Research Article

The Adsorption Properties of *Bacillus atrophaeus* Spores on Single-Wall Carbon Nanotubes

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An adsorption equilibrium and a kinetic study of *Bacillus atrophaeus* on Single-Wall Carbon Nanotubes (SWCNTs) were here performed to provide the basis for developing biosensor devices for detecting threatening micro-organisms in water supply systems. *B. atrophaeus* spores and carbon nanotubes were subjected to a batch adsorption process to document their equilibria and kinetics. Here, commercial nanotubes were either studied as received or were acid-purified before adsorption experiments. The *Bacillus* spores appear to show higher affinity towards the purified nanotubes than to the as-received nanomaterial. The effective diffusivity of the spores onto the purified nanotubes was found to be approximately 30 percent higher than onto the as-received nanotubes. It seems that the removal of amorphous carbon from the as-received nanotubes through a purification process yielded an intimate nanotubes-spore interaction as revealed by transmission electron microscopy. Freundlich model successfully correlated the adsorption equilibrium data for the nanotubes-spore interaction. Transmission electron micrographs showed extensive contact between the *Bacillus* and the purified nanotubes, but the association appeared less intimate between the spores and the as-received nanotubes.

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1. Introduction

Carbon nanotubes (CNTs) are one of the most promising materials in the areas of electronics, sensors, bio-manipulation, and medicine due to their unique mechanical, electrical, and chemical properties [1–4]. Indeed, a large amount of research on the area of sensors has positioned single-wall carbon nanotubes (SWCNTs) as one of the most capable structures for detecting diverse chemical and biological agents [5]. Recent studies have also shown the potential of carbon nanotubes as amperometric sensors for detecting analytes in solution without the requirement of any mediator due to their high electron transfer rates [6]. It has been shown that CNTs can act as a conducting channel for electrons towards redox active centers resulting in sensing materials for components such as cytochrome c, horseradish peroxidase, myoglobin, and glucose oxidase [7–10]. Li et al. [11] have studied the sensing properties of polymer-coated nanotubes for detecting hydrochloric acid (HCl) vapors.

They have shown that coated nanotubes are capable of sensing concentrations of HCl at levels as low as 2 ppm.

Detection of ammonia in water has been studied by Pantarotto et al. [12], and they have shown that the concentration can be measured based on the covered area of the CNTs by the ammonia. Current developments on nanotubes-based sensors performed by Nanosensors, Inc. [13] have shown that these nanomaterials can detect pathogenic microorganisms such as *E. coli* or *Salmonella* in food supplies. Studies carried out by Grüner [14] on sensors have shown that CNTs represent a real-time acquisition material with the potential of measuring intramolecular vibrations, anharmonic relaxations as well as conformational changes of the sensed media. Major challenges remain, however, in making devices that differentiate between adsorbed species in complex mixtures and provide rapid forward and reverse response. A recent work, based on the adsorption properties of different CNTs-bacterial systems, has shown that the adsorption kinetic is strongly dependant on the microorganism involved [15].

Here, it was reported that the adsorption of *S. aureus* upon as-received CNTs was considerably faster than that exhibited by *E. coli*. Evidently, the size of the microorganism influenced the rate of adsorption upon the carbonaceous material [15]. A research work carried out by Upadhyayula [16] on the adsorption equilibrium of *B. atrophaeus* upon commercial CNTs has also shown that the kinetics can be successfully predicted by the well-known Freundlich adsorption equation. These results are encouraging since predictive outcome on sensing signal may be simulated by established adsorption parameters. The study of *B. atrophaeus* (a nonpathogenic spore-forming bacterium) and CNTs system appears to be a good representation media of the interaction of nanotubes and lethal microorganisms such as *B. anthracis*. The rapid detection of microbial pathogens is of great interest due to the threats that they represent as a biological weapons [17]. We have shown that spores of *Bacillus* can survive in chlorinated tap water and can be recovered and identified on typical household water filters [18]. To date, the analytical detection methods of pathogenic contaminants on drinking water systems are complex and time consuming (between six hours to two days) [19]. Thus, the development of a pathogen detection device based on carbon nanotubes would represent a promising sensing platform.

