Cellular, particle and environmental parameters influencing attachment in surface waters: a review

Chunyu Liao  
*Iowa State University*, cyliao@iastate.edu

Xiao Liang  
*Iowa State University*, liang628@iastate.edu

Michelle L. Soupir  
*Iowa State University*, msoupir@iastate.edu

Laura R. Jarboe  
*Iowa State University*, ljarboe@iastate.edu

Follow this and additional works at: [http://lib.dr.iastate.edu/abe_eng_pubs](http://lib.dr.iastate.edu/abe_eng_pubs)

Part of the Agriculture Commons, Bacteriology Commons, Biochemical and Biomolecular Engineering Commons, Bioresource and Agricultural Engineering Commons, and the Water Resource Management Commons

The complete bibliographic information for this item can be found at [http://lib.dr.iastate.edu/abe_eng_pubs/652](http://lib.dr.iastate.edu/abe_eng_pubs/652). For information on how to cite this item, please visit [http://lib.dr.iastate.edu/howtocite.html](http://lib.dr.iastate.edu/howtocite.html).

This Article is brought to you for free and open access by the Agricultural and Biosystems Engineering at Digital Repository @ Iowa State University. It has been accepted for inclusion in Agricultural and Biosystems Engineering Publications by an authorized administrator of Digital Repository @ Iowa State University. For more information, please contact digirep@iastate.edu.
Cellular, particle and environmental parameters influencing attachment in surface waters: A review

C. Liao\textsuperscript{1,3}, X. Liang\textsuperscript{2}, M.L. Soupir\textsuperscript{2,*}, L.R. Jarboe\textsuperscript{3}

\textsuperscript{1} Interdepartmental Microbiology Program, Iowa State University, Ames, IA, USA
\textsuperscript{2} Agricultural and Biosystems Engineering, Iowa State University, Ames, IA, USA
\textsuperscript{3} Chemical and Biological Engineering, Iowa State University, Ames, IA, USA
* Corresponding Author, Michelle L. Soupir, Department of Agricultural and Biosystems Engineering, 3358 Elings Hall, Iowa State University, Ames, IA 50011, U.S.A. E-mail: msoupir@iastate.edu; phone: 515-294-2307

Abstract

Effective modeling of the fate and transport of water-borne pathogens is needed to support federally required pollution-reduction plans, for water quality improvement planning, and to protect public health. Lack of understanding of microbial-particle interactions in water bodies has sometimes led to the assumption that bacteria move in surface waters not associated with suspended mineral and organic particles, despite a growing body of evidence suggesting otherwise. Limited information exists regarding the factors driving interactions between microorganisms and particles in surface waters. This review discusses cellular, particle, and environmental factors potentially influencing interactions and in-stream transport. Bacterial attachment in the aquatic environment can be influenced by properties of the cell such as genetic predisposition and physiological state, surface structures such as flagella and fimbriae, the hydrophobicity and electrostatic charge of the cell surface, and the presence of outer-membrane proteins and extracellular polymeric substances (EPS). The mechanisms and degree of attachment are also affected by characteristics of mineral and organic particles including the size, surface area, charge, and hydrophobicity. Environmental conditions such as the solution chemistry and temperature are also known to play an important role. Just as the size and surface of chemical particles can be highly variable, bacterial attachment mechanisms are also diverse.

Introduction

Nearly 15\% of waters across the United States are classified as impaired because of elevated pathogen levels, determined by presence of indicator organisms such as \textit{E. coli} and enterococci (USEPA 2009). To prevent waterborne disease outbreaks and identify when there is a critical threat to public health, better models of the fate and transport of pathogenic organisms to drinking and recreational water supplies are needed. Existing models generally perform poorly when predicting environmental fate and transport of microorganisms, and one reason is the...
lack of understanding regarding the movement of organisms and interactions with environmental particles (Russo et al. 2011). Previous researchers have provided comprehensive reviews of the presence of pathogens in a range of water systems (Pandey et al. 2014) as well as the fate and transport of pathogens in the environment (Jamieson et al. 2004b; Benham et al. 2006; Pachepsky et al. 2006; Bradford et al. 2013); and many of these previous works raise awareness of the limitations in our understanding of microbe-particle interactions and the resulting impact on water quality.

Here we consider cellular, particle and environmental factors and the role they may play in aqueous transport of microorganisms. In general, the microorganisms considered here are representative of bacterial pathogens associated with waterborne diseases. Further, we have focused the discussion on the initial attachment which occurs prior to the changes in surface protein expression, morphology and signal transductions which lead to biofilm formation. Section 1 provides background on waterborne disease outbreaks and the legislation and strategies put in place by the regulatory agencies to reduce microbial pollution of the waters of the United States. Next we define attachment and discuss the implications of attached organisms in the environment and describe laboratory-based methodologies for enumerating attached microorganisms. The third section describes in detail cellular properties potentially influencing attachment, including flagella, fimbriae, outer-membrane proteins (OMP), polysaccharides, and growth phase and state. The fourth section considers the role of particle properties on attachment, including size, surface charge and charge density, organic matter content, surface area, and hydrophobicity. In section 5, the potential impact of environmental factors, including ionic strength, temperature, and pH are described. This review highlights the conflicting evidence regarding role of specific parameters that drive microbe-particle interactions and also identifies the need for a comprehensive study of these parameters to improve understanding of and modeling of pathogen fate and transport in waters.

