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Influence of Functionalization MWCNTs Using Acid Treatment on Gram Negative and Gram Positive Bacteria

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In this work, the effect of chemical acids (like, mixture of H_2SO_4, HNO_3 and H_2O_2) on the surface functionalization of multiwalled carbon nanotubes (F-MWCNTs) is illustrated. This effect leads to create functional groups like carboxyl (-COOH) and hydroxyl groups on the surface as well on the side walls of MWCNTs. The degree of covalent functionalization was examined by using Fourier transformed infrared spectroscopy (FTIR) which proved the formation of oxygen containing groups such as C=O and COOH. Transmission electron microscopy (TEM) used to characterize the morphological of surface modification on the MWCNTs structure due to oxidation by acid treatment during functionalization process. The antibacterial action of functionalized F-MWCNTs are tested for their efficiency in destroying the pathogenic bacteria, Gram negative (E. coli) and Gram positive (S. aureus) bacteria, in addition to evaluate of bacterial adsorption on F-MWCNTs that are attributed to their fibrous shape with high aspect ratio. The results obtained indicate that modified F-MWCNTs were able to remove from 80-90% of E. coli at large exposure times while a higher removal of bacteria (up to 100%) of S. aureus was achieved when F-MWCNTs was used under the same conditions and improved antibacterial action of F-MWCNTs for the removal of Bacteria from water using modified F-MWCNTs.

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1. Introduction

Carbon nanotubes (CNTs), including multi-wall carbon nanoutubes (MWNTs) and single-wall carbon nanotubes (SWNTs), have attracted considerable attentions in various nanotechnological applications, such as biological, biomedical, biosensors, electrochemical super capacitors, field-emitting devices, pharmaceutical, etc. due to their unique features such as one dimensional tubular structure, outstanding mechanical, electronic, thermal, and chemical properties since their discovery in 1991 [1].

However, lack of solubility of raw carbon nanotubes and the difficult manipulation in almost all solvents have imposed great limitations to the use of CNTs because of their high van der Waals interactions, surface area and high aspect ratio. A chemical functionalisation can be applied to overcome on these limitations, and offers the possibility of modifying the surface of the nanotubes [2].

The chemical functionalisation is useful to clean CNTs from catalyst particles and amorphous carbon; however, it causes the opening of tube terminations and the formation of defective site along the tube sidewalls. These sites become rich of oxygenated functionalities such as carboxylic groups (-COOH) and these functional groups make CNTs more hydrophilic and suitable for the adsorption of relatively low molecular weight and polar contaminants [3].

Antimicrobial materials such as CNTs are designed to remove, or at least prevent the growth of microbial species (e.g. bacteria), and are beginning to make a good effect on public health. With a rapid development of new biomedical devices and implants, antimicrobial surfaces are particularly important [4, 5].With the large scale production of carbon nanotubes and their wide applications, their antimicrobial activities have attracted much attention. In the recent years the cytotoxicity studies of MWCNTs expand from human cells to single celled organisms (bacterial cells) [6]. Also, it is found that cytotoxic nature plays an indirect role in improving the microbial sorption efficiency because of fibrous shape of CNTs that offer simultaneous capture and deactivation of pathogens, whereas other carbon-based filters offer only capture of pathogens [7-8].

In this work, the functionalisation F-MWCNTs were exhibited their antimicrobial activities to (*E. coli*) and (*S. aureus*) respectively. The rate of viable cell number is determined by the plating method which suggests that the antimicrobial activity of acid treated MWCNTs becomes higher with increasing time of shaker incubator. Therefore, the shaker incubator condition was efficient to promote the removal of a high concentration of *E. coli* and *S. aureus* from aqueous solution and could be a promissory method for deactivated bacteria.

2. Experimental work

The materials used in the paper represented by concentrated H_2SO_4 and nitric acid (3:1) from local markets, were used as the functionalization media. Raw-Multi-walled carbon nanotubes, MWNTs with Purity (> 95wt%, outside diameter: 5-15 nm, Inside diameter: 3-5 nm and Length: ~30µm /USA). All chemicals were used as-received without further purification. MWCNTs (0.1 g) were dispersed in a mixture of a concentrated H_2SO_4 and HNO_3 (3:1) under ultrasonication technique for 30 min to produce oxidized MWCNTs (F-MWCNT). The samples were washed with deionized water (D.I) and then dried at 70°C for 24 h.

Transmission electron microscopy (TEM) observations of the raw and acid treated-MWNTs were performed on EM208, Philips model (Al-Nahrien University, Iraq). The samples preparation was prepared by a tiny drop of well dispersed samples that are sonicated with ethanol solution for 15 min and was placed onto the carbon coated (TEM) copper grid and viewed under (TEM). The morphology was determined with acceleration voltage of 40-90 kV. The scanning scanning microscopy (SEM) measurements were performed with VEGA EasyProbe (nanotechnology and advanced material research centre, University of Technology, Iraq) operated at 200 V to 30kV. The samples were sonicated with ethanol solution for 15 min and small drop of these samples are placed on glass slide allowed to dry. The surfaces of the raw and acid treated-MWCNTs were coated with silver paste coated to make the samples conductive at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge at the surface.

