Colloids and Surfaces B: Biointerfaces 118 (2014) 126–132

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Characterization of the cell surface properties of drinking water pathogens by microbial adhesion to hydrocarbon and electrophoretic mobility measurements

Jonathan Popovici[a,1], Colin P. White[b,2], Jill Hoelle[c], Brian K. Kinkle[a], Darren A. Lytle[c,*,3]

[a] Department of Biological Sciences, University of Cincinnati, 2100 Clifton Ave., Cincinnati, OH 45220, United States
[b] Pegasus Technical Services Inc., 46 E. Hollister St., Cincinnati, OH 45219, United States
[c] U.S. Environmental Protection Agency, ORD, NMRML, WSWRD, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, United States

ARTICLE INFO

Article history:
Received 10 December 2013
Received in revised form 13 March 2014
Accepted 19 March 2014
Available online 28 March 2014

Keywords:
Electrophoretic mobility
Hydrophobicity
Surface
Pathogen
Drinking water

ABSTRACT

The surface characteristics of microbial cells directly influence their mobility and behavior within aqueous environments. The cell surface hydrophobicity (CSH) and electrophoretic mobility (EPM) of microbial cells impact a number of interactions and processes including aggregation, adhesion to surfaces, and stability of the cells within the aqueous environments. These cell characteristics are unique to the bacterial species and are a reflection of the large diversity of surface structures, proteins, and appendages of microorganisms. CSH and EPM of bacterial cells contribute substantially to the effectiveness of drinking water treatment to remove them, and therefore an investigation of these properties will be useful in predicting their removal through drinking water treatment processes and transport through drinking water distribution systems. EPM and CSH measurements of six microbiological pathogen or surrogate species suspended in phosphate-buffered water are reported in this work. Two strains of *Vibrio cholerae* were hydrophobic, while three strains of *Escherichia coli* were hydrophilic. *Bacillus cereus* was categorized as moderately hydrophobic. The strains of *E. coli* had the highest (most negative) EPM. Based on the measurements, *E. coli* species is predicted to be most difficult to remove from water while *V. cholerae* will be the easiest to remove.

1. Introduction

The cell surface characteristics of microorganisms play an important role in numerous processes including aggregation, adhesion to surfaces, biofilm formation, uptake of chemicals or antimicrobial agents, and stability within aqueous systems [1–6]. Multiple cell surface characteristics contribute to the overall tendencies for microorganisms to interact with their environment. Hydrophobic attractive forces and electrostatic charge interactions at cell surfaces are major contributors [7–11]. Cell surface hydrophobicity (CSH) reflects the tendency for the cell to be attracted to other hydrophobic surfaces, and subsequently leave the aqueous state. Low CSH has been associated with heightened suspension stability of cells within aqueous suspensions, while cells with high CSH have a low affinity for the aqueous environment [12–14]. The CSH of microorganisms can have significant implications on the effectiveness of water treatment processes (e.g. coagulation, flocculation, sedimentation, filtration) to remove pathogenic microorganisms from drinking waters. For example, previous research has demonstrated that higher attachment rates to quartz sand filtration media are associated with hydrophobic bacteria [15]. These findings suggest that the CSH characteristics of microorganisms can be useful in predicting their filtration efficiency in drinking water treatment processes. Additionally, CSH can govern the initial stages of cell adhesion and biofilm development on surfaces [16–18]. It is probable that CSH is involved in these processes on surfaces throughout drinking water distribution systems (e.g. pipe walls, storage tanks, sediment, etc.). Established biofilm in distribution systems can contribute substantially to a reduction in water quality (e.g. taste and odor), corrosion issues, and disinfectant demand [19–22]. There are also concerns that pathogenic species of microorganisms have the capability of

* Corresponding author. Tel.: +1 513 569 7432.
E-mail addresses: popovici.jonathan@epa.gov (J. Popovici), white.colin@epa.gov (C.P. White), lytle.darren@epa.gov (D.A. Lytle).
[1] Tel.: +1 513 569 7386.
[2] Tel.: +1 513 569 7708.
[3] Tel.: +1 513 569 7432.

http://dx.doi.org/10.1016/j.colsurfb.2014.03.033
0927-7765/

colonizing biofilms on various surfaces throughout distribution systems [23]. Researchers have developed a variety of methods for evaluating bacterial CSH including water contact angle measurement (CAM), hydrophobic interaction chromatography (HIC), salt aggregation test (SAT), and microbial adhesion to hydrocarbon (MATH) [24–27]. Of these methods, MATH is one of the most widely used [28]. In the MATH method, a layer of hydrocarbon is added to a suspension of planktonic cells in solution (e.g. aqueous buffer). They are then vortexed for an established amount of time, after which the layers are left to separate. While hydrophobic strains of bacteria will adhere to the hydrocarbon layer and leave the aqueous buffer phase, hydrophilic strains will remain in the aqueous phase. CSH is evaluated by calculating the percent reduction in optical density of the suspension buffer.