1. Background

Waterborne disease outbreaks

Waterborne disease outbreaks are often caused by ingestion or dermal contact with water contaminated by pathogenic microorganisms such as bacteria, protozoans, or viruses, present in human or animal excrement. Pathogenic organisms cause illnesses including but not limited to common gastroenteritis, diarrhea, typhoid fever, and dermatitis (Rosen 2001; Pond 2005). As the most well-known waterborne diseases worldwide, enteric and diarrheal diseases contribute to the death of more than 5 million people every year around the globe. In developing countries, diarrhea is one of the leading causes of childhood death and is attributed to unsafe drinking water, lack of sanitation, and insufficient clean water for hygiene (WHO 2008). Waterborne diseases also occur in developed countries with modern water and
sanitation systems. Between 2007 and 2008 the United States had 36 documented outbreaks of waterborne disease associated with drinking water and 134 outbreaks due to water-related recreational activity (CDC 2011). The largest waterborne disease outbreak in United States history occurred in 1993 in Milwaukee, WI, when over 400,000 people became ill due to Cryptosporidium in the city's drinking water supply.

**Regulatory strategies in the United States**

Water pollution is a global concern which requires ongoing evaluation and revision of water resource policies from the international to national and local levels. Water pollution regulation have evolved over the last 120 years in the United States. The River and Harbors Appropriation Act of 1889 first made it a misdemeanor to discharge refuse matter of any kind into navigable waters. In 1948, the Federal Water Pollution Control Act was the first major law in the U.S. to address water pollution. It was amended in 1972, to what is now known as the Clean Water Act (CWA), which established the basic structure for regulation of pollutant discharges into the waters of the United States. The act established the goals of eliminating releases of high amounts of toxic substances into water, eliminating additional water pollution by 1985, and ensuring that surface water would meet standards necessary for sport and recreation by 1983 (USEPA 1972). The CWA has been successful in regulating discharges of pollutants into the waters of the United States and in setting water quality standards for surface waters. The general water quality conditions are reported to Congress and the public every 2 years by National Water Quality Inventory Report (305(b) report).

One challenge in meeting the goals of the CWA resides in the sources of pollution. Contamination of surface water bodies is often due to a combination of point and nonpoint sources of pollution. Point source (PS) pollution is that which originates at a single identifiable source. Point sources include industrial facilities, municipal wastewater treatment plants and other government facilities, and animal feeding operations. The National Pollutant Discharge Elimination System (NPDES, Clean Water Act Section 402) is a permit-based system for regulating the discharge from point sources into navigable waters. Nonpoint source (NPS) pollution originates from non-regulated point sources and is often generated in the upland areas of a watershed and transported to water systems during hydrologic events. Major activities resulting in bacteria transport to surface water from NPS include land application of manure from animal feeding operations, direct deposit by grazing animals (Soupir et al. 2006), wildlife, and private septic systems.

**Fecal indicator organisms and water quality standards**

*E. coli* and enterococci are typically used as fecal indicator bacteria to predict when a risk to human health is present. Commonly found in the lower intestine of warm-blooded animals, *E. coli* is a gram-negative, rod-shaped bacterium while enterococci is a gram-positive
coccoci. Both are recommended as a freshwater indicator of fecal contamination by the U.S. EPA in 1986 (USEPA 1986), and are still considered to be the most reliable organisms used to indicate the presence of fecal pollution in environmental waters (Cabral 2010), and to indicate when a risk to human health is present. As the concentrations of indicator organisms increase, it is likely that other pathogenic organisms present in fecal material will also be in the water body, threatening human health (USEPA 2000). Exposure limits have been established to protect human health. For example, the EPA defines acceptable recreational limits as those that will result in eight or fewer swimming-related gastrointestinal illnesses out of every 1,000 swimmers (USEPA, 1986), with illness being defined as vomiting, diarrhea with a fever or disabling condition, or stomach ache or nausea in addition to a fever. Others have also identified a correlation between indicator organisms in water and gastrointestinal illness in humans (Cabelli 1983). For example, one study observed a significant correlation between increased gastrointestinal illness and indicator organisms at Lake Michigan beach, and a positive correlation for indicator organisms at Lake Erie beach (Wade et al. 2006). However, recent work by Edge et al. (2010) found that waterborne pathogens were detected in 80% of water samples with low *E. coli* concentration [less than 100 CFU (colony-forming units) 100mL⁻¹](Edge et al. 2012). The U.S. EPA recommends that the geometric mean concentration of *E. coli* for recreational activities should not exceed 126 CFU 100mL⁻¹ and the single sample maximum should not exceed 235 CFU 100mL⁻¹ (USEPA 1986).

Until recently, the U.S. EPA’s recommended water quality testing strategies were dependent upon culture-based methods, which are time-consuming and may pose potential health hazards (Myers et al. 2007). The Ambient Water Quality Criteria developed by the U.S. EPA in 1986 were recently revisited, and in 2012 the U.S. EPA established the new Recreational Water Quality Criteria (RWQC). Culturable *E. coli* (for freshwaters) and culturable enterococci (for marine waters) remain the two measures of recreational water quality, but quantification of enterococcus populations by quantitative real-time polymerase chain reaction (qPCR) methods was added as an acceptable alternative to measuring CFU in a water sample. Detection of pathogens and pathogen indicators via qPCR is promising due to the rapid turnaround time and strong correlation with health outcomes (Wade et al. 2008; Colford et al. 2012), analytical costs are high and specialized laboratory equipment is required. Furthermore, qPCR is unable to differentiate between viable and non-culturable organisms.

