Int. J. Nanosci. Nanotechnol., Vol. 8, No. 1, March 2012, pp. 3-10

Separation of Salmonella Typhimurium Bacteria from Water Using MWCNTs Arrays

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(Received:20 Dec. 2011 and Accepted: 10 March 2012)

Abstract:
In this study, Salmonella Typhimurium bacteria removal from polluted water has been investigated using multiwall carbon nanotubes arrays. Experimental results reveal that the contact time, the bacterial concentration and the weight of multiwall carbon nanotubes arrays have positive significant effects on the bacteria removal efficiency. Increasing the contact time and the weight of multiwall carbon nanotubes arrays enhances the removal efficiency which can be the result of the aggregation increase between bacteria cells and carbon nanotubes. Scanning electron microscopy images demonstrate that the multiwall carbon nanotubes arrays capture the bacteria cells by the sieve mechanism without any specific effect on the bacteria cell morphology. Furthermore, the impact of the compressing and crushing of carbon nanotubes arrays on the bacteria cell removal efficiency were studied. Results show that crushing process enhances the bacteria removal efficiency and also increases the loss of carbon nanotube arrays by transportation with water. However, compressing process does not have any significant effect on the bacteria removal efficiency in comparison to the primary samples, and also decreases the loss of carbon nanotubes. These observations suggest that compressed carbon nanotubes arrays can be an appropriate choice for separation of salmonella bacteria from polluted water.
Keywords: *Water Treatment, Multi Wall Carbon Nanotubes Array, Salmonella Typhimurium Bacteria, Removal Efficiency.*

1. INTRODUCTION

There is a big challenging area for accessing the safe drinking water all over the world because of decrease in drinking water sources. Biological contaminations such as bacteria, viruses and algae have polluted some parts of water. Therefore, water disinfection is necessary to protect people from pathogens. Recently, several disinfection methods have been developed for water treatment. The most common methods are chlorination, ozone inactivation and UV treatment. Recent studies have shown some disadvantages of these methods, most importantly: increasing the microorganisms resistance, growing the biological contaminations again after treatment processes and increasing human allergies to pathogens [1, 2]. These reasons justify finding new processes for water disinfection. Developing novel technologies especially in the area of nanoscale science and engineering may solve many of these problems. Water quality can be greatly improved using nanosorbents, bioactive [3] and catalytic nanoparticles [4]. One of the new nanomaterials is carbon nanotube (CNT). CNTs have been suggested for water disinfection, due to their high surface area, porous structure and cytotoxicity properties [5-8]. There are some experimental studies

which have investigated the interactions between CNTs and various pathogen agents. These studies illustrate that there is an excellent potential for CNTs as microbial capturing agents.

In 2007, Kang *et al.* [6, 9] suggested that CNT size (diameter) plays an important role on the inactivation of bacteria (E. coli K12) cells. They reported that CNTs with short diameter and high purity content have high antimicrobial activity because of their vast interactions with the bacterial cells. After that Brady *et al.* in 2008 [10] demonstrated that E.coli cells are completely retained on the SWNT filter due to size exclusion. They observed that E. coli cells are effectively inactivated upon contact with the SWNTs. In another study, adsorption capacities of Bacillus subtilis spores on pristine SWNTs and two adsorbent media (powdered active carbon and nanoceram) have been reported by Upadhyayula et al [11]. Their results showed that adsorption of B. subtilis spores is 27–37 times greater than powdered activated carbon and NanoCeram™. This is a convincing proof of high microbial affinity of CNTs due to their fibrous size and accessibility of external surface area which has not been seen in other two adsorbents.

Also, Akasaka *et. al*. [12] demonstrated that the precipitation of Streptococus mutans bacteria on MWCNTs with 30 nm diameter (semidispersible) is greater than both the completely dispersed SWCNTs and weakly dispersed MWCNTs by diameter of 200 nm. Arias *et al*. [13] indicated that the antimicrobial activities of SWCNTs on adsorption of salmonella bacteria can be enhanced by increasing their concentration and treatment time. Moreover, Tianjia *et al.* [14] observed that the removal efficiency of Bacillussubtilis var niger improves by increasing the CNT filler loading on the membrane supports.

In the other hand, MWCNT filters perform better than SWCNT filters. In another study on Salmonella bacteria, the CNT's length parameter has been studied. Yang *et al.* [15] suggested that longer SWCNTs have higher aggregated with Salmonella cells due to less aggregation of CNTs with each others. As a result, all studies show that some parameters of CNTs such as length, diameter, surface area, concentration, number of layers (single or multi wall) and impurity content play a fundamental role in their disinfection properties. But according to our knowledge, in all of these studies, the adsorption capacities of different bacteria cells on non-array CNTs have been investigated.

