Minimal Influence of Material Surface Properties on Initial Bacterial Attachment to Built Environment Surfaces

AUTHOR DETAILS

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ABSTRACT

Biofilms in the built environment (BE) can harbor pathogens and have been linked with negative health outcomes, particularly in hospital environments. The formation of biofilms requires bacterial cell attachment on surfaces, such as hospital plumbing, which can have varying properties, including roughness, wettability, chemistry, and charge. Despite the importance of bacterial attachment to surfaces, the role of multiple surface properties has been minimally investigated. Using seven materials with differing surface characteristics, this work considers the initial attachment of *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis,* and *Staphylococcus aureus* to investigate the impact of several surface characteristics. Initial attachment was evaluated using column experiments and compared to batch experiments in which bacterial growth on coupons was monitored. The attachment of all bacterial species was not influenced by material surface properties, with similar attachment seen across materials tested.

Bacterial cell envelope morphology affected attachment, with gram-negative species displaying greater attachment than gram-positive species. Attachment efficiency (α) was found to be a good predictor of bacterial attachment, with greater sensitivity than batch tests. Establishment of commensal communities should be the focus for limiting pathogens in the BE, as engineering surfaces to reduce microbial attachment appears to offer limited benefit.

KEYWORDS

bacteria, attachment efficiency (α), materials, surface properties, cell envelope morphology

INTRODUCTION

Pathogen presence in the built environment (BE) poses a risk to human health. This is particularly relevant in settings such as hospitals, where hospital-acquired infections represent a significant burden on the healthcare system. The annual cost of hospital-acquired infections is as high as $9.8 billion[1] in the United States healthcare system, and premise plumbing has been shown to support the growth of pathogenic bacteria and contribute to between 7% and 40% of infections[2,3]. This pathogen growth often occurs in the form of biofilms, which can serve as a protective barrier in extreme environments[4]. For example, bacteria established in hospital plumbing may migrate from these biofilm reservoirs, up towards the sink drain, where droplet dispersion during faucet operation can lead to infection[5]. The formation of bacterial biofilm involves several steps: (1) attachment, (2) microcolony formation, (3) matrix formation, (4) macrocolony formation, also known as maturation, and (5) dispersal[6]. The attachment of bacterial

cells to surfaces is therefore an integral step to the establishment and reestablishment of biofilms. The use of antimicrobial chemicals, including those to coat surfaces, may increase the prevalence of antimicrobial resistance[7,8]. The current work explores the impacts of surface properties on initial bacterial attachment, with the possibility of manipulating surface properties to limit bacterial attachment as a chemical-free strategy for combating biofilm formation without increasing antimicrobial resistance.

Bacterial attachment and biofilm formation have been studied previously in various contexts, including medical devices[9,10,11], membrane biofouling[12], and marine biofouling[13]. Surface properties play a role in attachment through attractive (van der Waals) and repulsive (electrostatic) forces. Although numerous studies have investigated the impact of material properties on bacterial attachment or adhesion, most of these studies have focused on the impact of a single surface property, and efforts to assess the impact of multiple surface properties are essential to advance knowledge[14]. Environmental sampling of the BE has largely failed to consider the impact of any material property on the microbes of the BE, let alone multiple properties[15]. This further amplifies the need to understand the collective impact of multiple surface properties on bacterial attachment and the microbiome of the BE.

In previous studies, the quantification of bacterial adhesion or attachment has been studied using batch tests, where bacteria were put in contact with materials for a defined time period, and cells remaining on the materials' surface after rinsing were quantified. While such an approach yields useful information concerning the tendency of bacteria to establish themselves on a given surface, it does not provide kinetic information on the attachment process and is far from real-life conditions (i.e., fluid dynamics, high contact surfaces, limited nutrients). Alternatively, the concept of attachment efficiency has been used extensively in colloid science to quantify the affinity of

70 particles for surfaces in a kinetic context, where α can have values between 0 and 1, and describes
71 the likelihood of a particle attaching upon interacting with a surface. This approach has been used
72 for predicting particle removal in water treatment[16,17,18], bacterial transport in bioremediation[19],
73 and the fate and transport of nanomaterials[20,21,22,23]. Surface attachment efficiency, α, is the ratio
74 between the number of successful attachments and the number of collisions with the surface and
75 is typically a function of surface properties (of both the particle and the surface) and environmental
76 conditions. Combined physical and hydrodynamic models for particle motion, α, can be used to
77 determine rates of attachment in porous media[24]. It has also been used to determine aggregation
78 rates[25], with applications in natural[26,27] and engineered systems[28].

