

Adsorption kinetics of *Escherichia coli* and *Staphylococcus aureus* on single-walled carbon nanotube aggregates

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ABSTRACT

Batch adsorption studies to determine adsorption kinetics of *Escherichia coli* (*E.coli*) K12 and *Staphylococcus aureus* (*S.aureus*) SH 1000 bacterial cells on single-walled carbon nanotube aggregates were performed at two different initial concentrations. The diffusivity of *E. coli* cells in single-walled carbon nanotube aggregates obtained was 6.54×10^{-9} and 8.98×10^{-9} cm²/s, whereas that of *S. aureus* was between 1.00×10^{-7} and 1.66×10^{-7} cm²/s respectively. In addition to batch adsorption studies, electron microscopy studies were also conducted. The results suggest that diffusion kinetics of bacterial cells is concentration dependent as well as bacteria dependent. Diffusivity of *S. aureus* is two orders of magnitude greater than *E. coli* cells. This proves to be beneficial from an adsorption perspective where it is desired to filter microorganisms (water pretreatment and wastewater post treatment) and from nanotube biosensor perspective where it is desired to simultaneously capture and detect biothreat agents in a shorter span of time.

Key words | carbon nanotubes, diffusivity, microbial adsorption, ultramicrotomy, water treatment

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INTRODUCTION

Bacterial adsorption studies on single-walled carbon nanotubes provide an insight on developing treatment-based and sensor-based applications in water and wastewater treatment industry. There are three major areas pertaining to water and wastewater plants where the use of carbon nanotube adsorption technology is foreseen. First and foremost, carbon nanotubes can be used as biosensors to concentrate and detect biothreat pathogens in drinking water treatment systems. In recent years, drinking water treatment plants have become highly susceptible to bio-terrorism attacks (Nuzzo 2006). When a biothreat pathogen is introduced into a drinking water system, there is a likely chance that it is not detected with existing systems (Horman 2005; Nuzzo 2006). This is due to the fact that current detection systems are purely designed to detect sewage-based

contaminations and not the biothreat pathogens (Nuzzo 2006). Moreover, most of the biothreat pathogens are colourless, odourless and tasteless (Horman 2005), which makes their detection almost impossible. Thus it is important to deploy a sensor comprising of a material such as single-walled carbon nanotubes which can concentrate and detect the pathogens almost instantaneously. Another application of single-walled carbon nanotubes is to detect upsets caused by toxic contaminants upfront in a wastewater treatment plant (D'Souza 2001; Timur *et al.* 2007). A certain indicative strain of bacteria is made to adsorb on the surface of carbon nanotubes and the corresponding activity of the adsorbed bacteria is monitored to detect the presence of toxic materials (Timur *et al.* 2007). Finally, single-walled carbon nanotube filters can possibly be used as seawater pretreatment to

remove bacteria from raw seawater and thereby reduce biofouling problems of reverse osmosis membranes.

Single-walled carbon nanotubes possess antimicrobial properties (Lee *et al.* 2004; Kang *et al.* 2007) that enable them to effectively concentrate and deactivate pathogens from contaminated water. When bacteria come in physical contact with nanotubes, they penetrate through the cell membrane, disrupt its activity and eventually destroy the cell viability (Lee *et al.* 2004; Narayan *et al.* 2005; Kang *et al.* 2007). It was proposed that the cylindrical shape of nanotube fibres coupled with a high aspect ratio are mainly responsible for the death of bacterial cells. (Kang *et al.* 2007). Typically fibrous media provide a larger accessible surface area for bacterial adsorption than powdered or granular adsorbents and, the higher the aspect ratio, the higher is the accessible surface area (Suzuki 1991; Kang *et al.* 2007). Single-walled carbon nanotubes have aspect ratios greater than 2000 (Ounaies *et al.* 2003) and are expected to adsorb bacteria efficiently. Srivatsava *et al.* (2004) provided qualitative confirmation that microorganisms are adsorbed on carbon nanotubes. Gu *et al.* (2005), Huang *et al.* (2004) provided the basis for functionalisation of carbon nanotubes using carboxylated ligands and antibodies to detect pathogens.