Therefore, in this study, multi wall carbon nanotubes arrays have been applied to disinfect water from Salmonella bacteria. This is the first time that CNTs arrays have been used for water treatment from Salmonella bacteria. Also, every year, three million people die across the world owing to infection with this bacteria [16]. Thus, removing the bacteria from drinking water is an important issue. Some important parameters such as the weight dependence of CNTs arrays, bacterial concentration, treatment time dependence and the structure of carbon nanotubes (crushed and compressed) on the bacterial removal efficiency have been investigated.

2. MATERIALS AND METHODS

2.1. Multi wall carbon nanotube arrays

Commercially available MWCNT arrays synthesized by thermal chemical vapor deposition method were purchased from Carbon Tarara Technologies (Iran). These MWCNTs had the average length size of 1 mm and average outer diameter of 100 nm. The scanning electron microscopy (SEM) images of these CNTs arrays were prepared by a field emission scanning electron microscopy (FESEM, S4160). Also, thermo gravimetric analysis (TGA) of CNTs arrays was performed by Shimadzu Japan 50 thermo-gravimetric analyzer to characterize the weight loss during oxidation of sample in air by heating up to 1000°C and the heating rate of 10°C/min.

2.2. Bacterial strains and culture conditions

Stock of strains of salmonella typhimurium purchased from quality control center of Iran. The stock of bacteria obtained from blood culture patient. Salmonella typhimurium has been diagnosed after diagnosis biochemical information such as glucose, lactose, +H2, +mobility, citrate, sulfur, lysine and decarboxylase by Anti serum center. The blood agar culture was used for surety of bacteria purification and Mac Conkey culture was applied to view colonies typically.

2.3. Filtration process

At first, CNTs arrays were washed by acetone and dried at 37°C for 24 hr. All glass wares and samples were sterilized by autoclaving at 120°C for 15 min. Then, the Salmonella typhimurium was cultured on a blood agar plate at 37°C for 24 hr. The cultured bacteria were suspended in ten milliliters broth solution to reach the concentration of 1.5×10^8 cfu/ml, according to McFarland 0.5 turbidity standard. A portion of cultured bacteria was diluted to the concentration of 1.5×10^2 cfu/ml. Next, CNTs array s were packed into the Pasteur pipette to prevent flow from being restricted. Five milliliters of each bacterial concentration were passed through the packed bed CNTs arrays at 25°C and neutral pH. Finally, 0.01 ml of permeate samples were spread on a blood agar plate and incubated at 37°C for 24 hr for counting the surviving bacteria colonies using an optical microscope. At all steps, the removal efficiency of CNTs arrays was calculated using equation (1) [10]:

$$\text{Removal Efficiency} = \left(\frac{1 - C_{withCNT}}{C_{notCNT}}\right)\times 100 \quad (1)$$

where $C_{withCNT}$ and C_{notCNT} are the concentration of bacteria after and before contacting with CNTs arrays, respectively.

To regenerate CNTs arrays, they were washed with formaldehyde and acetone. The removal efficiency of CNTs arrays versus contact time was examined using a batch process. Three milliliters of each bacterial suspension containing 1.5×10^8, 1.5×10^6 and 1.5×10^4 cfu/ml were added to three cylindrical vessels for testing and similarly three samples were selected as the control vessels. Then, 0.05g of CNTs arrays were added to the vessels receptacle bacteria cells. Each sample was cultured after 10 min.

2.4. Compressing and crushing of CNTs arrays

As carbon nanotubes are porous materials, each factor that affects the pore volume is important and especially has impact on the adsorption process. Among effective parameters, compressing and crushing are two important ones.

To compress CNTs arrays, the specific weight of CNTs arrays was placed into the mold of device under pressure of 6 tons. This procedure was repeated for various weights of CNTs to produce CNT arrays tablets with different thicknesses. The diameter of all constructed tablets was1 cm.

Crushing process is a good method to decrease CNTs self-assembly after drying process. The specific weight of CNTs arrays was crushed into a mortar for 5 minutes. For both compressed and crushed systems, the filtration process was done for one bacteria concentration of 1.5×10^4 cfu/ ml with 0.05, 0.1 and 0.15 gr of CNTs arrays.

2.5. Statistical analysis

Two-way analysis of ANOVA was used to identify the statistical significance of the influential parameters such as CNTs arrays weight and bacterial concentration on the removal efficiency.