79 In this study, we explore the initial attachment of bacteria to relevant, well-characterized
80 BE materials to fill the gap in assessing multiple surface characteristics' impact on bacterial
81 attachment. We used model bacteria (*Escherichia coli* K-12 and *Bacillus subtilis* ATCC1174) and
82 environmentally isolated bacteria (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) to
83 investigate their attachment to seven BE materials (acrylonitrile butadiene styrene, high-density
84 polyethylene, high-impact polystyrene, polycarbonate, polyvinyl chloride, polyvinylidene
85 fluoride, and stainless steel) in representative water chemistry.

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88 **MATERIAL AND METHODS**

89 **Material Preparation**

90 Pre-production pellets of six polymers: acrylonitrile butadiene styrene (ABS), high-density
91 polyethylene (HDPE), high-impact polystyrene (HIPS), polycarbonate (PC), polyvinyl chloride
92 (PVC), and polyvinylidene fluoride (PVDF); and stainless steel (SS) spheres were used in this

work. ABS, HDPE, HIPS, PC, and PVDF were purchased from McMaster Carr (Elmhurst, Illinois). PVC was purchased from Alphagary (Pineville, North Carolina). SS was purchased from MSE Supplies (Tucson, Arizona). To prepare the materials, 250mL batches of pellets/spheres were washed in 70% ethanol. After ethanol washing, the pellets/spheres were rinsed thoroughly with deionized water three times. Pellets/spheres were then air dried in a laminar flow hood and stored in an autoclaved, airtight container until use.

Material Characterization

The water contact angle (WCA) was measured to determine the surface wettability of the materials, using the sessile drop method with an optical tensiometer (Attension Theta Flex, Biolin Scientific; Phoenix, Arizona). Surface roughness was measured by 3D optical/ laser confocal profilometry (VK-X3000, Keyence; Itasca, Illinois) using a 50x objective, with images 208µm × 278µm collected for each material type. For cylindrical pellets (ABS, HIPS, and PC), images were taken of both the flat surface (top of cylinder) and the curved surface (lateral surface). The roughness of cylindrical pellets (ABS, HIPS, and PC) is reported as the weighted average of the flat and curved surfaces of the materials, assuming the flat surface covers one-third of the total surface area, and the curved surface covers the other two-thirds of the total surface area. The surface charge was measured as zeta potential using a solid surface zeta potentiometer (SurPass, Anton Paar; Austria). The surface charge was measured across a pH range from 2.7 to 7.8, a Boltzmann curve was fit to the zeta potential data, and the zeta potential at pH 7.5 was determined. Fourier transform infrared spectrophotometry (FTIR) (Nicolet iS50, ThermoFisher Scientific; Waltham, Massachusetts) was used to evaluate surface functional groups. Thirty-two scans were

performed over the spectral range of 3600-400 $cm^{-1,}$ and spectra processing was performed using Spectragryph[29].

Bacterial Preparation and Characterization

The four model organisms used in this study were *Escherichia coli* K-12 (*E. coli*), an environmental isolate of *Pseudomonas aeruginosa* (*P. aeruginosa*) from a hospital sink P-trap, *Bacillus subtilis* ATCC 1174 (*B. subtilis*), and an environmental isolate of *Staphylococcus aureus* (*S. aureus*) from a hospital sink basin. The model organisms were grown in liquid cultures in Lennox Luria Broth (LB) media (Sigma-Aldrich; St. Louis, Missouri) to a stationary phase at 37°C with shaking. After reaching the stationary phase, the liquid cultures were centrifuged in 50mL tubes at 3000xg and 4°C for 10 minutes. The nutrient media was aspirated from the tubes, and the pelleted bacteria were resuspended in EPA Moderately Hard water (EPA Mod Hard).

Cell surface charge was measured by zeta potential, calculated from electrophoretic mobility measurements using a Malvern Zetasizer Nano ZS (Malvern; United Kingdom). Cell hydrophobicity was determined using water contact angle measurements using the sessile drop method with an optical tensiometer (Attension Theta Flex, Biolin Scientific; Phoenix, Arizona)[30].