**Water quality improvement plans**

Although pollution control technologies have been implemented by many point sources such as municipal wastewater treatment plants, of the assessed river and stream miles in the United States, 54% still do not meet the national goal of “fishable and swimmable” (USEPA 2009). Under Sections 303 (d) and 305 (b) of the Clean Water Act, states, territories, and authorized tribes are required to identify impaired waters and to develop water quality
improvement plans for these waters based on a calculation of a total maximum daily load (TMDL). TMDL is a calculation of the maximum amount of a pollutant that a waterbody can receive while still meeting water quality standards. Once established, the TMDL is used to set limits on allowable discharges to meet water quality standards by identifying and quantifying both PS and NPS contributing to the problem. The calculation of a TMDL is as follows:

\[ \text{TMDL} = \text{WLA} + \text{LA} + \text{MOS} \]

where WLA is the wasteload allocation from PS of pollution, LA is the load allocation from NPS of pollution, and MOS is the margin of safety which accounts for the uncertainty in the response of the waterbody to loading reductions.

One method often used to set TMDL load allocations among point and nonpoint sources is through watershed-scale water quality modeling when there are multiple impairments existing. A typical TMDL plan often must address multiple pollutants, including bacteria, sediment, nitrogen, and phosphorus. Following approval of a TMDL plan, implementation plans are developed to recommend best management practices (BMP) and other management strategies (USEPA 2008) to reduce pollutant loading to waters.

Accurate modeling of bacterial fate and transport in the environment is critical to successful TMDL implementation plans. Watershed scale, water quality models such as the Soil and Water Assessment Tool (SWAT) may be used to predict bacterial fate and transport (Borah and Bera 2003; Borah et al. 2006; Gassman et al. 2007), but most models simulate bacterial transport as unattached cells, mainly due to insufficient data on bacteria partitioning fractions (Jamieson et al. 2004a; Paul et al. 2004; Soupir et al. 2008). However, microorganisms move through the environment in both a planktonic state and attached to suspended soil and organic particles (Jeng et al. 2005; Hipsey et al. 2006; Pachepsky et al. 2008; Liu et al. 2011). A lack of understanding of the mechanisms of bacterial attachment to particles in eroded soil, stream-bottom sediments, and suspended sediments leads to inaccuracy in modeling bacterial transport at the watershed scale – and therefore to inaccurate TMDLs.

2. Attachment

Definition

Broadly speaking, bacteria in waters can exist in two general states: free-floating or particle-attached. Free floating bacteria could be planktonic single cells or part of a microbial aggregate (Fletcher 1991; Rivas et al. 2008). Attached bacteria are associated with a free-floating mineral or organic particle and might also be immobilized, such as through the formation of a biofilm or some other physical attachment to a surface. The effective prediction of the fate and transport of bacteria through watersheds depends on the ability of our models to represent these various states.
Observations support the presence of microorganisms in both free-floating and particle-attached states in streams. For example, the fecal indicator bacteria in stream bottom sediments have been reported as 10-10,000 times higher than in the overlying water column (Davies and Bavor 2000; Bai and Lung 2005). Bacteria are also known to attach to metal or plastic solid surfaces, such as those used in wastewater treatment plants and medical devices, through the formation of biofilms (Davey and O'Toole G 2000; Dunne 2002; Flemming 2002). Bacterial attachment to sediment particles in a watershed is a dynamic process affected by the bacterial properties, particle surface properties, and environmental factors that regulate those properties (Goulter et al. 2009). It has been reported that bacteria attached to surfaces are more resistant to environmental stresses such as ultraviolet radiation and antibiotics, influencing their survival and ultimate fate in surface water systems (Gerba and McLeod 1976; LaLiberte and Grimes 1982; Burton et al. 1987; Davies et al. 1995; Russo et al. 2011).

Bacteria are frequently found attached to or living in close association with particle surfaces (Hipsey et al. 2006; Pachepsky et al. 2008). The mechanism of bacterial attachment in aquatic environments was summarized by Fletcher (1996), Figure 1. The book chapter stated that to establish contact with the surface, bacteria first must overcome hydrodynamic boundary layer and repulsive forces as they approach the surface. As the bacterium moves closer to the surface (~10-20 nm, depending on pH, ionic strength, and the dominant ions in solution), mainly repulsive electrostatic interactions occur since most often both the bacterium and surface carry the same net charge. When the bacterium is ~2-10 nm from the surface, both repulsive and attractive electrostatic interactions may occur simultaneously since the bacterium and the particle can carry both positive and negative charge at local sites. At shorter distances, water adsorbed at the surface can act as a potential barrier for attachment, while hydrophobic functional groups at the cell surface may help reduce the barrier by displacing the layer of water. Once the bacterium overcomes the hydrodynamic boundary layer and repulsive forces as they approach the surface (<1 nm), a variety of chemical interactions, include ligand exchange reactions, ion bridging, and van der Waals forces can promote attachment.

If the bacterium is not repelled by the surface, then bacterial attachment can be divided into two categories: reversible attachment and irreversible attachment. Reversible attachment refers to cells that are loosely attached via a single pole (flagella or pili). Rotation of the single pole results in a spinning motion of the bacteria, and the bacterium may readily detach and return to the planktonic phase. If bacteria attach to surface along their long axis, the spinning motion decreases and polysaccharide expression increases, making the attachment irreversible (Petrova and Sauer 2012). However, the irreversibility is conditional. For example, in the development of a biofilm, bacteria reproduce after attaching to a surface and some of the cells can then detach from the cluster (Esty 2010). Furthermore, rod-shaped bacteria can attach to
the surface: 1) at one end by their polar flagella, 2) along their length but still having slight Brownian movement, or 3) along their length but with no mobility (Meadows 1971).

