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Inhibition of biofilm growth on polymer-MWCNTs composites and metal surfaces

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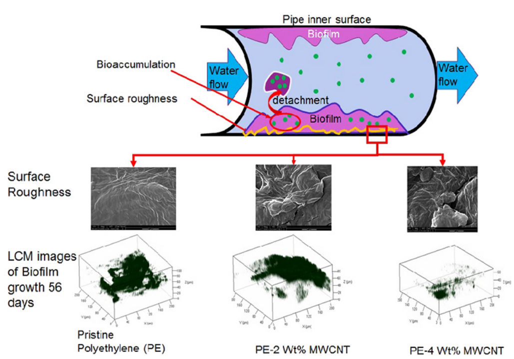
HIGHLIGHTS

- The growth of bacterial biofilms on metal, and nano-polymeric surfaces were studied.
- Surface roughness, hydrophobicity and charge affected bacterial attachment and biofilm growth.
- Carbon nanotube (CNT)-polymer composite surfaces showed lower biofilm growth than pure polymer.
- Stainless steel and CNT-polyolefin surfaces were effective in controlling biofilm growth on the surface.

GRAPHICAL ABSTRACT



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ABSTRACT

There is an increased interest in incorporating multi-wall carbon nanotubes (MWCNTs) into polymer matrices to control the adhesion of bacteria to surfaces and the subsequent formation of biofilm growth on the surface of water pipes, food packages, and medical devices. Microbial interactions with carbon nanotube-polymer composites in the environment are not well understood. The growth of *Pseudomonas fluorescens* (gram-negative) and *Mycobacterium smegmatis* (gram-positive) biofilms on copper, polyethylene (PE), polyvinyl chloride, and stainless steel was compared with growth on MWCNT-PE composites in order to gain insight into the effect of the surface properties of nanomaterials on the attachment and proliferation of microorganism which could result in the engineering of better, non-fouling materials. A statistical analysis of the biofilm growth showed a significant impact of materials for both *P. fluorescens* ($p < 0.0001$) and *M. smegmatis* ($p = 0.00426$). Biofilm growth after 56 days on PE compared to biofilm growth on copper surfaces decreased by 46.4% and 34.9% for *P. fluorescens* and *M. smegmatis*, respectively. Biofilm growth on PE-multiwall-carbon-nanotubes (MWCNTs)-composites surface compared to PE decreased by 89.3% and 29% for *P. fluorescens* and *M. smegmatis*, respectively. Bacterial species ($p < 0.0006$) and surface roughness ($p < 0.0001$) were important factors in determining the attachment and initial biofilm growth rate. The interactions between cells and material surface could be attributed to the complicated and collective effect of electrostatic forces, hydrophobic interactions, and hydrogen/covalent bonding. Further study is needed to determine whether or not there is a difference between the cell attachment in the exponential growth phase and the stationary, or decay, phase cells.

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1. Introduction

Bacterial populations exist in two general forms in an aquatic environment; freely existing in a bulk solution, or planktonic, and as a unit that is attached to a surface or within the confines of a biofilm, or sessile. Biofilms are surface-associated and highly-stratified microbial communities consisting of dense, hydrated clusters of bacteria embedded in a hydrogel matrix of an extracellular polymeric substance (EPS). EPS is composed of extracellular DNA, proteins, and polysaccharides (Sahle-Demessie and Tadesse, 2011; Sheng and Liu, 2011; Stewart and Franklin, 2008; Vu et al., 2009). The development of a biofilm helps microbes to increase their resistance to antimicrobial agents because of a physiochemical feature of the EPS matrix and the transfer of extrachromosomal elements to susceptible organisms within the biofilm (Donlan, 2001; Flemming et al., 2007). Bacterial biofilms create protective conditions that support the regrowth of pathogens, viruses and protozoa. Therefore, their eradication and mitigation are essential to the reduction of medical and public health risks (Batte et al., 2003; Percival et al., 2000). For many industrial sectors, biofilms have been a detriment that has resulted in significant increases in cleaning and maintenance costs. Indwelling medical devices and implants associated with biofilms lead to an increased risk of infection and patient morbidity (Abdallah et al., 2014; Costerton et al., 1999; Donlan, 2001; Flemming and Wingender, 2010). The contamination of food-processing equipment caused by biofilms could cause foodborne illnesses or infections (Abdallah et al., 2014). Biofilms in the drinking water distribution systems are correlated with water quality issues, including color, odor, taste, and the corrosion of pipe materials, which can spur both heterotrophic bacteria and *coliform* regrowth and *Legionellosis* outbreaks (Abdel-Nour et al., 2013; Flemming et al., 2002; Percival et al., 2000; Simoes and Simoes, 2013; Wingender and Flemming, 2011). For these applications, and others, controlling the growth of biofilm is necessary to minimize the potential negative consequences.