Bacterial Attachment in Dynamic Column Conditions

The column testing setup was modified from Rogers et al.[24] and was organized as follows: dry pellets/spheres were packed into a glass column (25mm x 150mm, Diba Omnifit EZ Chromatography Column, Cole Parmer; Vernon Hills, Illinois) to the 11mm line. Deionized water

was pumped into the column from the bottom by a syringe pump until the column was filled with water. The column effluent passed into an in-line ultraviolet-visible spectrophotometer (Evolution 201 UV-visible Spectrophotometer, CAT# 912A0883, ThermoFisher Scientific; Waltham, Massachusetts). The bacteria sample was passed directly into the spectrophotometer, bypassing the column by means of T-connectors, to obtain a maximum absorbance at 600nm for the sample (the initial bacteria concentration, C_0). The tubing was flushed with deionized water to remove all of the bacteria from the system. Then, roughly 3 pore volumes of the background solution (EPA Mod Hard) were passed through the column to equilibrate the packing material with the background. The bacteria sample was passed through the column at a flow rate of 2.95mL/min, and the absorbance was recorded at approximately every 10 seconds for at least 4 pore volumes.

A tracer study using potassium nitrate was performed to confirm the integrity of the column. Column experiments were also carried out using positively charged aminated 1μm silica particles to calculate a. The details of the tracer study and positive particle column experiments are included in the Supporting Information.

Calculation of Attachment Efficiency (α)

Colloid filtration theory was used to describe bacterial attachment using α. α can be expressed as a function of experimentally observed concentrations of particles in the column effluent (C) and influent (C_0)[31]:

$$\alpha = -\frac{2}{3}\left(\frac{d_c}{(1-\varepsilon)L\eta_0}\right)\ln\frac{C}{C_0} \quad (1)$$

where d_c is the diameter of the collector (pellet/sphere), ε is the porosity of the column, L is the column length, and η_0 is the predicted single-collector contact efficiency.

Using the aminated 1μm silica particles as an assumed α = 1 particle, due to their positive charge, the value of α was determined using Eq. 2, with the values of C/C_0 taken from the steady-state phase of the column data. Experiments for α were conducted in duplicate, and the standard error is reported to compare between experiments.

$$\alpha = \frac{\ln \frac{C}{C_{0_{bacteria}}}}{\ln \frac{C}{C_{0_{silica}}}} * \left(\frac{1}{\frac{L_{bacteria}}{L_{silica}} * \frac{1 - \varepsilon_{bacteria}}{1 - \varepsilon_{silica}}} \right) \quad (2)$$

Bacterial Attachment in Static Batch Conditions

Batch attachment experiments were carried out as an additional method for determining the attachment of the model bacteria to the materials tested[32,33]. A known mass of the test material was incubated with 2mL of the bacterial solution in 5mL glass test tubes at room temperature on an orbital shaker at 150rpm for 10 minutes. After the incubation period, the bacterial solution was removed from the test tube, the material was rinsed with 1× phosphate-buffered saline (PBS) (Corning; Manassas, Virginia) three times to remove planktonic bacteria, and the material was placed into a 15mL centrifuge tube containing 5mL of PBS. Then, the material was vortexed for 1 minute, bath sonicated for 10 minutes, and vortexed for 1 minute to remove attached bacteria. The removed bacteria were quantified by single plate serial dilution spotting (SP-SDS)[34,35].

Statistical Analysis

Statistical analysis was conducted for the α results as a function of material and organism. To test for significance, Kruskal-Wallis and subsequent Dunn's multiple comparisons tests were

184 performed in R for changes in material and organism. The tabulated results of the statistical tests
185 are included in the Supporting Information.
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188 **RESULTS AND DISCUSSION**
189 **Characterization of Material Surface Properties**
190 The materials were selected for their range of expected properties and prevalence in the
191 BE. HDPE and PVC are commonly used for pipe materials, in both distribution systems and
192 premise plumbing[36]. PVDF is used in high-purity water systems, such as Ultrapure water, due to
193 its chemical and biological stability[37]. ABS is used in drain-waste-vent systems, which include the
194 drainpipes from sinks and other plumbing in the BE[38]. HIPS is found in many plastic surfaces in
195 the BE, particularly in packaging applications[39]. PC is another common plastic material in the BE,
196 with more resistance than HIPS[40]. SS was selected as a control material and is often used due to
197 its corrosion-resistant properties[39].
198 The materials were characterized for several surface properties to identify any differences
199 that may influence bacterial attachment, and as shown in Table 1, the materials studied presented
200 different surface properties. The properties that have been the focus of previous studies
201 investigating bacterial attachment to surfaces are water contact angle[41,42], roughness[42,43], and
202 surface charge[10,44].
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Table 1: Material Properties and Surface Characteristics