A Fourier transforms infrared (FTIR) spectra of raw and acid treated-MWCNTs were obtained using 8400S, Shimadzu model spectrophotometer (nanotechnology and advanced material research centre, University of Technology, Iraq). In addition, the changes in the morphology of both Gram negative and Gram positive bacteria can be studied using (SEM) VEGA EasyProbe, when they were interacted with treated -MWNTs.

The antibacterial activity of the acid treated-MWCNTs was tested against two bacterial strains of Staphylococcus aureus (*S. aureus*) and Escherichia coli (*E.coli*) supplied by (the stock collections of Laboratory of Biology/ Department of Applied Science, Technology University /Baghdad- Iraq) with the shake flask method. The bacteria were subculture on nutrient agar (N. agar) and incubated for 24 h at 37°C. The cells were suspended in 50 ml of normal saline 0.9% NaCl to yield a bacterial suspension of 10^7 colony forming units/ml (CFU/ml). The sample powder (3 mg of MWCNTs) was weighed and shaken in 9 ml of a bacterial suspension for various exposure times (1h,3h,24h) respectively. The suspension was serially diluted in normal saline and cultured on nutrient agar as shown in Fig. 1 (a, b) (using 100µl spread out on a solid agar) at 37°C for 24 h. Then the number of viable organisms in the plates was determined by multiplication of the number of colonies with the dilution factor, and the percentage reduction was calculated on the basis of the initial count.

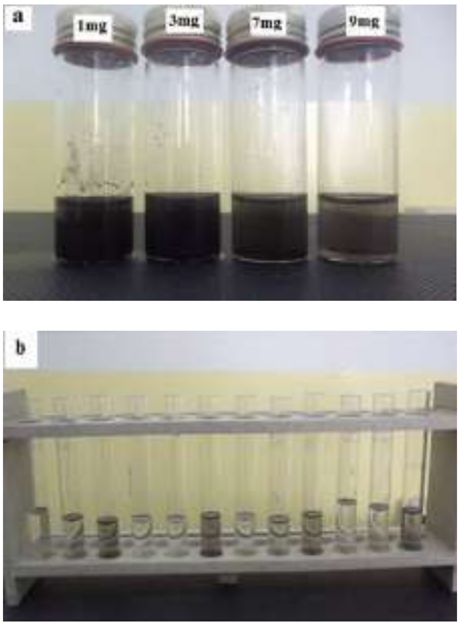


Fig. (1) photograph of preparation Antimicrobial material with a) different weight of F-MWCNTs in a solution of 0.9% NaCl , and b) diluted cell suspensions of each sample

3. Results and discussion

FTIR Spectrum software was used to generate a plot of absorbance (%) vs. wave number (cm^{-1}) in the wave number range about 500 to 4000 cm^{-1} for functionalized MWCNTs (F-MWCNT) by acid treatment (blue line) and raw-MWCNT (green line), respectively as shown in Fig.2(a, b). Here, one can observe typical bands that correspond to carboxyl groups -COOH and -OH produced on the functionalized MWCNTs (F-MWCNT) surface. As shown in Fig. 2(b) the peak at 1635 cm^{-1} is due to C=C stretching of the CNTs.

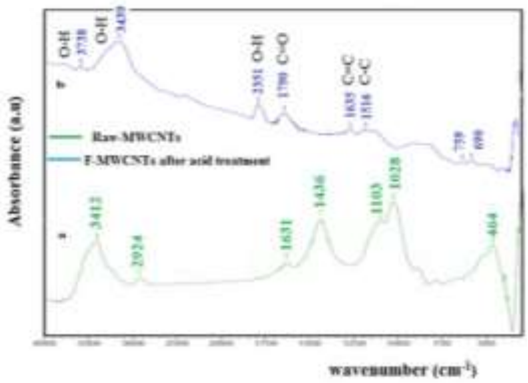


Fig. (2) FTIR spectra of raw-MWCNTs a) and b) functionalized MWCNT COOH with acid treatment

For treatment acid, broad absorption band has been seen at 3439 and 3738 cm^{-1} that attributed to the O-H stretch of the hydroxyl group. These bands can be ascribed to the oscillation of hydroxyl groups -OH. The carboxylic groups of F-MWCNTs were confirmed by FTIR with stretching bands of carboxylic acid groups at 1790 cm^{-1} as shown in Fig. 2 (b) and carboxyl groups on the surfaces of F-MWCNTs could be due to the partial oxidation of the surfaces of MWCNTs during treatment by mixture of acids. Some peaks were seen around 1516 cm^{-1}, that is originated from C-C bonding indicate the presence of the MWCNTs, however, despite of the damage, all the nanotubes still maintain their carbon network structure. The peak at 2351 cm^{-1} can be associated with the O−H stretch from strongly hydrogen-bonded –COOH.

Figure (3) shows the TEM images of pristine-MWCNT (a) and acid treated MWNT (b). In Fig. 3(a) long nanotubes with relatively well graphitized walls are observed. Furthermore, large agglomerates and closely packed MWCNTs are common in raw-MWCNTs, which become loosened and highly dispersed after acid treatment. By comparison; Fig. 3 (b) shows F-MWNTs with some damage caused by oxidation, with what appears to be short nanotubes produced by the breaking of some large nanotubes.