In real time applications in water treatment plants, pathogenic bacteria must have faster adsorption kinetics. When a biothreat pathogen is introduced in treated water, it is required to concentrate and detect the pathogen almost instantaneously. Thus it is important to measure diffusivities of bacterial strains adsorbed on carbon nanotubes. The present study is initiated to determine adsorption kinetics of *E. coli* and *S. aureus* on single-walled carbon nanotube aggregates at two different initial concentrations. Diffusivities of these microbes in single-walled carbon nanotubes are also measured. We have also performed transmission electron microscopy (TEM) and ultramicrotomy analysis of the adsorbed bacteria to visualise the binding and association of the bacterial cells onto the carbon nanotubes. Ultramicrotomy is widely used to image bacterial cell characteristics. Recently, the ultramicrotomy technique was extended to study the morphology of non-biological specimens such as rubber latex (Subramaniam *et al.* 2004). Ultramicrotomy may work well to visualise the association between large biological entities such as bacteria and mesoporous fibrous materials like carbon nanotubes.

The information on the association of bacteria with carbon nanotubes is important in designing a biosensor for specific applications in water and wastewater treatment plants.

MATERIALS AND METHODS

Adsorbent

The single-walled carbon nanotube samples (AP-Grade) were purchased from Carbolex, Inc. (Lexington, KY, USA), a commercial supplier of carbon nanotubes for research and development. These carbon nanotubes are synthesised with an arc-discharge method and are close ended nanotubes with an average diameter of 1.4 nanometres and lengths of 2–5 μm . The reported average size of single-wall carbon nanotube aggregates using similar arc-discharge technique is approximately 20 μm (Qian *et al.* 2003; Yu *et al.* 2003). The as received single-wall carbon nanotube adsorbent samples have a purity of $\sim 80\%$ carbon nanotubes with $\sim 10\%$ of catalyst impurities including nickel and yttrium and $\sim 10\%$ amorphous carbon.

Batch adsorption studies

Fresh bacterial cultures of *E. coli* and *S. aureus* are prepared from their respective stock cultures. The cultures were prepared in tryptic soy broth solution in sterile glass tubes. The growth of the bacteria is confirmed with a spectrophotometer by measuring the optical density of the solution. The optical densities of *E. coli* and *S. aureus* solutions were between 1.8 and 2.0. This freshly prepared bacterial solution is subsequently used for shaker experiments. Prior to the shaker experiments, the concentrated bacterial solution is aliquoted in 1.5 mL eppendorf tubes and centrifuged for 2 minutes. The supernatant broth solution is removed and the bacterial pellet formed at the bottom of the tubes is suspended in 0.85% saline solution and vortexed for 2 minutes. The same procedure of centrifuging and vortexing is repeated twice more to remove the broth content from the cells. The cells were prepared to be used for shaker studies and microscopic studies.

For shaker experiments, 100 mL of distilled water each (autoclaved) is taken in four flasks (duplicate samples two for *E. coli* and two for *S. aureus*) and bacterial solutions of

E. coli and *S. aureus* are added to the flasks. A blank (without addition of nanotubes) is taken as a control to determine the initial concentration of bacteria in the solution. The bacteria concentration of *E. coli* in the two flasks was approximately 4.5×10^8 CFU/ml (colony forming units) and that of *S. aureus* was 4.4×10^8 CFU/mL. To this solution, 0.1 g of carbon nanotubes is added to each flask and the flasks are mounted on a mechanical shaker and the contents are shaken at 6,000 rpm. One mL of supernatant is drawn at regular intervals and filtered via $2\ \mu\text{m}$ polycarbonate filter (Millipore, MA, USA). Then, 100 μL of the filtrate sample was taken and diluted to several orders less than the initial concentration. The dilution factor was varied from 10^{-1} to one factor less than the initial concentration, i.e. if C_0 is of the order of 10^7 CFU/mL, the upper dilution factor used to for plating is 10^{-6} and if C_0 is of the order of 10^{-6} CFU/mL, the upper dilution factor is 10^{-5} . From each diluted sample, 20 μL of sample was taken and inoculated three times on selective agar plates. McKonkey agar is used to enumerate *E. coli* cells and mannitol salt agar is used for *S. aureus* cells. The plates were then incubated at 37°C for 24 hours. The number of colonies grown on the plates was enumerated. The total number of colonies was counted according to the following equation:

$$\text{Number of colonies} = \frac{(\text{number of colonies for each dilution})}{[(\text{CFU})/\text{mL}]} = \frac{(\text{number of colonies for each dilution})}{(\text{dilution factor})/(\text{sample volume})}$$

The same experiment was repeated once more, both for *E. coli* and *S. aureus* with different initial concentrations.