**Significance of bacterial attachment in the environment**

Microbe-particle interactions play an important role in predicting the movement of microorganisms in waters. For example in streams, stable populations of microorganisms surviving and possibly multiplying in bottom sediments are resuspended during storms, and they can contribute fecal indicators and pathogenic organisms to the water column (LaLiberte and Grimes 1982; Rehmann and Soupir 2009). Between 20 and 35% of fecal coliforms, *E. coli*, and enterococci are associated with settleable particles (settleable being defined by a calibrated centrifugation technique), during normal flow conditions, and 30 to 55% during storms (Krometis et al. 2007). In the Neuse River Estuary an average of 38% of *E. coli* and enterococci in water samples was associated with settleable particulates (Characklis et al. 2005; Fries et al. 2006), while more than 80% of fecal indicators were associated with suspended sediments (defined as particles retained by a 0.6 µm cellulose membrane) in the Chesapeake Bay (Sayler et al. 1975). Field studies have shown that most resuspension of bacteria and particles occurs during the rising limb of the storm hydrograph (Nagels et al. 2002), that the supply of *E. coli* available for resuspension regenerates between storms (Jamieson et al. 2005b) and that *E. coli* and turbidity are highly correlated during artificial floods (Muirhead et al. 2004).

**Figure 1.** Forces involved in interaction between a cell and a negatively-charged surface: 1) when the distance is greater than 50 nm van der Waals forces may be present; 2) as the bacterium moves closer to the surface (10-20 nm), repulsive electrostatic interactions become present as well; 3) when the distance is 2-10 nm, both repulsive and attractive electrostatic forces may occur since both positive and negative
charge can be carried at local surface sites; 4) as the distance decreases to 0.5 to 2 nm, interfacial water barrier and hydrophobic groups can act simultaneously; 5) when the distance is smaller than 1 nm, chemical bonds such as receptor-ligand interactions, ion bridging, and van der Waals forces can promote attachment (modified from Fletcher, 1996 and Ojeda, 2012).

The lack of understanding about the mechanism driving microbe/particle interactions has led to various assumptions in modeling environmental transport. Dorner et al. (2006) assumed that data for the attached fraction of Cryptosporidium was representative of all microorganisms, while Wu et al. (2009) assumed an invariant attached fraction of 50%. Disagreement also exists among the methods used to mathematically represent bacterial attachment: Bai and Lung (2005) and more recently Russo et al. (2011) assumed attachment occurs as a linear and reversible adsorption process based on previous studies (Scholl and Harvey 1992; Chapra 1997), while Jamieson (2005a) argued that irreversible adsorption is more representative of attachment in low-ionic strength freshwater systems. Rehmann and Soupir (2009) initially assumed all E. coli were attached to sediment particles to predict the fate and transport of microbes. However, decreasing the attached fraction to 45% was one of several approaches that improved the match between observed and predicted values. To predict resuspension in streams, Pandey et al. (2012) recently developed a model based on measurable properties of the flow, sediment, and organisms. However, to reduce uncertainty in the model’s predictions requires improved quantification of the attached bacteria in sediments.

Measuring bacterial attachment in experimental contexts

Despite the need to quantify attached and unattached cells, a standard protocol to distinguish between unattached and attached bacteria does not currently exist. During the last decade, separation techniques such as settling, filtration and centrifugation have been used for research purposes (Schillinger and Gannon 1985; Henry 2004; Characklis et al. 2005; Jeng et al. 2005; Muirhead et al. 2005; Krometis et al. 2009). Settling is a straightforward method to isolate those cells attached to large particles, since they settle much faster than unattached, suspended cells. For example, with a simple estimation using the Stoke’s Law, E. coli attached to 63 to 125 μm quartz particles settled in 5 minutes (Liu et al. 2011). Filtration allows quantitative and hierarchical separation as particles of different sizes are retained via sieving the suspension through membranes of a known pore size (USEPA 2005). Unattached bacteria are those which pass a certain screen size, such as 8 μm (Krometis et al. 2009) which is comparable but larger than the typical size of an individual E. coli cell [1.1 to 1.5 μm in width and 2 to 6 μm in length (Grismer 2006)]. Multiple-screen filtration separates suspended solids into different particle sizes. In 2008, Soupir et al. utilized a series of screens and filters (35 mesh screen, 230 mesh screen, 8 μm and 3 μm polycarbonate filters) to quantify the percentage of attached E. coli cells in surface water runoff samples. Filtration has its limitation, since the
filtered solution contains not only unattached bacteria but also other small colloidal particles and bioflocculated clumps of comparable size (Soupir et al. 2008).

Another method to quantify sediment-attachment of bacteria is centrifugation (Faegri et al. 1977; Schillinger and Gannon 1985), which allows one to measure the concentration of unattached cells in the supernatant, and calculate the attachment fraction by comparing with total concentration (Soupir et al. 2008). However, the difficulty of determining an appropriate centrifuge time and speed to separate unattached cells from sediment particles with attached cells is a major drawback, especially when they have similar diameters (Henry 2004).