3. RESULTS AND DISCUSSIONS

3.1. Thermo Gravimetric Analysis

Figure 1 shows TGA and differential thermo gravimetric (DTG) curves of CNTs arrays. From this figure, it can be found that significant weight loss of the sample start from 522°C and continue until the stable plateau region at 800°C. The first significant peak occurs at 584°C. This observation shows that the starting oxidation temperature of the sample is different from that in high- purity graphite. In pure graphite, the first weight loss starts from approximately 630°C with maximum oxidation rate at 850°C [17]. This difference between our CNTs arrays and pure graphite is mainly due to the presence of amorphous carbon in the CNTs sample [18, 19]. Amorphous carbon has the highest reactivity rather than CNTs with the maximum oxidation rate at approximately 500°C. Therefore, the weight loss below 600°C indicates the presence of amorphous carbon in the sample.

The overall weight loss during thermal treatment is 95%. This shows that 5% of the CNTs mass is catalyst. Moreover, no weight increase was observed during thermal treatment. This indicates that there is not any metal particles oxidation [20, 18]. Therefore, in the sample, the catalyst particles

are completely encapsulated and away from any external or tip catalytic particles.

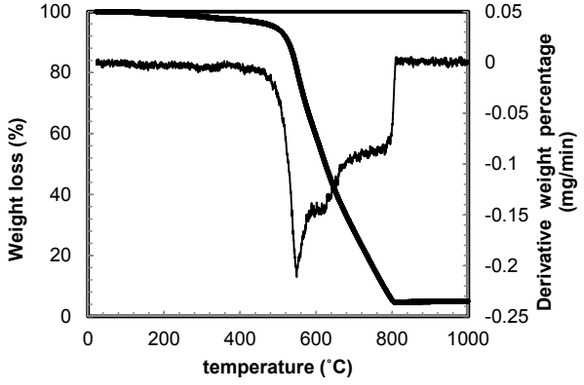

Figure 1: *Mass loss curve (full line) and derivative (broken line) obtained from TGA experiments for the MWCNTs arrays sample.*

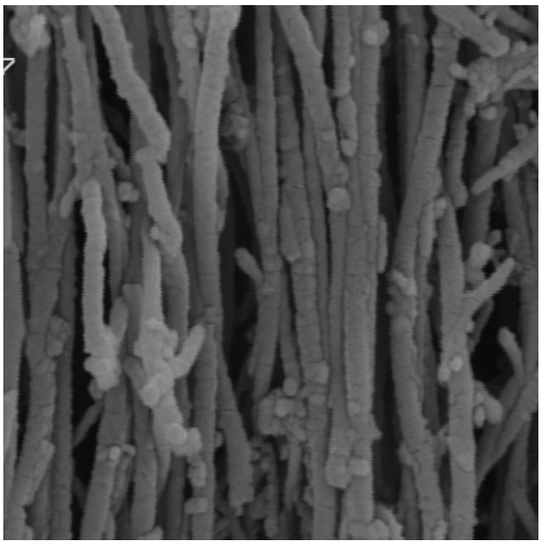

Figure 2: *Scanning electron micrography of MWCNTs array .*

3.2. Scanning Electron Microscopy

Figures 2 and 3 show SEM images of CNTs arrays before and after contact with bacterial cells. Figure 2 indicates the regular structure of CNTs arrays. Figure 3a and 3b demonstrate that bacteria cells are trapped among the CNTs arrays bundles. It can be due to the interactions of bacteria cells with the external surfaces of CNTs arrays. Also, Figure 3b indicates that there are no major changes in the morphology of the bacteria cells after incubating with CNTs arrays. These SEM images reveal that CNTs clusters only capture the bacteria cells due to sieve mechanisms without any damage of the cell wall. This observation differs from other studies [9,10]. Using non-array CNTs have shown that CNTs rupture cell wall–membrane due to toxicity mechanisms such as oxidative stress [21] and physical damage [6,9,10] while this observation has not been observed here.

