence of flagella was not correlated with soil transport at a flow rate of 2.5 cm h^{-1}. This is a somewhat surprising result in the light of the aquatic literature which suggests that high fluid flows promote the secretion of bacterial slime layers and the development of active biofilms on solid surfaces (Geesey *et al.*, 1977; Ladd *et al.*, 1979).

VI. EFFECTS OF SATURATED *VERSUS* UNSATURATED CONDITIONS

As pointed out earlier, the further away from saturation a soil is, the thinner is the water film. But at the same time, additional surface for sorption is produced. A number of investigators have provided ample evidence of sorption to the gas–water interface (GWI) in unsaturated soils. The observation is supported by evidence from surface layer films found in open water bodies. Guerin (1989) found that the total heterotrophic bacteria and humic materials were concentrated in an estuary microlayer, and particulate amino acids were found to adsorb to the sea surface microlayer (Hendricks and Williams, 1985). In lakes, organic N, P, and C in the microlayer are enriched by factors of 1.6–45 (concentration in the microlayer divided by concentration in the subsurface), and bacteria by factors of 6.4–10.7, compared with subsurface water (Sodergren, 1993). In the ocean near sewage outfalls, Plusquellec *et al.* (1991) found bacterial surface enrichment of 32–341, and many of the surface bacteria may be attached to particles, which in turn are concentrated in the surface microlayer. Harvey and Young (1980) found that the degrees to which bacteria were concentrated into the surface microlayer were linearly dependent on surface concentration of particulate material. There may be also a phylogenetic discrimination by the microlayer. Hardy and Apts (1984) found not only that the sea surface microlayer is enriched in total microalgae, but also that it contains distinct types of algae compared with the bulk water.

While adhesion of microorganisms to the surface microlayer in open water bodies has a strong enriching factor over the bulk water, it may be that the attachment of microorganisms to the GWI and soils may have an even greater enrichment factor. In open water, the microorganisms are subjected to a number of stress factors such as ultraviolet light, airborne pollutants, and rapid temperature and salinity changes that are not found or are not as severe in soil (Lion and Leckie, 1981). Gas phases (i.e., bubbles) are often present in contact with particles in porous media and may act as additional immobile adsorptive surfaces.

Bacterial adsorption to the GWI may be an important and often overlooked factor affecting adhesion in transport of bacteria in porous media (Mills and Powelson, 1996; Powelson and Mills, 1996, 1998; Wan and Wilson, 1994; Wan *et al.*, 1994). Wan and Wilson (1994) employed etched glass micromodels of porous media to observe colloidal polystyrene beads, clay particles, and bacteria concentrating at the air–water interfaces under flow conditions. In these experiments, sorption appeared to increase with particle hydrophobicity, solution ionic strength, and positive electric charge of the particles. The authors suggested that initial adsorption was due to van der Waals and electrostatic interactions, followed by essentially irreversible adsorption due to capillary forces.

Thus, sorption to the GWI is both extensive and very strong; Wan and Wilson (1994) predicted that "for relatively hydrophobic strain of bacteria, even a small amount of residual gas can dramatically reduce . . . transport" of particles through the porous media. In another study, bubbles moving through a microscope field were observed to sweep glass and polymer surfaces clean of adsorbed bacteria (Pitt *et al.*, 1993).

It is not know if GWIs and particle surface–water interfaces (SWIs) interact with bacteria in a similar manner. The two interfaces are likely to react differently in several ways. The SWI generally has an electrostatic component that may dominate adsorption, unlike the essentially non-charged GWI. Although not conclusively demonstrated, it is likely that that the bacterial association with the GWI is dominated by hydrophobic effects. If so, surfactants may have particularly strong effects on adsorption at the GWI.

