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Adsorption kinetics of Escherichia Coli on different Carbon Nanoforms

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Adsorption of Escherichia coli (E. Coli) bacterial cells on different carbon nanoforms (i.e. Single-walled carbon nanotube (SWCNT), Multiwalled Carbon nanotube (MWCNT), graphite and mixed Fullerene) aggregates is studied. The diffusivities of pure cultures of E. Coli cells in SWCNT aggregates, MWCN aggregates, Graphite aggregates and Mixed Fullerenes was observed to be 1.5×10^{-9} cm^2/s, 0.55×10^{-9} cm^2/s, 0.8×10^{-9} cm^2/s, and 1.016×10^{-9} cm^2/s, respectively. In addition to batch adsorption studies, optical microscopy studies were also performed. The results suggest that diffusion kinetics of bacterial cells depends on the concentration and average diameter of the nano-carbon aggregates and also on the type of material used. Diffusivity of E. Coli. in SWCNT was observed to be highest and is about three times greater than for MWCNT, about two times greater than for graphite and about 1.5 times greater than for Fullerene aggregates. SWCNT seems to be best candidates (amongst the other materials studied) for adsorption of microorganisms – paying their way for application towards microorganisms filters and for biosensors (where it is desired to simultaneously detect and capture bio-threat agents).

1. **Introduction :** Nano Carbons as major building blocks of the newly emerged nanotechnology have captured a tremendous attention in research since their discovery in the early 1990s. These nano carbons possess unique optical, electrical, mechanical, and thermal properties [1 - 3]. Carbon nanotubes (CNT), exhibit a number of extraordinary physical properties, such as enormously small size and high aspect ratio (>1000), and a well-off spectrum of electrical properties [4]. Exploiting these unique features for developing bioelectronics, biosensors, and circuitries have produced the rapidly developing fields of nanobioelectronics and nanobiotechnology [5]. Other studies have also shown that CNTs possess strong antimicrobial activity. Kang et al. [6] reported the first direct evidence showing that SWNTs exhibited strong antimicrobial activity to bacterial cells by direct contact using E. coli as a model microorganism. The same group discovered that the diameter of CNTs was a key factor governing their antimicrobial activity and those SWNTs were much more toxic to bacteria cells than MWCNTs [7]. There has been massive interest in exploiting their amazing properties for numerous biological and environmental applications [8 - 9]. For example, CNTs have been used for constructing biosensors and bio-devices for detection of bio-molecules and biological cells [10 - 15]. Both the potential toxic effects and the promising applications of CNTs have been reported recently. To improve reactivity and sensitivity, functionalizing CNT sidewalls with specific bio/chemical molecules ensure better chemical bonding between the nanotube and a specific chemical species as well as improve the selectivity of absorption process. Bacterial adsorption studies on single-walled carbon nanotubes provide insight on developing treatment based and sensor based applications in water and waste water 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 bioterrorism attacks [16]. When a biothreat pathogen is introduced into a drinking water system, there is a likely chance that it is not detected with existing systems [16 - 17]. This is because of the fact that current detection systems are purely designed to detect sewage based contaminations and not the biothreat pathogens [16]. Moreover, most of the biothreat pathogens are colorless odorless and tasteless [17] 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 [18 - 19]. 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 [18]. 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 [6, 19] 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, disrupts its activity and eventually destroys the cell viability [6, 20, 21]. It was proposed that the cylindrical shape of nanotube fibers coupled with a high aspect ratio are mainly responsible for death of bacterial cells [6]. Typically fibrous media provide larger accessible surface area for bacterial adsorption than powdered or granular adsorbents and higher the aspect ratio, higher is the accessible surface area. [6, 22]. Single-walled carbon nanotubes have aspect ratios greater than 2000 [23] are expected to adsorb bacteria efficiently [24] provided qualitative confirmation that microorganisms get adsorbed on carbon nanotubes. 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* on different nano carbon aggregates. Diffusivities of these microbes in single-walled carbon nanotubes are also measured. We have also performed optical microscopy analysis of the adsorbed bacteria to visualize the binding and association of the bacterial cells onto the carbon nanotubes. The information on association of bacteria with carbon nanotubes is important in designing a biosensor for specific applications in water and wastewater treatment plants.

2. Materials and Methods :

Adsorbent : The nano carbon samples (e.g. SWCNT, MWCNT, graphite and mixed fullerene) were purchased from **GSI Creos** (Japan), a commercial supplier of carbon nanotubes for research and development. The SWCNT has an average diameter of 1.4 nanometers and lengths of 2 - 5 μm. The as received single-wall carbon nanotube adsorbent samples have a purity of ~ 80 % carbon nanotubes with ~ 10 % of catalyst impurities including nickel and yttrium and ~ 10 % amorphous carbon. MWNTs, with a powder density of 2.1 g/cm^3 at 20 ^{o}C. It is also reported that the MWNTs are on average 20 - 40 nm in diameter and 50 μm in length. Mixed Fullerene(C_{60} and C_{70}) has a average diameter of 10 – 20 nanometer. Graphite powder has a particle size of 20 – 25 nm. Escherichia Coli. sample was collected from the environmental laboratory of department of Aquaculture, Bangladesh Agricultural University, Mymensingh. Average length was about 2 μm to 5 μm.