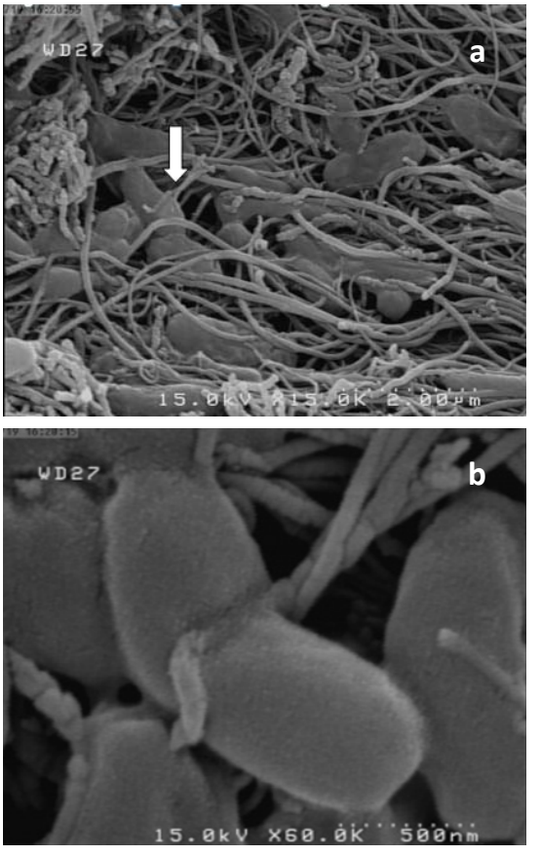

Figure 3: *Scanning electron micrography of MWCNTs array, (a) MWCNTs array with bacterial cells, (b) Evidence of capturing the bacteria cells by the sieve mechanism.*

3.3. Important parameters on the removal efficiency

3.3.1. Bacteria concentration and MWCNTs arrays weight

Figure 4 shows the bacteria removal efficiency versus the bacterial concentration at three different CNTs arrays weight. By variation the bacterial concentrations and weight of CNTs arrays, the

removal efficiency of CNTs arrays significantly changes (p-value<0.05). At low bacterial concentrations such as 1.5×10^2 and 1.5×10^3 cfu/ml, the removal efficiency is more than 90%. But by increasing the bacterial concentration, the removal efficiency decreases. This is may be because of the saturation of the effective mesopore sites of CNTs arrays surfaces by the bacterial cells. This shows that at low bacterial concentrations, changing the weight of CNTs arrays does not have any significant effect on the removal efficiency.

Another effective factors on the removal efficiency is the weight of CNTs arrays. From Figure 4 it can be observed that by increasing the weight of CNTs arrays from 0.05 to 0.15g at constant bacterial concentration of 1.5×10^5 cfu/ml, the removal efficiency improves from 46% to 72%. The dependence of the weight of CNTs arrays on the removal efficiency is probably due to the variation of the bacteria cells-CNT aggregation. By increasing the weight of CNTs arrays, the number of available tubes for aggregation with the bacteria cells increases that enhances the effective sites and contact surfaces for the adherence of bacterial cells. Therefore, the aggregation between bacteria cell and CNT arrays increases. These observations confirm the high ability of CNTs arrays for removal of low bacterial concentrations from polluted water. The weight-dependence of CNTs arrays on the removal efficiency is in agreement with previous studies [10, 12, 14]. It means that CNTs arrays are similar to non array SWCNTs and MWCNTs, in that, all of them have the same interaction behavior in bacteria removal efficiency regarding to the weight dependence.

3.3.2. Contact time effect

In the next stage, the treatment time effect on the number of survival bacteria cells has been investigated by incubating bacteria cells with 0.05g CNTs arrays for 30 min. Figure 5 shows the statistical effect of contact time on the number of viable cells. At the initial contact time, the ratio of viable cells rapidly decreases, but after 30 min, it decreases smoothly until it becomes constant. At the initial stage of contact time, a large number of active vacant sites are available for adsorption of bacteria cells and after a period of time the remaining vacant surface sites of the CNTs arrays become lower.

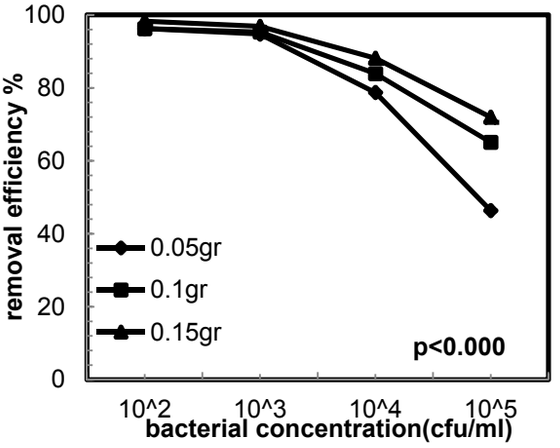

Figure 4: *The removal efficiency of Salmonella typhimurium by MWCNTs arrays at different weight of CNTs arrays and bacterial concentration .*