Electron microscopy analysis

One mL of bacteria solution adsorbed on carbon nanotubes (not the supernatant) sample is taken from each flask after conclusion of the batch adsorption experiment and collected into four 1.5 mL eppendorf tubes. Two eppendorf tubes contained *E. coli* cells adsorbed on carbon nanotubes and two were *S. aureus* cells on nanotubes. One mL of these samples was used for TEM analysis while the rest was reserved for TEM-Ultramicrotomy analysis.

For TEM imaging, approximately 5 μL solution was taken from the eppendorf tube containing adsorbed *E. coli* reserved for TEM analysis. The sample is directly deposited

on Formvar coated nickel grids (Electron Microscopy Sciences, PA, USA) and viewed under a Hitachi H7650 TEM machine (Hitachi Technologies, CA). For *S. aureus* the same procedure was followed except that the cells were fixed using 1% phosphate buffered formaldehyde solution for approximately 2 hours prior to the examination under TEM.

For ultramicrotomy analysis, the sample in the tubes was allowed to settle down naturally. After 15 minutes the supernatant was then carefully removed from the tubes using 1 mL disposable glass pipette. The contents in the tubes were then fixed by adding 2.5% cacodylate buffered (pH 7.2) glutaraldehyde and refrigerated overnight at 4°C . The fixed samples were then washed in cacodylate buffer and post-fixed in 1% osmium tetroxide (OSO_4) for 1 hour at 4°C . The tissue samples were dehydrated in a graded series of ethanol (50, 70, 80, 90 and 100%) for 10 minutes each and treated with acetone twice for 5 minutes each. Following the acetone treatment, the samples were infiltrated with 1:1 (vol/vol) mixture of acetone and Spurr's low viscosity epoxy resin embedding media (Spurr 1969) and incubated overnight on a rotator at room temperature. On the following day, the samples were infiltrated further with a mixture of acetone and Spurr's media (1:3 volumetric ratio) for 6 hours on a rotator at room temperature followed by an overnight incubation in 100% Spurr's at room temperature. Finally, the samples were embedded in 100% Spurr's and cured in a 60°C oven for 24–48 h.

A Leica UC-6 ultramicrotome and a diamond knife were used to make ultrathin sections (70–80 nm) of the specimen blocks. The sections were collected on a 200 mesh Formvar coated nickel grids, stained with uranyl acetate (Epstein & Holt 1963), followed by lead citrate (Reynolds 1963), and examined under a Hitachi H7650 TEM (Hitachi High Technologies, CA). Images were captured using a side mount AMT XR-60 digital camera.

RESULTS AND CONCLUSION

Adsorption kinetics

The amount of bacteria adsorbed on a given adsorbent is calculated by the following equation:

$$M_t = [(C_0 - C_t)V]/m \quad (1)$$

where M_t is the amount of bacteria adsorbed on a given adsorbent (CFU/g); C_0 is the initial concentration of the bacteria in the feed solution (CFU/mL); C_t is the concentration of bacteria in solution at a given time t (CFU/mL); V is the total volume of the feed solution (mL); and M is the mass of the adsorbent (g).

At adsorption equilibrium bacteria concentration in the feed C_t becomes the equilibrium concentration C_e and adsorbed amount M_t becomes $M_{\max}$.

A plot between $M_t/M_{\max}$ and time (t) is called the fractional uptake curve (Do 1998) is drawn for two different concentrations of *E. coli* and *S. aureus* and are shown in Figure 1.