Batch Adsorption Studies : Fresh bacterial culture of *E.coli* was prepared from its stock. The culture was prepared in Trypton Soya Agar in sterile Petri dishe. The Petri dishe was incubed at 32 ^{o}C temperature in a incubator. From that cultured solution E. Coli. suspension was prepared by mixing 150 mg of cultured E.Coli. in 200 mL of Physiological salaine (0.9 % NaCl in deionized water). This freshly prepared bacterial solution was subsequently used for shaker experiments. Prior to the shaker experiments, the concentrated bacterial solution was aliquoted in 1.5 ml test tubes. The cells were prepared to be used for shaker studies and microscopic studies. Before performing the experiments all the test tubes, Petri dishes, L shaped glass rods, micro pipettor tips, beakers were sterilized in a dry sterilizer at 170 ^{o}C temperature for one hour. For this batch adsorption experiment, 10 ml of contaminated solution was taken in five test tubes. The bacterial concentration of E.coli in the test tubes were approximately 4.5 x 10^7 CFU/ml (Colony Forming Units). To these solutions, 0.01 grams of carbon nanotubes (SWCNT, MWCNT, fullerene and graphite) were added to each test tube. One test tube is kept free of material as control to

measure the actual concentration of the solution. Now these four samples were dispersed using a vortex mixture at the rotating speed about 1200 rpm. One ml of supernatant was drawn at regular intervals using a micro pipettor from the four test tubes and swelled to the Petri dishes (agar plates) using a L-shaped glass rod for culture. The dilution factor was varied from 10^{-4} to one factor less than the initial concentration, i.e. if C0 is of the order of 10^7 CFU/ml, the upper dilution factor used to for plating is 10^{-6}. The plates were then incubated at 32 °C for 24 hours. The number of colonies grown on the plates was enumerated using a colony counter. The total number of colonies at the test solutions were counted according to the following equation.

Concentration of E. Coli at the test tube of order h (C_h) = (Average concentration of E. Coli. at 10^l) × $10^{(h-l)+1}$

C_h= Concentration of E. Coli at stock, CFU/mL

C_{avgl} = Average concentration of E. Coli at 10^l, CFU/mL

C_{avgl} = (No. of E. coli at 10^l +.1× No. of E. coli at 10^{l+1} + .01×No. of E. coli at 10^{l+2})/n

Where -
n = number of sample data
h = Dimension no. of the concentration for the test tube where the material was mixed
l = Dimension no. of the concentration for the test tube where the C_{avg} is to calculate

Here, if h = 9 and l = 5

$$C_9 = C_{avg5} \times 10^{(9-5)+1}$$

The same experiment was repeated one more times for the *E.coli* contaminated solution with different initial concentrations by using the same nano carbons.

Optical Microscopy Analysis : 1 ml of bacteria solution adsorbed on carbon nanotubes (not the supernatant) sample is taken from each test tube after conclusion of the batch adsorption experiment and collected into four 1.5 ml eppendorf tubes. Four eppendorf tubes containing *E.coli* cells adsorbed on nano carbons (SWCNT, MWCNT, graphite and Fullerene) and one is only stock solution. One drop of each sample was used for gram staining. These stained samples were used for optical microscopy analysis.

3. Results and Discussion :

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

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

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) ; m is the mass of the adsorbent (g).

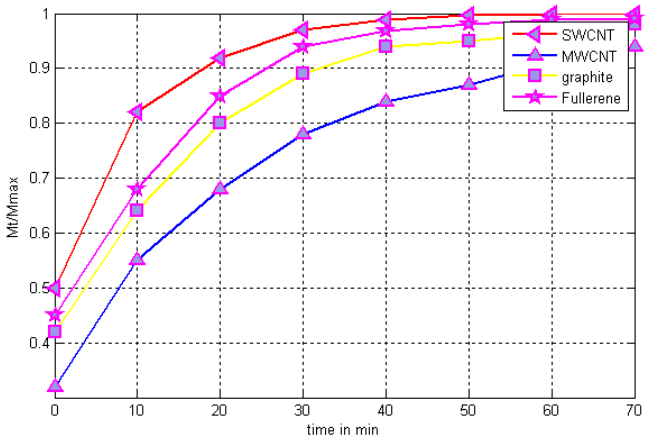

Figure (1) ; Fractional uptake curve of *E.coli* on SWCNT, MWCNT, graphite, fullerene Aggregates.