Because microbial adhesion and biofilm formation are very dependent on the surface properties of materials (Kanematsu and Barry, 2015; Renner and Weibel, 2011), the selection and modification of materials has emerged as a promising way to control the biofilm in various applications (Kanematsu and Barry, 2015; Percival et al., 2000; Simoes and Simoes, 2013). Many engineered nanomaterials have exhibited biocidal properties and they can be incorporated into larger mesoscopic and macroscopic systems. These composite materials are of particular importance in the biomedical application associated with biofilm prevention on implant and medical device surfaces (Cioffi and Rai, 2012; Diallo et al., 2008; Dror-Ehre et al., 2010; Fabrega et al., 2009; Kim et al., 2007; Secinti et al., 2011; Sheng and Liu, 2011). MWCNT- embedded polymer composites have improved physical, thermal, electrical and mechanical properties (Dong et al., 2012). Polymer-carbon nanotube (CNTs) composites have also emerged as a novel and promising class of nano-enabled materials that exhibit a substantial antimicrobial effect and can be used for a wide range of applications (Allaker, 2010; Cioffi and Rai, 2012; Dong et al., 2012, 2014; Li et al., 2008).

The antimicrobial effect of the CNTs depends heavily on their dispersivity in suspended form (Dong et al., 2012). To maintain the dispersivity of the CNTs, they are blended in to coat porous substrates such as filters, textiles, and membranes. Or, they are added to polymers to form nanocomposites (Cioffi and Rai, 2012; Dong et al., 2014; Upadhyayula and Gadhamshetty, 2010). CNTs have been reported to build highly efficient filters for removing bacteria cells and viruses (Guan and Yao, 2010; Vecitis et al., 2011). There is an increased interest in using polymer-CNT composites to manage health risks associated with biofilms because they can provide the advantage of combining the unique properties of CNTs with the useful properties of polymer materials (Dong et al., 2014). Even though polymer nanocomposites have attracted considerable interest for mitigating biofouling (Yin and Deng, 2015), there are few published studies on their potential applications for minimizing biofilm formation in the drinking water distribution systems. Biofilm growth is influenced by physical, chemical and biological processes that include adhesion to the surface and cell-to-cell cohesion. Biofilms found in the natural environment primarily contain gram-negative bacteria such as *Pseudomonas* and *Flavobacterium*-like species (Flemming et al., 2002; Lechevallier et al., 1987; Percival et al., 1998). These include groups from *Proteobacteria* such as *Pseudomonas aeruginosa* and *P. fluorescens* (Schmeisser et al., 2003). *Mycobacterium* species, which are regarded as one of the primary reasons for pipe encrustations, have also been found in the natural environment. (Du Moulin and Stottmeier, 1986). Both *Pseudomonas* and *Mycobacterium* species are known as opportunistic bacterial pathogens associated with water-borne diseases (Flemming et al., 2002; Percival et al., 2000).

The goal of this study was to gain an understanding of the feasibility of materials selection or modification on controlling biofilm growth. A rigorous evaluation of the potential inhibition of biofilm formation and growth by polyethylene-MWCNT (PE-MWCNTs) composites was conducted. The study compares biofilm growth on pristine polymers with PE containing 2 wt% and 4 wt% MWCNT with growth on copper, polyvinyl chloride (PVC) and 304 stainless steel, which are commonly used materials for water systems. The surfaces of coupons were characterized by using a contact angle goniometer, surface electrokinetic analyzer, Fourier transform infrared spectroscopy (FTIR) and a scanning electron microscope (SEM) to evaluate the impact of surface hydrophobicity, zeta potential, functional groups, and roughness on the biofilm-surface attachment. The role of different surface properties was correlated to the adhesion and growth of two types of biofilms, *P. fluorescens* and *M. smegmatis*. The coupons, which were constructed of the different materials, were kept on the vertical holder of a biofilm reactor. The temporal changes of the biofilm were measured using a confocal laser scanning microscope (CLSM).

2. Materials and methods

2.1. Biofilm cultivation

For this study, *P. fluorescens* and *M. smegmatis* were selected to be cultivated and studied because both bacteria are prevalent and representative biofilms in the natural environment. *P. fluorescens* is a gram-negative, rod-shaped bacillus, acid-fast negative bacteria, whereas *M. smegmatis* is a gram-positive, club-shaped bacillus, acid-fast bacteria. The gram-negative bacteria have an outer membrane which consists primarily of lipopolysaccharides while the gram-positive bacteria have peptidoglycan as its outer layer. Both bacteria form fast-growing biofilms and are readily cultivable in most synthetic or complex laboratory media.

Lyophilized microorganisms of *P. fluorescens* and *M. smegmatis* were obtained from MicroBiologics, Inc. (St. Cloud, MN). The strains of both species were injected and cultivated in the CDC biofilm reactors (continuous stirred-tank reactor) which were acquired from BioSurface Technologies, Inc. (Bozeman, MT, USA). The CDC biofilm reactor has been widely used in biofilms research and is regarded as a promising way of biofilm cultivation and sampling (Martinez-Gutierrez et al., 2013; Pérez-Díaz et al., 2015, 2016). Each of two biofilm reactors has a total volume of 1 L with an effluent spout of liquid volume of 400 mL. Each reactor consists of eight polypropylene coupon holders suspended from a ultrahigh molecular weight polyethylene ported lid. Each coupon holder can accommodate three 12.7 mm diameter coupons, allowing for a total of 24 coupons per batch. The materials represent the most commonly used materials in most process industries and water treatment systems where biofilm formation is vital (Cioffi and Rai, 2012; Percival et al., 2000; Zacheus et al., 2000). The biofilm reactors have glass lids and side-arm discharge ports. The liquid growth media and bacterial strains were circulated through the biofilm reactor using a magnetically driven baffle stir bar. A sampling of the biofilms on the coupons was