If the fractional uptake ($M_t/M_{\max}$) of the adsorbate (bacteria) on nanotubes is greater than 70%, the effective diffusivity D_e (cm^2/s) of the bacteria in nanotubes can be estimated from a macropore diffusion model developed by Ruthven (1984).

$$\frac{M_t}{M_{\max}} \cong 1 - \frac{6}{\pi^2} \exp\left(-\frac{\pi^2 D_e t}{R_p^2}\right) \quad (2)$$

This solution for the fractional adsorption uptake is used to correlate the adsorption kinetics data. A plot of $\ln(1 - (M_t/M_{\max}))$ vs. time is obtained for *E. coli* and *S. aureus* and is shown in Figure 2. The slope of the fitted straight line is $(-\pi^2 D_e/R_p^2)$ and the intercept of $\ln(6/\pi^2)$ from which the effective diffusivity D_e in macropores of carbon nanotubes can be calculated.

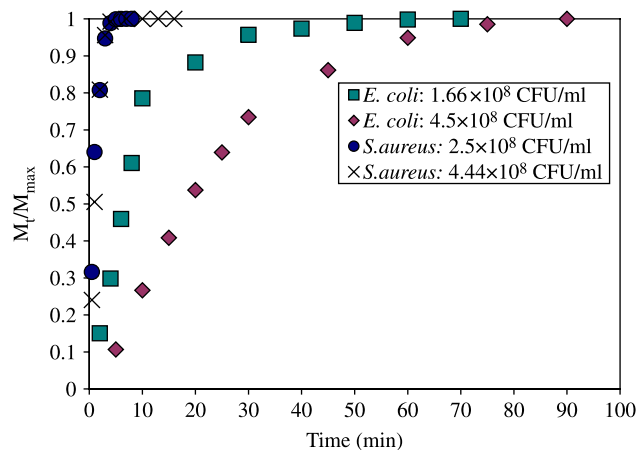


Figure 1 | Fractional uptake curve of *E. coli* K12 and *S. aureus* SH 1000 on SWCNT aggregates.

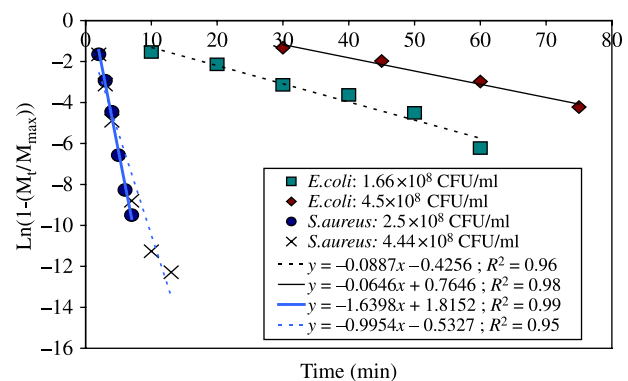


Figure 2 | Correlation of adsorption kinetics data with diffusion model for *E. coli* K12 and *S. aureus* SH 1000 on SWCNT aggregates at 25°C.

The diffusion time constant (D_e/R_p^2) and corresponding diffusivities (D_e) obtained for *E. coli* and *S. aureus* at two different initial concentrations is calculated and reported in Table 1.

The results obtained in Table 1 indicate that diffusion of *S. aureus* in nanotubes is two orders of magnitude higher than *E. coli*. This could be due to the smaller size and shape of *S. aureus* cells because it is generally believed that adsorption of bacteria with size less than $1 \mu\text{m}$ is higher than that with size greater than $1 \mu\text{m}$ (Stevik et al. 2004). Typical bioparticle diffusivity in carbon nanotubes is $10^{-7} \text{ cm}^2/\text{s}$ (Zhou et al. 2006). Our diffusivity data closely match this value.

TEM images of *E. coli* and *S. aureus* cells adsorbed on single wall carbon nanotube aggregates are given in Figures 3(A) and 4(A) and the corresponding ultramicrotomy images are given in Figures 3 (B) and 4 (B).