The above techniques to distinguish between unattached and attached bacteria have been used for several decades. However, in all the above methods, a time consuming and labor intensive assessment of the total number of bacteria present is needed to calculate the extent of attachment (e.g., the ratio of attached bacteria to total bacteria). The attached and unattached fractions are typically assessed via plate counting techniques, which involves serial dilutions and colony counting, or microscopic counting. Furthermore, to disperse bacteria from sediment and organic matter particles, different approaches have been applied to detach bacteria from particles. Chemical agents such as Tween 85 and sodium pyrophosphate have been used to weaken the hydrogen bonding, van der Waals, electrostatic, and chemical forces that tie cells and particles together (Amalfitano and Fazi 2008; Soupir et al. 2008). Physical techniques may be used to mechanically disrupt the physical entrapment of bacteria in small pores of particles. Physical disruption methods include blending, hand or orbital shaking, sonication probe, or submerging the sample in an ultrasonic bath. The traditional separation methods mentioned above have limitations in that they can cause cell damage, even though bacteria die-off by filtration, Tween, and sonication has been previously found to not be significant (Krometis et al. 2009); they are also labor intensive because they require diluting, plating, and colony counting.

A new method using flow cytometry was recently developed to distinguish between unattached bacterial cells and those that are attached to small particles (Liang et al. 2014). Flow cytometry is a technique used in particle counting and property analysis (Vital et al. 2010). The particles which can be applied include microorganisms, nuclei, and latex beads (Brown and Wittwer 2000). *E. coli* treated with a fluorescent nucleic acid stain can be distinguished from mineral particles. After attaching to particles, *E. coli* cells have a larger side scatter signal, which can be distinguished from the fluorescent signal of free *E. coli*. This method is limited in its application to environmental samples because bacterial species cannot be easily distinguished from each other; however, fluorescent *in-situ* flow cytometry could be a potential solution (Lange et al. 1997). And particles greater than 30 µm cannot be processed by the flow cytometer.
3. Bacterial properties influencing attachment

Physical structures expressed by bacteria and the chemical characteristics of the microbial surface are both important factors influencing bacterial attachment (Figure 2). In this section we discuss bacterial surface structures and compounds, including flagella, fimbriae, outer member proteins (OMP), and polysaccharides. Surface features expressed by a cell influence both the chemical and physical characteristics of that cell’s surface. Such characteristics include hydrophobicity, surface charge and sphericity.

Bacterial surface structures

The contribution of microbial physical structures to attachment to mammalian tissues has been well characterized (Garcia and Le Bouguénec 1996; Mitsumori et al. 1998; Nowrouzian et al. 2001; Rendón et al. 2007; Axner et al. 2011). However, the role of these surface structures in attachment to environmental particles is less understood given the conflicted results reported among studies. Figure 1 is a schematic summary of bacterial surface structures and cell properties that involved in attachment. The arrangement of surface structures was modified from previous study (Lepek and D'Antuono 2005). Surface structures influence attachment not only through active motion (like flagella and pili), but also through contributing to the cell surface chemical properties. Here we briefly summarize the current state of the knowledge of the relationship between these surface structures and attachment to abiotic surfaces.

Flagella

The most well-recognized bacterial surface feature is the flagella. These long, flexible filaments can propel the bacterial cell through aqueous environments (Berg and Anderson 1973; Silverman and Simon 1974), with directional guidance provided by the chemotaxis system. Flagella are important for motility of free-floating bacteria (Lowe et al. 1987). Contrary to expectations, flagella have also been shown to be important in the attachment process. Specifically, flagella are essential for initiation of surface attachment to biofilms but not for further biofilm development (Pratt and Kolter 1998). The explanation for this is that the motility endowed by flagella increases the chance that a bacterial cell will contact a surface. In natural water systems, bacteria might be actively swimming toward sediment particles to initiate attachment rather than passively depending on turbulent flow.

Generally, it is believed that flagella facilitate the initial interaction of the bacterial cell with the surface to which it will attach (Van Houdt and Michiels 2005). In a study of the attachment of Sphingomonas wittichii and E. coli to amorphous silica, Gutman et al. (2013) found that motility is a critical factor in determining bacterial adhesion. They proposed that
Flagella contribute to attachment via two possible mechanisms: overcoming repulsive forces at the interface by movement of the flagella and improving distribution of bacteria on the particle surface. However, it has been shown that flagella are not involved in attachment to some specific surface types, such as stainless steel (Rivas et al. 2007). Thus, flagella clearly play a role in bacterial adhesion at least to some types of surfaces.

**Figure 2.** Schematic summary of bacterial properties relate to attachment to surfaces. Surface structures influence attachment not only through active motion (like flagella and pili), but also through contributing to the cell surface chemical properties. Bacteria surface structure is also variable over different growth phases and states.

**Fimbriae**

Fimbriae, also known as pili, are short, thin appendages (3-10 nanometers in diameter, several micrometers in length) on bacterial cell surfaces. Unlike flagella, pili are not generally involved in motility, but instead directly mediate attachment. While only a few flagella are produced by any one cell, pili are typically concentrated on the bacterial cell surface (Rendón et al. 2007). Pili are well-studied in the context of pathogenesis, such as the P pili of uropathogenic *E. coli* (Nowrouzian et al. 2001; Mulvey 2002; Rendón et al. 2007; Axner et al. 2011). Pili contribute to pathogen-host interaction in that the proteins at the end of the pili can bind to specific sugar subunits on the host tissue. Curli fimbriae are distinct from standard fimbriae in that they have amyloid characteristics (Shewmaker et al. 2009). More than 20 different fimbriae types associated with *E. coli* have been identified (Cassels and Wolf 1995; Kaper et al. 2004).
It has been hypothesized that bacteria in the environment can attach to soil particles through a similar recognition mechanism since abundant carbohydrates, including both polysaccharides and monosaccharides, are present in soil (Safařík and Šantrůčková 1992). A number of studies found that the most common fimbriae for *E. coli*, the type I fimbriae, is necessary for adhesion to abiotic polymer surfaces, such as polystyrene (Prigent-Combaret et al. 2000; Cookson et al. 2002). Several studies have also indicated that the curli fimbriae of *E. coli* can promote surface interactions (Cookson et al. 2002; Pawar et al. 2005; Macarisin et al. 2012). One study has suggested that pili influence attachment by contributing to the hydrophobic nature of the cell surface rather than by directly binding to a specific surface receptor (Otto et al. 1999). It is clear at this time that at least some pili, fimbriae, and curli may contribute to bacterial attachment to abiotic surfaces, although the mechanisms of this contribution remain largely uncharacterized.