Material	WCA[a] (°)	Roughness[a] (nm)	Surface Charge at pH 7.5 (mV)	Surface Functional Groups by FTIR
ABS	92.2 ± 4.5	503.9 ± 160.2	-8.6	C-H; C-H_2; C=C; C≡N
HDPE	108.9 ± 2.3	769.0 ± 474.7	-13.2	C-H_2
HIPS	107.2 ± 6.8	258.2 ± 62.7	-7.9	C-H; C-H=C-H_2; C-H; C=C
PC	78.2 ± 2.9	266.0 ± 167.3	-9.8	C-O; C=O; C-H; C-O-C; O-C-O
PVC	89.4 ± 3.7	156.6 ± 37.3	-12.0	C-H; C-H_2; C-Cl
PVDF	95.4 ± 4.2	405.4 ± 124.5	-9.1	C-H; C-H_2; C-F; C-F_2
SS	49.9 ± 3.3	50.9 ± 9.9	-18.7	-

[a]The error is the standard deviation for all measurement methods.

Surfaces with water contact angles greater than 90° are generally considered hydrophobic, while those with water contact angles less than 90° are generally regarded as hydrophilic[45]. The selected materials range from hydrophilic (SS) to hydrophobic (HDPE and HIPS), with several materials falling near the 90° threshold. Surface roughness ranged from very smooth (SS = 50.9 ± 9.9 nm) to rough (HDPE = 769 ± 474.7 nm). The standard deviation for the roughness measurements describes the heterogeneity of the surfaces, with regions of particularly high roughness visible on some surfaces (Figures S.3.1-S.3.2). The surface charge was negative for all tested materials, ranging between –7.9 mV and -18.7 mV. The surface chemistry was measured using FTIR, and the FTIR spectra are provided in Figure S.6.1 in the Supporting Information. The plastics evaluated in this study (ABS, HDPE, HIPS, PC, PVC, and PVDF) exhibited a range of functional groups, consistent with their polymer composition. ABS, HDPE, and HIPS surfaces showed non-polar groups, with ABS also showing nitrile (C≡N) groups associated with

222 acrylonitrile. PC presents more oxygen-containing surface functionality that makes the surface
223 more polar, which corresponds with the lower water contact angle than the other plastics. PVC and
224 PVDF surfaces are chemically distinct, with the presence of chlorine and fluorine on the surface,
225 respectively. Stainless steel lacks organic functional groups, explaining the lack of signal found
226 from the FTIR measurement of SS. Interestingly, the material properties measured in this study
227 showed that while surface charge was similar across materials, the most hydrophilic material (SS)
228 had the lowest surface roughness, while the material with the greatest roughness (HDPE) was the
229 most hydrophobic.
230
231
232 **Effect of Material Surfaces on Initial Bacterial Attachment**
233 The parameter α was used to evaluate the initial attachment of bacteria to the selected BE
234 materials. Two lab-derived bacteria (*E. coli* and *B. subtilis*) and two bacteria isolated from the
235 hospital environment (*P. aeruginosa* and *S. aureus*) were selected for this study to compare the
236 initial attachment behavior to bacteria with different cell morphology (Gram-negative vs. Gram-
237 positive), and different pathogenicity (lab-derived vs. hospital isolates).
238 To determine the value of α for bacterial cells attaching to BE materials, column
239 experiments were performed for all four bacterial species using the seven different characterized
240 materials. Breakthrough curves, where the bacterial cells become observable in the column
241 effluent, are shown in the Supporting Information. The attachment efficiency to the seven materials
242 was evaluated for all four bacterial species (*E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*). The
243 measured normalized concentrations (C/C_0) and calculated α values are shown in Table 2. For all
244 materials and bacteria, α is low with values below 0.02.
245

246 **Table 2:** Calculated Values of α for Four Bacterial Species to Various Materials