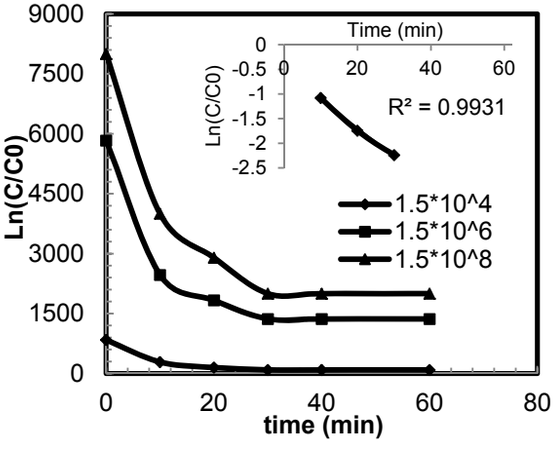

Figure 5: *Number of survival viable bacteria cells after contacting with MWCNTs arrays at 25°C and neutral pH .*

This rapid reduction of the viable cells at the initial time suggests that CNTs arrays have an effective adsorption potential and high capacity to remove bacteria from water. Also, the logarithmic reduction of viable cells for 1.5×10^8, 1.5×10^6 and 1.5×10^4 cfu/ml are 0.27, 1.24 and 2.25, respectively. These values indicate that the removal of the bacterial cells is more than 90%. The inset graph in Figure 5 shows the dependence of the logarithmic value

of the normalized residual bacteria cells against the time. This linear dependence is in agreement with the bacterial decay theory [22].

3.3.3. Compressing and crushing effects

Finally, compressing and crushing effects of carbon nanotubes arrays on the bacterial removal efficiency at three different weight of carbon nanotubes arrays and fixed bacterial concentration of 1.5×10^4 cfu/ml has been investigated. The results of bacterial removal efficiency for primary, crushed and compressed samples are shown in Figure 6. Results indicate that compressing process does not have any effect on the bacteria removal efficiency, while crushing process has a positive effect on the bacteria removal efficiency. Crushing process increases the removal efficiency of the bacteria by increasing the contact surfaces of the bacteria cells with CNTs. Further analysis show that the loss of crushed carbon nanotubes arrays increases about 13% compared to the primary sample. This value decreases to 3.3 % for compressed CNTs in comparison to the primary sample. Therefore, the use of compressed carbon nanotubes arrays can be a better choice for water disinfection instead of primary CNTs arrays.

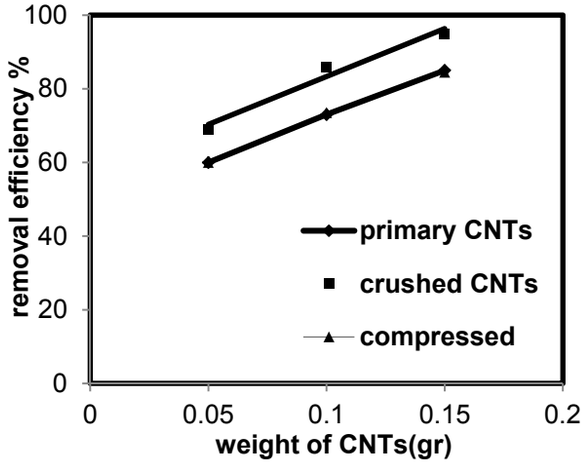

Figure 6: *The removal efficiency of Salmonella typhimurium by primary, crushed and compact of MWCNTs arrays at different weight of CNTs arrays .*

4. CONCLUSIONS

In summary, this study shows the high ability of CNTs arrays for removing the Salmonella typhimurium across a wide range of bacterial concentrations. Analysis of permeate samples and SEM images of CNTs indicate that Salmonella typhimurium cells were completely retained by CNTs arrays due to the adherance of bacteria cells to CNTs arrays via physical sorption and size exclusion such as seive mechanism. In addition, the removal efficiency increases at higher weight of CNTs arrays and lower rate of bacterial concentrations. Also, a high rate of removal efficiency is observed at high bacterial concentration when the treatment time increases. Moreover, compressing process does not have any effect on the bacteria removal efficiency, but it decreases the loss of CNTs arrays by transportation with water. So, using compressed CNTs arrays can be a better choice for water disinfection. These properties of CNTs arrays in omitting Salmonella typhimurium cells from water can be attributed to both adequate dispersion and regular structure of CNTs arrays. The lower lost of CNTs arrays because of their long lenght is another important parameters which recommend CNTs arrays as a novel choice for water treatment.

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