In order to build an effective sensor device based on nanotubes, it is critical that these nanomaterials are free of their metal catalyst as well as of impurities such as amorphous carbon material. It has been shown that presence of amorphous carbon severely reduces the electrical properties of the nanostructures [20]. Additionally, elimination of amorphous carbon is a critical step for subsequent surface functionalization [21]. Indeed, by functionalizing their surface structures, specific chemical groups can be covalently attached to the nanotubes in order to address them into specific sensing applications. Undoubtedly, study of the physical attachment of biological organisms onto carbon nanotubes has to be initially performed in order to characterize their adsorption properties. The purpose of this research program is to study and compare the adsorption properties of *B. atrophaeus* onto as-received and purified SWCNTs for the development of a biothreat nanosensing devices.

2. Experimental Methodology

2.1. Absorbent Material. SWCNTs purchased from Carbolex, Inc. (Lexington, Ky, USA) were studied in this research program. These nanostructures were synthesized through an arc-discharge method yielding nanotubes with a diameter around 1.4 nm. A thermogravimetric analysis, performed in air by Carbolex, Inc. on these raw nanotubes, indicated that this material contained between 20 to 30 wt% of amorphous carbon. In this study, removal of amorphous carbon and impurities was performed by introducing 0.5 gram of carbon nanotubes into a 100 mL of a 2.6 M HNO_3 solution. The acid solution containing the nanotubes was placed in a shaker for 48 hours rotating at 150 r.p.m. at room temperature. Subsequently, the CNTs-acid solution was vacuum-filtered through a 5 μm pore size polycarbonate membrane, and the

filtrate washed with approximately 2 liters of deionized water. In this paper, both the as-received and the purified nanotubes were considered for the adsorption equilibrium and kinetics study.

2.2. Absorbate Material. *B. atrophaeus* (formerly *B. subtilis* var. niger) was received from the U.S. Army Proving Grounds, Dugway, Utah, courtesy of Patricia Cox. Here, a stock culture was prepared following the purification method previously reported on *B. atrophaeus*-carbonaceous system [15]. The spores were stored in multiple capsules of 2 mL each at 4°C. These spores presented an elliptical shape with dimensions of 0.3 μm $\times$ 1 μm . The Schaeffer Fulton spore-staining method was also performed on the spores in order to identify the ratio between spores (nongrowing) and vegetative (growing) forms. Indeed, it is important to consider the amount of spores on each stage, since the adsorption and sensing mechanisms might be influenced by the spore/vegetative ratio. In this study, the ratio of spores to vegetative form was found to be around 2 : 1.

2.3. Adsorption Stage. Shaker experiments were performed to determine the kinetics and adsorption equilibrium of *B. atrophaeus* onto the as-received and the purified SWCNTs. Here, 0.1 gram of the as-received CNTs and 0.1 gram of the purified CNTs were added to different 250 mL flasks containing 99 mL of sterilized deionized water. Then, 1 mL of a spore solution of *B. atrophaeus* at a pre-established concentration was added to each flask at 1.3×10^4 , 4×10^5 , and 6×10^6 CFU/mL. (CFU: Colony Forming Unit). The flasks were then placed in a mechanical shaker at 200 r.p.m. at room temperature. Two milliliters of each CNTs/spore system were sampled at regular intervals and filtered through a 2 μm polycarbonate filter paper (Millipore, Mass, USA). The filtrates (containing unattached cells) of the CNTs/spore samples were inoculated on Tryptic Soy Agar (TSA) plates and incubated at 37°C for 24 hours, and the number of colonies grown on the plates was counted. The absorbed amount of spores onto the CNTs was calculated by a mass balance of the total spores in the solution before and after adsorption. The final concentration of the solution and the maximum adsorbed amount give the respective adsorption equilibrium concentration and adsorption capacity for the spores. The kinetic and equilibrium study performed in this research program was carried out on duplicates based on three different initial spore concentrations.

2.4. Microscopic Analysis. Optical analysis using a Hitachi S-3200 SEM and a Hitachi H 7650 TEM was performed on the as-received and purified CNTs to characterize their morphology. Microscopic observations of the CNTs/spore systems after the batch experiment were also carried out to elucidate their interaction.