Bacteria	Material	C/C_0[a]	α[a,b]
E. coli	ABS	0.84 ± 0.054	0.018 ± 0.0066
	HDPE	0.86 ± 0.046	0.016 ± 0.0055
	HIPS	0.83 ± 0.027	0.015 ± 0.0022
	PC	0.87 ± 0.039	0.015 ± 0.0054
	PVC	0.86 ± 0.023	0.014 ± 0.0026
	PVDF	0.88 ± 0.041	0.019 ± 0.0067
	SS	0.91 ± 0.014	0.012 ± 0.0021
P. aeruginosa	ABS	0.84 ± 0.0074	0.017 ± 0.0015
	HDPE	0.86 ± 0.0038	0.016 ± 0.00087
	HIPS	0.84 ± 0.0030	0.014 ± 0.00042
	PC	0.87 ± 0.0034	0.015 ± 0.00013
	PVC	0.86 ± 0.040	0.014 ± 0.0043
	PVDF	0.90 ± 0.0045	0.015 ± 0.00062
	SS	0.87 ± 0.0071	0.019 ± 0.0011
B. subtilis	ABS	0.98 ± 0.014	0.0016 ± 0.0014
	HDPE	0.97 ± 0.013	0.0031 ± 0.0012
	HIPS	1.00 ± 0.0016	0.00012 ± 0.00012
	PC	0.98 ± 0.017	0.0019 ± 0.0019
	PVC	0.99 ± 0.00056	0.0012 ± 0.000087
	PVDF	0.94 ± 0.037	0.0089 ± 0.0053
	SS	1.00 ± 0.0033	0.00045 ± 0.00045
S. aureus	ABS	0.96 ± 0.0024	0.0043 ± 0.00024
	HDPE	0.95 ± 0.0011	0.0051 ± 0.000078
	HIPS	0.97 ± 0.0051	0.0026 ± 0.00046
	PC	0.93 ± 0.031	0.0089 ± 0.003
	PVC	0.96 ± 0.0046	0.0041 ± 0.00051
	PVDF	0.93 ± 0.018	0.0099 ± 0.0027
	SS	0.95 ± 0.0083	0.0071 ± 0.0012

247 [a]The error is the standard error based on duplicate measurements. [b]α values were compared for

248 columns of the same bacterial species.

The low attachment found during this study can be explained by repulsive electrostatic forces between negatively charged surfaces and negatively charged bacterial cells[46,47]. Column experiments and attachment efficiency (α) have been used previously to evaluate the transport and attachment of bacteria in porous media[48,49,50], and these studies have found higher attachment than this study. McCaulou et al. found α values between 0.04 and 0.4 for the interaction between bacteria and different quartz surfaces[48]. The largest α values found were between negatively charged bacteria and coated surfaces with a positive charge. Deshpande et al. showed that *P. fluorescens* had an α value of 0.094 when interacting with silica surfaces[49]. Increased ionic strength increased the attachment of *P. fluorescens*, up to 0.206 at ionic strength of 3 x 10^{-2}M, which has also been seen with attachment of other biological particles[24,50].

Usually, hydrophilic surfaces, with increased wettability, led to increased attachment in previous studies. Bruzaud et al. found that more wild-type *P. aeruginosa* cells attached to a stainless steel surface with WCA of 61° (attached cells ≅ 9 x 10^4 CFU/cm^2) than to a superhydrophobic stainless steel with WCA of 147° (attached cells ≅ 3 x 10^3 CFU/cm^2)[41]. Similarly, Liu et al. found increased attachment of *E.* coli on hydrophilic titanium (WCA = 42.0°) and stainless steel (WCA = 65.8°) surfaces, compared to hydrophobic Ni-P-PFTE surfaces (WCA 108.0° and 117.8°)[51]. Conversely, another study found that the near-wall velocity of bacteria was reduced when interacting with hydrophobic surfaces, promoting adhesion to the surface[52]. Rough surfaces have also been shown to lead to increased microbial attachment. Mu et al. showed that bacterial densities were low on smooth surfaces, with values between 2-3 x 10^{-2} cells/µm^2, but as roughness increased, bacterial density increased to 5-10 x 10^{-2} cells/µm^2 [42]. Bohnic et al. found that for surfaces with roughness ranging from 0.07µm to 5.8µm, attachment of bacteria, measured as crystal violet (CV) absorbance, increased[43]. For *E. coli*, CV absorbance increased from 0.0267

± 0.024 to 0.3117 ± 0.054. This trend was also seen for *P. aeruginosa* and *S. aureus*, with increases from 0.0517 ± 0.072 to 0.9417 ± 0.11, and 0.0537 ± 0.019 to 0.2697 ± 0.068, respectively. Yang et al. found a positive correlation between bacterial attachment and surface roughness above a threshold of 6 nm[53]. However, according to Kruskal-Wallis tests, none of the α values were significantly affected by changes in material (Tables S.4.1-4.5.8). Additionally, when bacterial attachment is plotted against the surface properties of the material, no trend is observed. The results showed that material properties in this study did not significantly impact initial bacterial attachment, contrary to previous studies.