Polystyrene beads have been used to elucidate some of the basic mechanisms of particle interaction with the GWI. Butt (1994) directly measured the force between hydrophobic particles in water and air bubbles. The particles snapped into the air bubble by a process that is irreversible based on thermodynamic considerations. The figures presented by Butt (1994) indicate that the force necessary to pull a 20 μm hydrophobic particle (contact angle 110–120°) away from the air–water interface into water was about 1000 nN, while suspension agitation (probably Brownian motion) appeared to provide less than 2 nN; once the particle is stuck in the GWI, it is there permanently. Williams and Berg (1992) observed polystyrene beads accumulating and aggregating at the surface of a water drop. They found that the beads arrived at the GWI at a constant rate over 1 h period and that the rate increased with increasing salt content. Beads aggregated at the GWI surface at salt concentrations that were only 1% of that required for aggregation of the beads in the bulk suspension.

Organic matter, especially surfactant compounds, have important effects on microbial adsorption at the GWI. Marshall (1976) suggested that hydrophobic interactions likely dominate at the GWI and therefore hydrophobic bacteria should be more attracted to the gas phase and that surfactants should reduce adsorption. Powelson *et al.* (1991) found that dissolved organic matter increased the transport of MS2 virus in unsaturated soil columns by nearly an order of magnitude, and offered several possible explanations for the effect. First, organic matter may reduce surface tension, thereby reducing the strength of attachment of surface-adsorbed virus proteins. Almost all organic substances found in natural waters reduce the interfacial tension (Lion and Leckie, 1981). Hunter and Liss (1981) reported that adsorption of surface-active species on lake water microlayers can reduce the surface tension by 40%. MacRitchie and Alexander (1963) found that the adsorption rate of proteins at air interfaces declined logarithmically with surface tension. Second, the increase in virus transport (i.e., the decrease in sorption) with added dissolved organic matter might be due to the effect of surface-active organic molecules on the structure of water. In pure water, layers thicker than 1 μm from a surface can be considered to have properties of the bulk liquid (Clifford, 1975). The presence of surface-active organic films, however, can cause water molecules next to the interface to orient into ice-like, clathrate, lattice structures, resulting in additional stabilization of the boundary layer to a depth of 50 μm (Hardy *et al.*, 1987). It is possible that the stabilization reduces access of the partly hydrophobic virus to the GWI. Third, the presence of organic matter may inhibit virus sorption because organic matter may compete with virus particles for GWI adsorption sites (Trouwborst *et al.*, 1974).

Powelson and Gerba (1995) reviewed several studies that compared microbial concentrations in porous media after exposure to water saturated and unsaturated conditions and found that, in every case, recovery of microbes was less in unsaturated conditions. Some authors (Boyd *et al.*, 1969; Kibbey *et al.*, 1978) reported a correlation of loss of microbes with the degree of unsaturation, and Powelson and Gerba deemed that observation consistent with the hypothesis of strong sorption to the GWI. Later work (Powelson and Gerba, 1995; Powelson *et al.*, 1990, 1993; Wan and Wilson, 1994) directly attributed the loss of the microbes to adsorption to the GWI. From a practical point of view, bacterial adsorption to a GWI in soil may be detrimental by slowing the transport of organisms intended to degrade a pollutant or may be beneficial by slowing the transport of pathogens to drinking water aquifers. In the vadose zone, it may be possible to "chase" pollutants spilled on the ground surface with bacteria capable of degrading the chemicals by minimizing bacterial adsorption to GWIs.

VII. SUMMARY

Soil microorganisms live in a particle-surface-dominated environment, and the microbes exist there associated either with the solid–liquid interface or the gas–liquid interface (GLI). They do so because it is advantageous for them; the particles attract and concentrate energy sources and nutrients, but they also associate with surfaces because the thin water films leave little space otherwise in which they can exist. To this end, there appears to be very little contribution to soil activities by unattached bacteria. Initial attraction to surfaces is largely a combination of electrostatic and hydrophobic effects, but irreversible attachment occurs at points where opposing charge centers appear (as when mineral grains are coated with metal oxides) or when the organisms attach via microfibrils (fimbriae) or form slime layers. Microbes likely move from location to location in soils under unsaturated conditions by association with GLIs that sweep the organisms along as the GLI moves under moisture tension gradients.

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