3. Results and Observations

3.1. Microscopic Analysis. Initial evidence of the as-received material under the SEM showed bundles of aggregated

FIGURE 1: Scanning electron microscope micrograph of the SWCNTs (a) as-received and (b) purified.

nanotubes with a continuous entangled appearance (see Figure 1(a)). The presence of these continuous fibers agrees with previous studies carried out by Liu et al. on SWCNTs [22], where it has been shown that it is difficult to identify the end and beginning of an SWCNT. In contrast, Figure 1(b) shows an SEM micrograph of the SWCNTs after being subjected to an acid purification. From the figure, it can be observed that the chemical treatment disentangles the nanotubes as well as removes impurities such as amorphous carbon. Figure 2 shows TEM micrographs of the as-received and purified SWCNTs. This figure supports the findings observed on the SEM analysis, where it can be seen that a considerable amount of amorphous material has been removed after the nanotubes have been chemically treated.

3.2. Adsorption kinetics and Equilibrium. For the adsorption kinetic studies, samples were taken at different time intervals from the spores-CNTs batch experiments and were filtered. The variation of adsorbed spores onto the CNTs was established by counting the cells in the filtrate (representing the non-adsorbed spores) and subtracting them from the initial concentration.

Adsorption curves of the *B. atrophaeus*/as-received CNTs and the *B. atrophaeus*/purified CNTs for the three different initial spore concentrations here studied are shown in Figure 3. Here, the average fractional adsorption uptake ($M_t/M_{\max}$) is plotted against time (t); where M_t and $M_{\max}$ are the adsorbed amounts per unit mass of adsorbent at a specific time and at infinity time, respectively. In this

FIGURE 2: Transmission electron microscope micrograph of the SWCNTs: (a) as-received and (b) chemically treated.

study, $M_{\max}$ was calculated from the last point of the kinetics study in the batch experiment. Figure 3 shows that the purified nanotubes yielded a higher adsorption uptake than that displayed by the as-received nanotubes. This is probably due to the fact that less amorphous carbon is present in the acid-purified nanotubes and, therefore, a more efficient adsorption process takes place on these purified CNTs. Indeed, it is interesting to note that in 1 minute of batch experiment, the treated nanotubes already adsorbed more than 80% of the spores. Figure 3 also shows that the maximum uptake increases at shorter times by decreasing the initial concentration of the absorbate material.

The effective diffusivity De of the spores onto the CNTs can be estimated using the following equation based on macropore diffusion process if the fractional uptake is greater than 70% [15]:

$$1 - \frac{M_t}{M_{\infty}} \approx \frac{6}{\pi^2} \exp\left(-\frac{\pi^2 De t}{r^2}\right), \quad (1)$$

where r is the absorbent particle radius, and t is the time. Indeed, it has been suggested in a previous study that the CNTs act as web during the bacteria nanotubes adsorption process [15]. Here, r is considered as the distance between carbon nanotubes aggregates, which is about $100 \mu\text{m}$ [16]. Hence, the diffusivity rate and the effective diffusivity (De) can be obtained from the slope of $\ln[(1 - M_t/M_{\infty})]$ against

FIGURE 3: Adsorption uptake curves of *B. atrophaeus* on “as-received” and purified single-wall carbon nanotubes at room temperature.

FIGURE 4: Correlation of adsorption kinetics data using a macropore diffusion model for the *B. atrophaeus* spores/nanotubes complex based on a spore initial concentration of 6×10^6 CFU/mL.

time (t); where the slope is given by $(-\pi^2 De/r^2)$ and the intercept by $\ln(6/\pi^2)$. Figure 4 shows the kinetic correlation for the *B. atrophaeus* and the two systems of CNTs here studied for the batch experiment based on the initial concentration of spores of 6×10^6 CFU/mL. From Figure 4, it can be observed that the effective diffusion rate of the spores onto the purified CNTs is more than 1.5 times greater than its as-received counterpart; this effect is probably due to the more intimate contact between the treated nanotubes and the spores. A conjecture that appears to agree with the TEM analysis.

Similar adsorption uptake curves were obtained with the other two concentrations of the absorbate here studied.

FIGURE 5: Diffusion time constant of the spores on as-received and purified nanotubes for different initial absorbate concentrations.

FIGURE 6: Adsorption equilibrium response of *B. atrophaeus* onto SWCNTs at room temperature. The solid lines represent the fitting of the Freundlich isotherm model to the experimental data.

Indeed, by plotting the diffusivity rate of the three sets of experiments based on the initial spore concentration (see Figure 5), it can be observed that the diffusivity rate of spores onto the purified nanotubes is higher than that shown onto the as-received nanotubes. The effective diffusivity was then determined from the data presented on Figure 5. Table 1 shows the effective diffusivity of the samples here studied. As expected, the effective diffusivity for the *B. atrophaeus*/purified nanotubes is higher than that shown by the *B. atrophaeus*/as-received nanotube system. These results are again encouraging since they suggest that nanotubes with higher percent of purity may be used to develop a faster detection platform.