Effect of Bacterial Cell Properties on Initial Attachment

The cell surface charge and hydrophobicity of the four bacterial species were measured, and the results are shown in Table 3, along with the cell envelope morphology and pathogenicity.

Table 3: Bacterial Cell Surface Characteristics and Pathogenicity

Species	Cell Surface Charge[a,b] (mV)	Contact angle (Hydrophobicity)[b] (°)	Cell Envelope Morphology	Pathogenic
E. coli	-30.12 ± 0.56	16.85 ± 1.67	Gram –	No
P. aeruginosa	-9.60 ± 0.50	38.28 ± 6.72	Gram –	Yes
B. subtilis	-31.74 ± 0.35	29.57 ± 1.17	Gram +	No
S. aureus	-21.37 ± 0.38	17.97 ± 0.59	Gram +	Yes

[a]Cell surface charge measured as zeta potential. [b]The error is the standard deviation for all measurement methods.

Figure 1 shows the results for α compared between bacterial species when pooled across all seven materials. There are statistically significant differences in α between both Gram-negative species (*E. coli* and *P. aeruginosa*) and both Gram-positive species (*B. subtilis* and *S. aureus*). The α results did not show a dependence on any of the other bacterial cell properties (surface charge, hydrophobicity, pathogenicity). The initial attachment efficiency of the Gram-negative species was found to be higher than the initial attachment efficiency of the Gram-positive species by an order of magnitude (0.016 and 0.016 compared to 0.0025 and 0.0060).

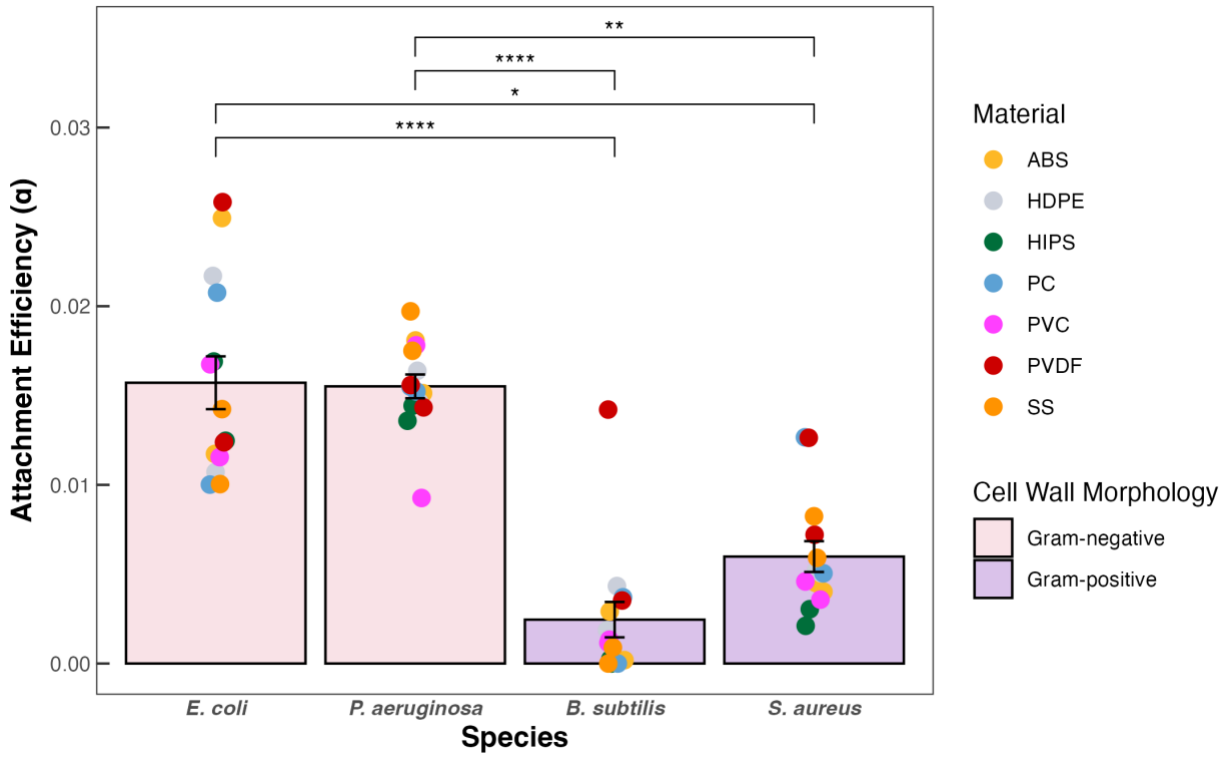

Figure 1: Average bacterial attachment efficiencies for different bacterial species across all materials tested. The error bars are the standard error for 14 measurements.