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 curves are drawn for of *E.coli* for four different materials and shown in Figures (1).

M_t /M_{max} = $[(C_0-C_t)\ V]/m$ where If the fractional uptake (M_t/M_{max}) of the adsorbate (bacteria) on nanotubes is greater than 70 % the effective diffusivity De(cm^2 /s) of the bacteria in nanotubes can be estimated from a macropore diffusion model developed by Ruthven (1984) **[25]**.

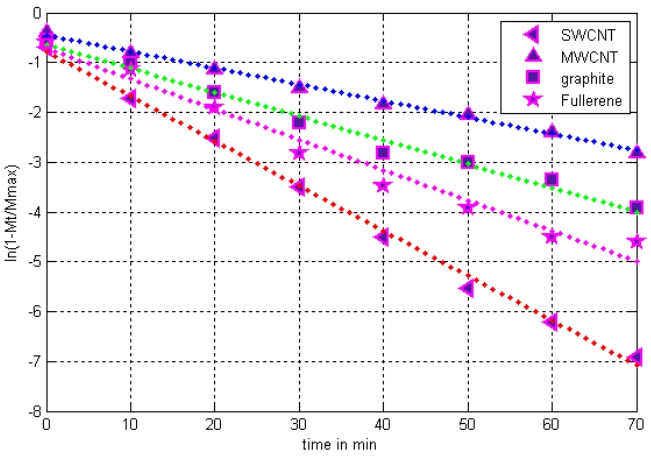

Figure (2) : Correlation of adsorption kinetics data with diffusion model for *E.coli* on different nano carbon aggregates at 25°C

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* which is shown in Figure (2). Effective diffusivity D_e in macropores of carbon nanotubes can be calculated. Diffusivities (D_e) obtained for *E.coli* is calculated.

Table (1.1) : Diffusion time constants and diffusivities of *E.coli* cells in different nano-carbon aggregates

Material	**$(D_e/R_p^2)(s^{-1})$**	**$(D_e)(cm^2s^{-1})$**
MWCNT	0.55×10^{-3}	0.55×10^{-9}
Graphite	0.8×10^{-3}	0.8×10^{-9}
Fullerene	1.0167×10^{-3}	1.016×10^{-9}
SWCNT	1.5×10^{-3}	1.5×10^{-9}

Diffusivity of E. Coli. in SWCNT is about three times greater than MWCNT, about two times greater than graphite and about 1.5 times greater than fullerene aggregates.

Results from Optical Microscopy : It appears from the optical images Figure (3 to 7) that *E.coli* cells colonize in presence of carbon nanotubes. From these figures we can confirm the adsorption phenomenon of *E. Coli*. with different nano-carbons.

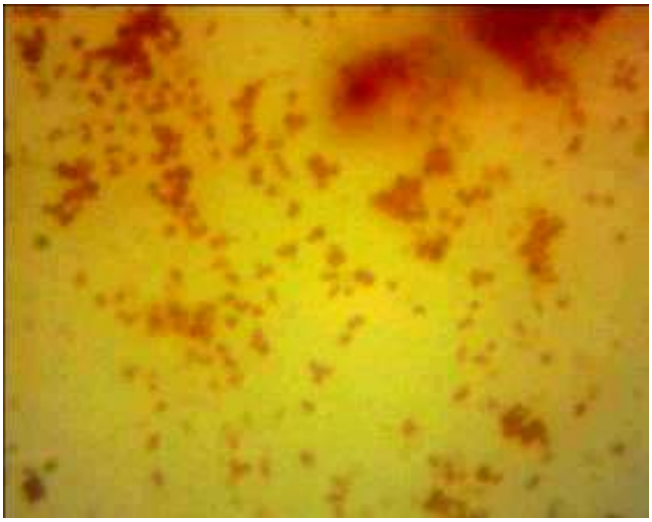

Figure (3) : Image of E.coli without mixing

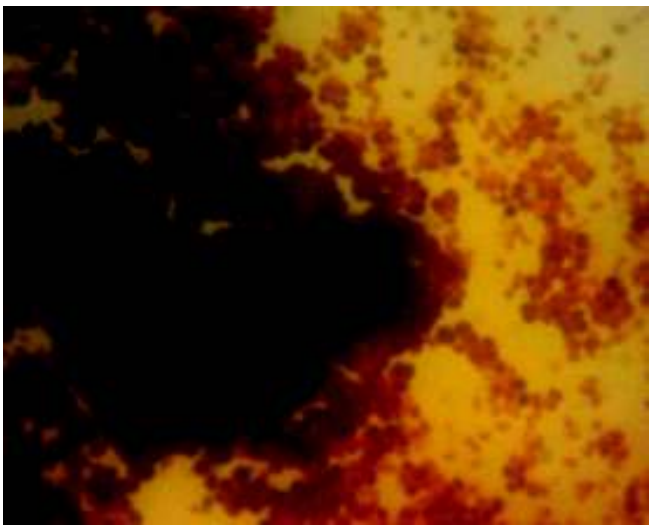

Figure (4) : *E.coli* adsorbed on SWCNT aggregates. with nano carbons.