The maximum adsorbed amount of absorbate material (M_{max}) upon the nanotubes and its corresponding concentration in the supernatant (C_e) are plotted on Figure 6. Freundlich adsorption isothermal equation was used to fit the component adsorption data for the two systems-based CNTs here studied. The Freundlich isothermal is given by

$$M_{max} = k_f C_e^{1/n}, \quad (2)$$

TABLE 1: Effective diffusivity of the *B.atrophaeus* spores onto purified and as-received SWCNTs for the three initial absorbate concentrations here studied.

Sample	Initial spore conc. (C_{ini})	Effective diffusivity D_e (cm^2/s)
<i>B. atrophaeus</i> /purified CNTs	1.33×10^4	3×10^{-10}
<i>B. atrophaeus</i> /as-received CNTs	1.33×10^4	2.33×10^{-10}
<i>B. atrophaeus</i> /purified CNTs	4×10^5	2.44×10^{-10}
<i>B. atrophaeus</i> /as-received CNTs	4×10^5	2.12×10^{-10}
<i>B. atrophaeus</i> /purified CNTs	6×10^6	2.14×10^{-10}
<i>B. atrophaeus</i> /as-received CNTs	6×10^6	1.38×10^{-10}

(a)

(b)

FIGURE 7: TEM picture of an isolated spores/untreated nanotubes complex following the shaker-batch interaction experiment. (a) After 1 minute. (b) After 50 minutes.

where k_f is the Freundlich adsorption coefficient, and n is the adsorption intensity parameter. Figure 6 shows a good fit between the isotherm and the experimental data (yielding r^2 higher than 0.98 for both systems). From the figure, the adsorption coefficient of the *B. atrophaeus*/purified CNTs is more than 3 times higher than the *B. atrophaeus*/as-received CNTs system. This again suggests that the removal of amorphous carbon increases the affinity of the nanotubes towards the spores.

3.3. TEM Analysis. Optical analysis of the bacillus-nanotubes systems following the batch experiments was carried out on a TEM to elucidate their interfacial interaction.

FIGURE 8: TEM picture of an isolated spores/purified nanotubes complex following a 1 minute shaking-batch interaction process.

Indeed, the sampled aliquots had to be diluted in order to be observed on the TEM. Typical micrographs of the CNTs-spores system are shown in Figure 7, where a strong interaction between both components can be observed. The figure shows the as-received nanotubes/bacillus complex after 1 and 50 minutes of shaking in the batch experiment. From the figure, it is observed that the interfacial contact increases at longer times, since more nanotube-spore material appears to be in contact after 50 minutes of shaking-batch interaction. Similar behavior was observed with the spore-purified CNTs system. Figure 8 shows the contact of the bacillus with the purified nanotubes after 1 minute in the shaker-batch experiment, and the amount of material interacting in this purified system appears to be higher than that shown with the as-received material. The micrograph shown in Figure 8 supports the kinetic data obtained in the present research work regarding the contention that there is stronger affinity

between spores and purified nanotubes compared to as-received nanotubes.

4. Conclusions

An adsorption equilibrium and a kinetic study of *B. atrophaeus* on SWCNTs have been here performed. The carbon nanotubes used in this study were investigated in its as-received condition as well as after being subjected to a chemical acid treatment. Evidence here presented demonstrates that the adsorption rate of spores onto purified nanotubes is faster than that shown onto untreated carbon nanotubes. Indeed, results have shown that the effective diffusivity of spores upon the nanotubes follows the same trend displayed by the adsorption rate. The Freundlich isotherm equation seems to fit well with the experimental data; here, the Freundlich adsorption coefficient of the *B. atrophaeus* on purified nanotubes was more than 3 times higher than that shown by its as-received counterpart. It has also been shown that the removal of amorphous carbon through the purification process evidently allows a more intimate interaction between the nanotubes and the spores. Optical analysis following spores-nanotubes interaction on a shaker-batch experiment supports the experimental data obtained during the kinetic process. Here, TEM micrographs showed higher interaction between the purified nanotubes and the spores than that exhibited between spores and the untreated nanotubes. This research work demonstrates the promising potential of building relatively short outcome biosensor devices based on diffusion parameters of microorganisms onto SWCNTs.

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