The electrostatic charge properties of bacterial cell surfaces are also important in predicting cell interactions within aqueous environments [29]. Many studies have evaluated the relationship between electrostatic properties of bacterial cells and their interactions within a variety of environments. These studies have indicated that the surface charge characteristics of microorganisms are directly related to multiple processes including attachment to a variety of surfaces, stable suspension within aqueous environments, transport through soil and contamination of food products and equipment, as well as virulence and pathogenicity [10,12,30–34]. Although the surface charge of bacterial cells in suspension cannot be directly measured, measurement of the electrophoretic mobility (EPM) reflects the surface charge and can be used to make relative comparisons of surface charge characteristics [35,36]. EPM is defined as velocity of the suspended cells in an applied charge field.

The process of bacterial adhesion to surfaces has been described as a multiphase process consisting of an initial reversible attachment stage that is mediated by the physico-chemical characteristics of the cells, suspension media, and attachment surface [37,38]. Similarly, the stability of the bacterial cells in aqueous environments is also associated with these factors. Later stages consist of surface conditioning and biofilm development, which leads to a state of irreversible attachment [39]. Insight into the initial stages of bacterial attachment to surfaces, bacterial cell flocculation, and other surface-to-surface interactions has been provided through the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloidal stability [40,41]. The classical DLVO theory explains the state of colloidal stability through the balance of the attractive Lifshitz–van der Waals forces, and the generally repulsive electrostatic forces of the surface ion double layer. The processes of coagulation and flocculation can be accomplished through manipulation of surface charge characteristics of the suspended particulates. Rather than increasing ionic strength, a common process utilized in water treatment is the addition of inorganic coagulants that function to neutralize the surface charge of suspended particles, including microorganisms, effectively reducing the energy barrier needed for hydrophobic forces to interact and aggregation to occur.

Marshall et al. suggested that DLVO theory could be applied to modeling the behavior of bacterial suspensions [37]. In such applications, the CSH and EPM represent attractive and repulsive forces, respectively, that are described in the DLVO approach. Numerous studies followed which incorporated the theory in evaluating the mechanisms by which microorganisms adhere to various surfaces, respond to the incorporation of coagulants, and migrate through granular porous media and geological structures [29,33,38,42–47]. Many of the factors influencing colloid coagulation and flocculation are upheld with respect to microbial suspensions, most significant of which are the increases in the rate of cell coagulation and adhesion with a reduction in surface charge [48–50]. These interactions, however, are subject to the unique surface characteristics of microorganisms that allow for variables not readily observed in colloidal particulate suspensions.

Given the importance of the surface properties of microorganisms to drinking water treatment effectiveness, distribution system biofilm development, mobility of microorganisms within the distribution system, and public health, systematic EPM and CSH measurements would be useful in predicting their behavior under those environmental conditions. Although CSH and EPM measurements have been reported for various bacteria of interest, usually only one of the parameters is measured, leaving only a partial expectation of the cell's tendencies. Furthermore, reported measurements of CSH and EPM are conducted in a variety of buffers, and under differing conditions and test protocols. The use of different protocols makes direct comparison of studies nearly impossible. There is an obvious need to systematically measure EPM and CSH of select microbes under controlled conditions. The objective of this study was to measure and report the CSH and surface charge characteristics (EPM) of six microbial pathogens and pathogen surrogates associated with drinking water contamination. Based upon the results, predictions about their tendencies to attach to surfaces and mobility through the aqueous environment will be made. The evaluations of CSH and cell surface charge were performed in various phosphate buffers to evaluate the influence of water chemistry on the indicated parameters.

2. Materials and methods

2.1. Bacterial culture and conditions

Bacterial isolates were chosen based on their importance as drinking water pathogens and surrogates of potential pathogens. Isolates were cultured from the United States Environmental Protection Agency's (EPA) culture collection (EPA, Cincinnati, OH) and consisted of 5 Gram negative strains and 1 Gram positive strain. The Gram negative bacterial strains used in this study consisted of two isolates of *Vibrio cholerae*, ATCC 14033 and C6706. *Vibrio cholerae* C6706 is an El Tor, Inaba strain that was isolated from a patient in Peru in 1991 [51]. Two environmental isolates of Gram negative *Escherichia coli*, EPA 8 and Lye, were used along with an O157:H7 (ATCC 35150) strain. The EPA 8 strain of *E. coli* was originally isolated in June 1997 from a water main break in Louisville, KY. Lastly, a strain of the Gram positive *Bacillus cereus* (ATCC 9592) was used. Culture preparation for the MATH assay and electrophoretic mobility measurements were performed under identical conditions. All cultures were grown aerobically at 35 °C and 100 rpm, shaking for a minimum of 18 h to stationary phase. The growth medium used for all strains was Nutrient Broth (BD Bioscience, Sparks, MD). The cells were then collected by centrifugation (Legend RT, Sorvall Instruments, Du Pont Co., Wilmington, DE) at 4150 × *g* for 15 min. Cultures were washed three times in a buffer specific to each experiment.

2.2. Buffer preparation

Potassium phosphate monobasic (KH_2PO_4) buffer was selected for use in evaluations of CSH and EPM based on its common use within the MATH assay. Experiments were conducted in KH_2PO_4 buffer not only to prevent excessive osmotic pressure on the suspended cells, but also because the suspension buffer used in MATH is constrained by minimum ionic strength requirements [28]. In order to prevent the interference of electrostatic interactions on measurement of CSH, it has been suggested that MATH should be performed at either the isoelectric point of the cells, or in high ionic strength solutions [52,53]. However, for the purposes of this study, measurement of CSH and EPM were conducted over a range of I