**Outer Membrane Proteins (OMPs)**

Microbial cells contain various membranes composed mainly of lipid bilayers. For some types of microbes, there is both an inner and an outer cell membrane. Both types of membranes contain proteins. OMPs of bacteria such as *E. coli* make up approximately half of the total outer membrane mass of the cell (Lin et al. 2002). The flagella and pili described above are essentially multi-unit polymeric filaments that project beyond the membrane. OMPs typically exist in a transmembrane form and consist of either monomers or dimers. These OMPs usually have activity that is restricted to the membrane, such as functioning as a transporter or part of a signal transduction system. However, some OMPs have been implicated in the attachment process. For example, it has been reported that expression of some OMPs, such as MipA and OmpX, could be induced under sessile (agar) growth compared to planktonic (broth) growth (Rivas et al. 2008), suggesting that these OMPs might play an important role in growth while attached. Other studies have also demonstrated the positive role of OMPs, such as OmpA and Ag43, in *E. coli* attachment to abiotic surfaces (Danese et al. 2000; Torres and Kaper 2003).

**Polysaccharides**

Lipopolysaccharides (LPS) are repeating sugar units attached to the cell surface that can extend for tens of nanometers (Matsuura 2013). These molecules have been shown to both increase or decrease microbial adhesion to various surface types (e.g., TiO₂, Al₂O₃, and SiO₂ model surfaces). For example, the O-antigens of some organisms promote hydrogen bonding with some oxide surfaces (Jucker et al. 1997), while in other cases the O-antigen portion of the LPS may shield charged functional groups at the cell surface and lead to significantly reduced electrostatic interactions (Walker et al. 2004). Parikh and Chorover (2007) demonstrated that the configuration of LPS components was strongly dependent on solution pH, ionic strength, electrolyte composition, and phosphate groups in the LPS.
Extracellular polymeric substances (EPS) are high-molecular-mass compounds secreted by microorganisms at the cell surface. While LPS are anchored to the cell surface, EPS are not. EPS are mainly composed of polysaccharides and proteins, but may also include other macromolecules such as DNA and lipids (Flemming and Wingender 2010). EPS play an important role in cell aggregation, cell adhesion, biofilm formation, and protection of cells from hostile environments (Dogsa et al. 2005; Nielsen et al. 2011). Matthysse (2008) reported that polysaccharides cellulose, colanic acid, and poly-β-1,6-N-acetyl-D-glucosamine (PGA) are essential for optimal binding of *E. coli* to plastic surfaces and to alfalfa sprouts (Matthysse et al. 2008). Agladze et al. (2005) claimed that EPS PGA is required for the initial attachment of *E. coli* to glass surfaces.

**Bacterial hydrophobicity, surface charge, cell size and sphericity**

The hydrophobicity of a bacterial cell is influenced by the relative abundance of hydrophilic and hydrophobic residues and structures on the cell surface (van der Mei 1991). Li and McLandsborough (1999) reported that of 22 *E. coli* O157 strains they tested, 9 were moderately hydrophobic, four were strongly hydrophobic and four were strongly hydrophilic. It has also been noted that hydrophobicity can vary by up to five-fold with the growth stage of the bacterial cells (Hassan and Frank 2004). On the basis of literature data, Liu et al. (2004) proposed a model of attachment which reflected a positive correlation between hydrophobicity and attachment to both hydrophobic and hydrophilic support surfaces. However, other studies, such as Bolster et al. (2009) reported no correlation between hydrophobicity and attachment of various *E. coli* strains to quartz.

The surface charge of a bacterial cell is the overall net charge carried by the cell. This charge is influenced by the composition and abundance of the cell membranes and surface structures (Goulter et al. 2009). The majority of bacterial cells have a net negative charge. Li and McLandsborough (1999) reported the surface charge of 22 *E. coli* isolates in 150 mmol L\(^{-1}\) PBS buffer (pH 7.4) based on measurement of zeta potential, which ranges from −4.9 to −33.9 mV. Ukuku and Fett (2002) measured the surface charge of *Salmonella*, *E. coli* and *Listeria monocytogenes* using an electrostatic interaction chromatography (ESIC) column packed with an ion exchange resin and sodium phosphate buffer (pH 6.8). They found that *Salmonella saphra* had the strongest negative charge, up to 50 times more than *E. coli*. The surface charge can affect attachment to particles by repelling similarly charged particles and by attracting particles with an opposite charge. Ukuku and Fett (2002) also found a negative correlation between attachment to cantaloupe rind and negative surface charge of *Salmonella* and *E. coli*. In the study of Rivas et al. (2007), twenty *E. coli* strains were examined for their attachment ability to stainless steel and no correlation was found with surface charge. Thus, the contribution of surface charge to attachment may vary according to the surface in question.
The spatial distribution of positive and negative charges on the cell surface is known as surface charge heterogeneity. Surface charge heterogeneity can decrease electrostatic repulsion at the local scale and increase the rate of irreversible particle attachments (Walker et al. 2005). Walker et al. (2005) approximated the change of charge heterogeneity through the measurement of surface charge density. The chemical heterogeneity of the bacterial surface, i.e., the kinds and abundance of charged functional groups in surface structures, is thought to change with cell growth phase (Huisman et al. 1996).