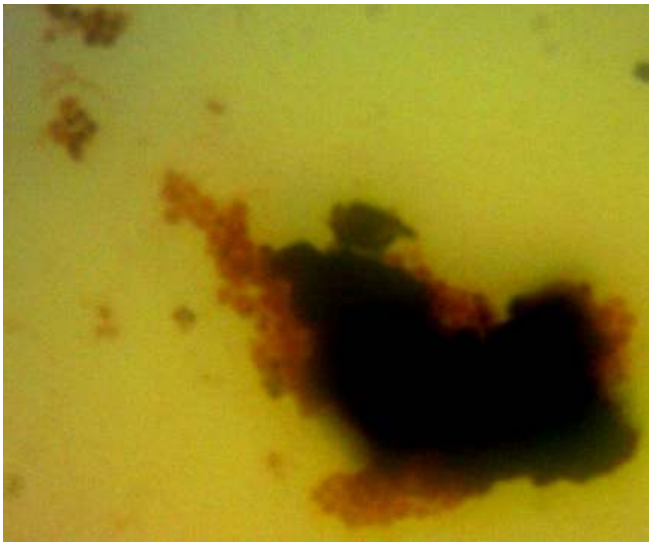

Figure (5) : *E.coli* adsorbed on Fullerene aggregates.

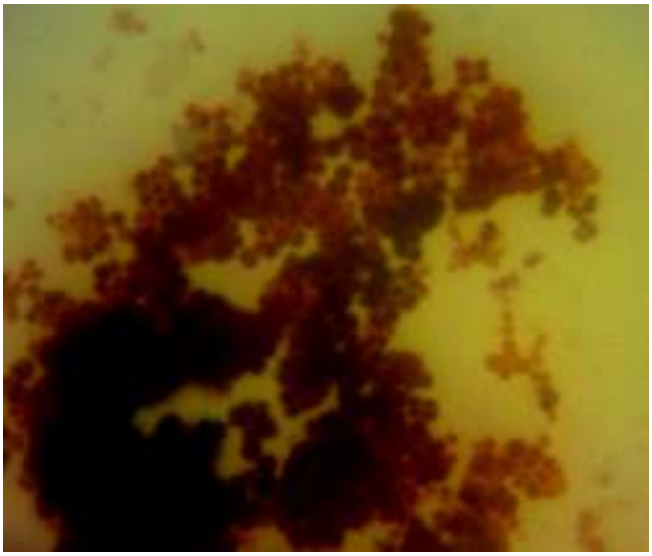

Figure (6) : *E. Coli* absorbed on MWCNT aggregates

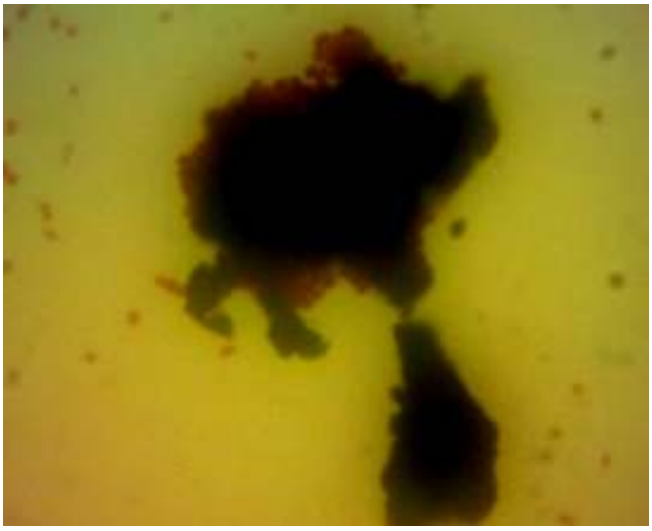

Figure (7) : *E. Coli* absorbed on Graphite aggregates.

4. Conclusions : The results suggest that diffusion kinetics of bacterial cells depends on the concentration and average diameter of the nano carbon aggregates and also on the type of material used. This proves to be beneficial from 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 bio-threat agents in a less span of time. Typical bioparticle diffusivity in carbon nanotubes is 10^{-7} cm^2 /s **[26]**. It is generally believed that adsorption of bacteria with size less than 1 µm is higher than that with size greater than 1 µm **[27]**. Our diffusivity data agrees with these concepts.

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