It appears from the TEM images (Figure 3(A)) that *E. coli* cells colonise in the presence of carbon nanotubes. Although the colonisation phenomena are observed with *S. aureus* cells, it can be seen that it is not as effective as it is in *E. coli* cells. One more observation is that the structural integrity of *E. coli* cells (Figure 3(A)) is still retained though

Table 1 | Diffusion time constants and diffusivities of *E. coli* and *S. aureus* cells in single-walled carbon nanotube aggregates at different initial concentrations

Bacterium	Initial concentration (CFU/mL)	$(D_e/R_p^2) (\text{s}^{-1})$	$D_e (\text{cm}^2/\text{s})$
<i>E. coli</i>	1.66×10^8	8.98×10^{-3}	8.98×10^{-9}
<i>E. coli</i>	4.5×10^8	6.54×10^{-3}	6.54×10^{-9}
<i>S. aureus</i>	2.5×10^8	0.166	1.66×10^{-7}
<i>S. aureus</i>	4.44×10^8	0.100	1.00×10^{-7}

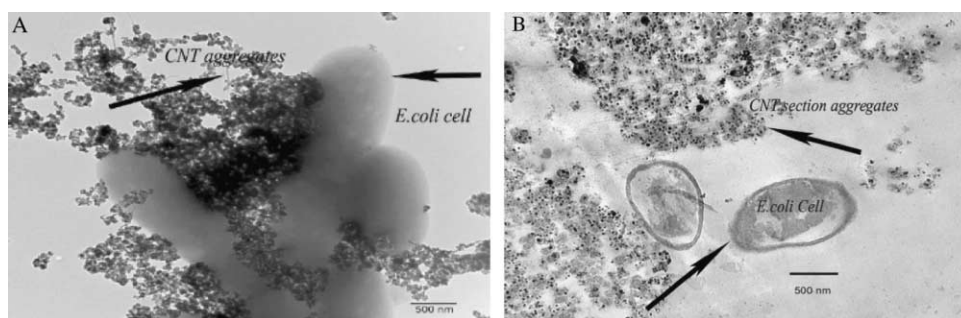


Figure 3 | (A) TEM image of *E. coli* adsorbed on SWCNT aggregates; (B) Ultrathin image of *E. coli* adsorbed on SWCNT aggregates.

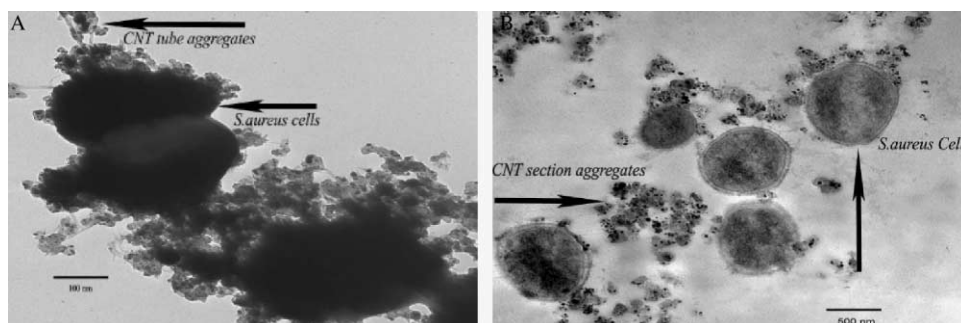


Figure 4 | (A) TEM image of *E. coli* adsorbed on SWCNT aggregates; (B) Ultrathin image of *E. coli* adsorbed on SWCNT aggregates.

the cell activity might have been lost. This is similar to what Lee *et al.* (2004) observed where the cell structure is intact but the viability of the cells is lost immediately after coming in contact with nanotubes. On the contrary, some other researchers (Narayan *et al.* 2005; Kang *et al.* 2007) depicted that the intracellular contents are released and the cell activity is completely disrupted when they are adsorbed on carbon nanotubes.

In conclusion, batch adsorption studies and microscopic studies show that *E. coli* and *S. aureus* in single-walled carbon nanotube aggregates has rapid diffusion kinetics. The diffusivity and kinetics of *S. aureus* are two orders of magnitude higher than *E. coli*. This suggests that the diffusivity is concentration dependent and bacteria dependent. The association of adsorbed bacteria with carbon nanotubes is also a function of type of bacterial strain. These points are important in designing nanotube biosensor for pathogen detection because effective diffusion length in a carbon nanotube biosensor depends on a type of bioparticle (Zhou *et al.* 2006) and it appears that nanotube sensor can provide selective differentiation of bacterial strains.

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