Colloid-filtration theory (CFT) (Nelson and Ginn 2005) states that the attachment rate of colloids to sediments is influenced by the size and shape of the colloid. Therefore, if microbes are considered to be the colloids, then the size and shape of the bacteria should influence the attachment rate. This size and shape can be influenced by the surface structures, such as pili, flagella, and EPS. Bacteria have a large diversity in size and shape. Typical bacterial cells are 0.5-5.0 micrometers in length. Shapes of bacteria include spheres (such as *Staphylococci* and *Pneumococci*), rods (such as *Pseudomonas* and *Escherichia*) and spirals (such as *Leptospira* and *Treponema*). It has been reported that the deposition rate for bacteria in a quartz bed was correlated with cell width and sphericity (Bolster et al. 2009). Lutterodt et al. (2009) measured sphericity of six *E. coli* strains obtained from soil from cattle farms and found that cell sphericity did not significantly correlate with attachment to quartz. Moreover, no relation was found between attachment to quartz and cell sphericity in a study of 54 *E. coli* strains mainly isolated from animals in a zoo (Foppen et al. 2010).

**Bacterial growth phase and state**

Actively growing microbial cells can differ dramatically from those in stationary phase (Huisman et al. 1996). These differences impact the cell surface structures, which can in turn alter the cell surface properties that influence attachment. Therefore the bacterial growth phase is an important variable when investigating attachment. For example, Walker et al. (2005) found that the deposition rate of microbes onto quartz grains was 14 times greater for stationary-phase cells than for mid-exponential phase cells. They also found that cells from these two growth phases had different charge densities. They argued that this difference in charge density represents a more heterogeneous distribution of charged functional groups on the surface of stationary-phase cells compared to the mid-exponential cells (Walker et al. 2005). This difference in cell surface properties was responsible for the difference in observed attachment.

Just as the cell surface properties can be significantly affected by the growth phase, these properties are also influenced by whether or not the cell is in the attached state. Properties such as hydrophobicity, surface charge, type of OMPs, and the expression of surface
structures can differ between cells grown in an attached and planktonic mode (Fletcher 1991; Tremoulet et al. 2002; Rivas et al. 2008). For example, Tremoulet et al. (2002) found increased expression of transporters on the cell surface when *E. coli* O157:H7 was grown in an attached mode using proteomic analysis. Thus the mode of growth can influence attachment through changes in cellular physiology. Using epifluorescence microscopy Rivas et al. (2007) tested the attachment ability of 20 *E. coli* strains to stainless steel surface after growing in planktonic (broth) and sessile (agar) culture. They found that some isolates of *E. coli* exhibited a greater ability for attachment following sessile growth, other isolates showed higher attachment ability following planktonic growth, and the rest of the isolates showed no significant difference in attachment abilities under the two different growth modes.

4. Particle properties: Size; surface charge and charge density; organic matter concentration; surface area; hydrophobicity

There are many different particles in the aquatic environment. They include but are not limited to silicates, layer silicates, calcium carbonate, oxides and oxyhydroxides, humified colloids, and particulate organic matter. Previous research has demonstrated that particle size plays an important role in bacterial attachment. For example, the fraction of bacteria attached to particles is typically negatively correlated with particle size (Fontes et al. 1991; Fuller et al. 2000; Bolster et al. 2001; Dong et al. 2002; Levy et al. 2007). In urban storm water runoff, fecal indicator bacteria were adsorbed predominantly to clay particles (<2 µm) (Muirhead et al. 2006). Bacteria are more likely to attach to small particles, partially because they have greater surface area available for attachment than larger particles with same mass and density (Oliver et al. 2007).

Particle surface charge and hydrophobicity are often reported to be important factors impacting the transport of particle-attached bacteria through porous media (Bolster et al. 2006; Jacobs et al. 2007), although in some studies these characteristics have been found to not be significantly correlated with bacterial attachment (Bolster et al. 2009). Over a wide range of pH values, both bacteria and silicate particles such as smectites or quartz have a net negative charge, and so charge-based attachment is likely to be retarded. However, even layer silicates have some uncharged and positively charged sites (Pachepsky et al. 2006). Bacteria with a net negative charge may thereby be attached to local sites on the mineral surface that carry a positive charge (e.g., at broken particle edges) (Bolster et al. 2009). Since the total area of positively charged sites is much smaller than the overall surface area of layer silicates, the attachment will occur only at low surface coverage (Song et al. 1994). Furthermore, such electrostatic forces combined with London dispersion forces can overcome the dominant electrostatic repulsion of bacteria and negatively charged sites on mineral particles (Mills 1981).

A study by Scholl et al. (1990) found that negatively charged bacteria were more likely to attach to positively charged surfaces, such as calcite, than to negatively charged surfaces,
such as clean quartz. Bacterial attachment is enhanced significantly in the presence of Fe-oxide and other metal-oxide coated particles because of increased abundance of positively charged sites (Ams et al. 2004), which can be explained by the concept of point of zero charge (pzc). The research from Hendershot et al. (1983) demonstrated that after coating with Al or Fe sesquioxide, the pzc of mineral surface increased (Hendershot and Lavkulich 1983). Thus at the same pH, the surface of Al or Fe sesquioxide coating mineral would have more positively charged sites than the uncoated mineral surface.