Gram-positive bacteria have a thick peptidoglycan layer that increases the rigidity of their cell envelope[54]. This rigidity may limit initial attachment due to an inability of the cell envelope

304 to adapt to nanoscale roughness present on the surface, leading to sub-optimal surface contact. The
305 rigid peptidoglycan layer of Gram-positive bacteria limits mechano-selectivity towards a surface,
306 compared to the more sensitive surface structures of Gram-negative bacteria[55]. The effect of cell
307 envelope rigidity was also shown in a study comparing the effect of zero-valent iron, where the
308 envelope of Gram-negative bacteria was more susceptible to the zero-valent iron adhering to the
309 cell membrane than that of Gram-positive bacteria[56]. Additionally, Gram-negative bacteria have
310 external lipopolysaccharides and adhesin proteins on their cell walls, which facilitate attachment
311 to surfaces[57].
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314 **Comparing Batch and Column Experimental Approaches for Measuring Bacterial**
315 **Attachment to Surfaces**
316 Column experiments have been utilized to evaluate the transport and attachment of bacteria
317 in porous media[48,49,50], but not, to our knowledge, to assess attachment to BE materials. The
318 attachment of *E. coli* and *B. subtilis* was evaluated using batch tests and compared to attachment
319 determined using column experiments. Comparing these two methods allowed us to place our
320 results in context with previous approaches and assess the suitability of column experiments for
321 determining initial attachment in the BE.