Particle hydrophobicity is a physical property that causes nonpolar substances to aggregate in aqueous solution and exclude water molecules. In aquatic environments, it is related to the surface Gibbs energy and the Van der Waals interaction between particles or between particles and bacteria (Van Loosdrecht et al. 1990). Bacterial attachment to environmental particles is also enhanced by surface hydrophobicity of the particles (Lindqvist and Bengtsson 1991; Johnson and Logan 1996; Fein et al. 1997; Ong et al. 1999; Yee and Fein 2001; Ams et al. 2004; Foppen and Schijven 2005; Bolster et al. 2010).

Both dissolved natural organic matter (DOM) and sediment organic matter (SOM) can affect bacterial attachment. For instance, it has been shown that *E. coli* survival in water bodies can increase after attaching to mineral aggregates with high sediment organic matter concentration (Sherer et al. 1992). However, under typical environmental pH, organic matter carries a net negative charge. Thus DOM could diminish bacterial attachment by sorption to the bacterial surface, increasing the net negative charge at the bacterial surface and increasing repulsion between bacteria and negatively charged particles (Johnson and Logan 1996). Zhao et al. (2014) showed that the attachment of bacteria to soil colloids that had been treated to remove natural organic matter was greater than the attachment to soil colloids with natural organic matter present, suggesting that natural organic matter inhibited bacterial attachment.

5. Environmental factors: Ionic strength, temperature, pH

In natural aqueous systems, bacteria attach to particle surfaces that are bathed by solutions in which ionic strengths may vary seasonally or in response to storms or flooding. When the ionic strength increases, the number of attached cells per unit area increases which can be explained by Brownian interactions between the bacterial surface and the particle surface (Morisaki and Tabuchi 2009) increase. As mentioned, under typical conditions (e.g., pH 7), both bacteria and some particle surfaces are negatively charged (Pachepsky et al. 2006). There are two opposite interactions: electrostatic repulsion and van der Waals attraction due to London dispersion forces. Whether or not bacteria attach to a surface depends on which interaction dominates (Morisaki and Tabuchi 2009). Because of their size and surface polymers, bacteria can be treated as soft colloidal particles, with lower surface electric potential than smooth colloidal particles (Marshall 1976). The low surface potential may reduce electrostatic repulsion between bacteria and particle surfaces, and the repulsion is further reduced as ionic
According to Derjaguin-Landaul-Verwey-Overbeek (DLVO) theory, the thickness of the electrical double layer of both particles and bacteria should decrease as the ionic strength increases, promoting the close approach of bacteria to particle surfaces (Lee et al. 2010). For example, the *E. coli* strain S17 showed a lower negative electrophoretic mobility as ionic strength increased from 1 to 1000 mmol L\(^{-1}\) (Janjaroen et al. 2013).

Temperature also has an impact on bacterial attachment by impacting cell properties and surface structures. *E. coli* O157:H7 grown in a nutrient rich medium at 15°C to the stationary phase showed less attachment to abiotic surfaces such as stainless steel, pure titanium, glass, and plastic than those grown at 25°C and 37°C (Tsuji and Yokoigawa 2012). The possible reason could be *E. coli* cells grown at lower temperatures tend to have high motility which may suppress attachment (Turner et al. 1996). Moreover, the temperature shift from 25 °C to 4 °C during growth had a negative impact on attachment to polystyrene surfaces because temperature shifts could induce alterations in the bacterial surface properties which could reduce attachment (Zeraik and Nitschke 2012).

In addition to ionic strength and temperature, bacterial attachment can also be influenced by pH. Environmental pH influences cell surface charge: when the environmental pH is lower than the pzc of the bacteria or particle, the surface would carry more positively charged sites while the surface would carry more negatively charged sites when the environmental pH is higher than the pzc. Mafu et al. (2011) also found that culture medium pH can impact *E. coli* attachment to both hydrophobic and hydrophilic surfaces due to the different expression level of fimbriae and curli under different growth pH.

**6. Concluding remarks**

Bacteria-particle attachment is a complex process involving numerous factors. While some bacterial and particle properties play major roles in the attachment process, others may play minor roles. There is extensive scientific literature addressing this topic, but also conflicting conclusions, making it difficult to identify the most important factors influencing environmental attachment. One reason for the apparent conflicting information is that the factors affecting attachment also affect one another. For this reason, the experimental design of research studies is complicated. To obtain reliable and valuable results, more systematic and standardized methods need to be applied. Another reason for apparent conflicts may be that limited strains of representative bacteria have been studied at one time, leading to a lack of diversity in terms of genotype. It has also been noted that some studies have been conducted with unrealistic conditions of pH or ionic strength and with model particles that do not always reflect the heterogeneity of environmental surfaces.
Future work is needed to focus on the mechanisms and extent of interactions among the different factors that contribute to attachment. Specific recommendations for future work include a comprehensive study of genetic attachment factors and particle characteristics on bacterial attachment under environmentally relevant conditions. Future study should confirm the genetic diversity of the isolate population prior to initiation of experiments. While this review has focused primarily on bacteria used to indicate the presence of waterborne pathogens, similar work is needed to better predict the movement of specific pathogens in the environment.

Acknowledgements

Funding for this work was provided by the National Science Foundation (CBET-1236510) and the Leopold Center for Sustainable Agriculture at Iowa State University (E2012-05). The authors would like to thank Dr. Michael Thompson for his thoughtful comments and suggestions.

Conflict of Interest

None declared.

References


Grismer, M.E. (2006) Vegetative Filter Strips for Nonpoint Source Pollution Control in Agriculture