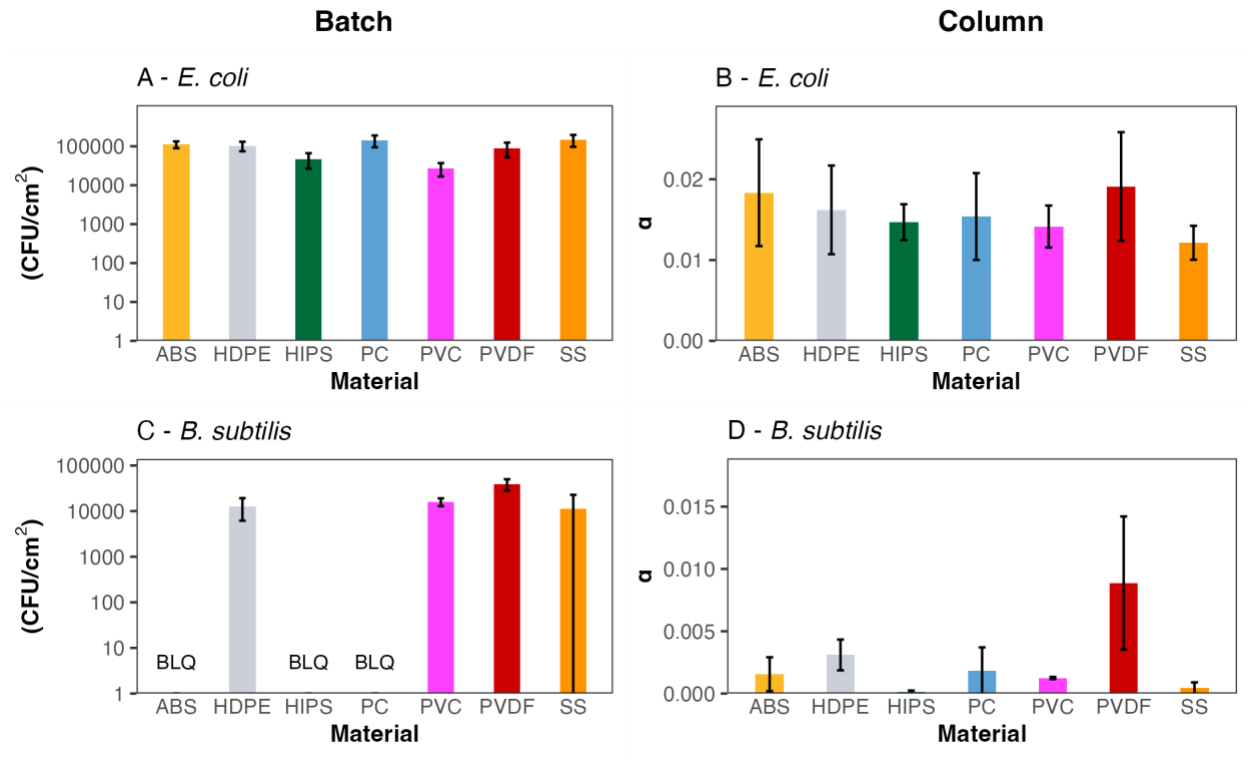

Figure 2: Attachment results from batch experiments (left panels) and column experiments (right panels). Panels A and B are results for *E. coli,* and Panels C and D are results for *B. subtilis*. The error bars for the batch experiment are the standard error for triplicate measurements. The error bars for the column experiment are the standard error for duplicate measurements. BLQ - below the limit of quantification, defined as 6 colonies for the SP-SDS method[34,35].

The results of attachment from batch experiments are similar to the results found by the column experiments when comparing attachment across the tested materials (Figure 2). No statistical difference in attachment was found between materials for both experimental methodologies, for both *E. coli* and *B. subtilis*. Interestingly, the column experiments had a higher sensitivity and were able to determine the attachment efficiency of *B. subtilis* for all seven materials, while the batch experiments were below the limit of quantification for three materials. Although the batch and column experiments had similar contact time between material and

bacteria, 10 minutes and approximately 7 minutes, respectively, the batch experiments involved no flow. Furthermore, the batch experiments involved a limited surface area for bacteria-surface interactions (1.19 ± 0.551 cm^2) compared to the column experiments (766 ± 188 cm^2). These factors, along with the real-time monitoring provided by the in-line spectrophotometer, are hypothesized to be reasons for increased sensitivity of the column experiments when compared to the batch tests. Previous batch tests have found similar numbers of attached cells compared to this study. Results for *B. subtilis* showed ~5.1 x 10^4 CFU/cm^2 and ~4.6 x 10^4 CFU/cm^2 attached to PE and PVC, respectively[32]. However, results for *E. coli* were lower than those for *B. subtilis*, contradicting the trend found in this study.

The comparison between column experiments and batch experiments, shown in Figure 2, indicates that column experiments and the calculation of attachment efficiency are an effective method for testing bacterial attachment to BE materials. An additional advantage of the column experiments is the ability to test attachment under flow conditions, which is particularly important for aqueous systems with flow, as it has been shown that adhesion is strengthened by shear stress[58]. Utilizing column experiments to evaluate bacterial attachment has its advantages but also limitations. Materials to be tested in this system must fit into a small diameter column to provide a high surface area to volume ratio, which may limit the variety of materials available for testing. Additionally, calculating α assumes a spherical collector material[59]– in our study, plastic pellets or stainless steel – further limiting the selection of materials upon which bacterial attachment can be evaluated using this methodology.

CONCLUSION

Overall, these results suggest that initial bacterial attachment to BE materials is uniformly low, with little to no dependence on any of the physico-chemical properties of the surfaces as characterized in this work. The initial kinetics of bacterial attachment appear to be much more dependent on the nature of the cell envelope (morphology, gram-negative vs. gram-positive). Column experiments and determination of attachment efficiency (α) can be an effective method to compare initial attachment across different bacterial species, particularly when the impact of fluid flow is relevant. Manipulating material properties to limit initial bacterial attachment may not be an effective strategy for reducing biofilm formation and the presence of bacterial pathogens due to the limited differences in material seen in this study. Future directions for limiting bacterial pathogen presence in the BE should focus on the establishment of beneficial bacterial communities, along with the effect of material properties on biofilm formation and cleaning efficiency.

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AUTHOR CONTRIBUTIONS

CRediT: **Kobi Talma:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing; **Nathan Bossa:** Conceptualization, Methodology, Supervision, Writing – review & editing; **Evan Hankinson:** Investigation; **Lijia Gao:** Visualization, Writing – review & editing; **Aicha El Kharraf:** Investigation, Writing – review & editing; **Mark Wiesner:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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The authors declare no competing financial interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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