The oxidation treatment could result in a high density of surface functional groups on the raw-MWCNTs; among the functional groups are carboxyl, hydroxyl, and carbonyl groups which lead to enhance the dispersion of the MWNTs and this might be due to the -OH groups formed in the acid treatment state that make hydrogen bonding with water molecules. The typical diameters of F-MWCNTs, after treatment, were about a few nanometers (10 to 25 nm). It was demonstrated that after being treated in chemical acid, there are sufficient –COOH, –CO and –OH groups produced on the surface of F-MWNTs, and the F-MWNTs become negatively charged.

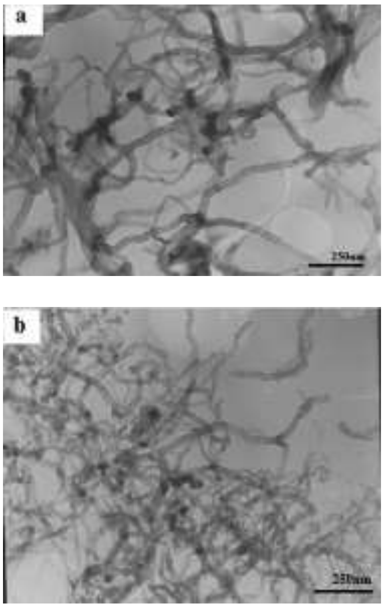


Fig. (3) TEM images of pristine-MWCNT (a) and acid treated MWNT (b)

In Fig. 4(a, b) it is observed that N. agar plates is incubated for 24h with 100µl samples and came from tests tube containing *E. coli* and *S. aureus* bacteria with treated-MWCNTs at various time of shaker incubator, respectively. The results showed a significant reduction of CFU/ml after increasing time of shaking for both two types and become higher for *S. aureus*. Since the rate of viable cell number was determined by the plating method of both *E. coli* and *S. aureus* bacteria and showed only limited colonies for *E. coli* as shown in Fig. 5(a) with rate about 80-90% and no colonies 100% for *S. aureus* from the initial numbers as shown in Fig. 5(b) indicating that the treated-MWNTs have obvious antimicrobial activity.

Furthermore, the results obtained in this section indicate that the number of deactivated *S. aureus* using F-MWCNTs is 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 µm is higher than that with size greater than 1 µm and F-MWCNTs becomes a good penetrate to the wall of adsorbed bacteria.

Moreover, Fig. 6(a,b,c,d) shows the SEM images of cells of bacteria interacted with treated-MWCNTs and incubated for 60 min in normal saline. From Fig. 6(a,b), some of cells are setting on the top of F-MWCNTs filters, representing excellent adsorption while the others are interacted with MWCNTs and penetrated viable cells and disrupted of the integrity of the cell membrane. Similarly, F-MWCNTs were captured *S. aurous* and wound around the curved surface of *S. aurous* as shown in Fig. 6(c,d).

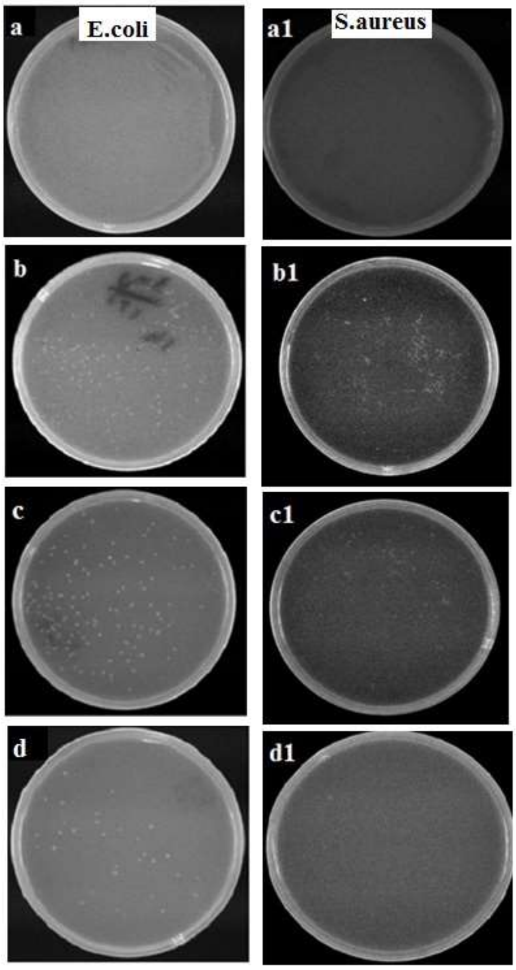


Fig. (4) Photographs of N. agar plate of test tubes with *E. coli* and *S. aureu* bacteria treated with 3mg/ml of MWCNTs incubated for 24 h at 37 °C after different exposure times using shaker incubator at a,a1) control, b,b1) after 1h, c,c1) after 3h and d,d1) after 24h treated with 3mg MWCNTs

Depending on the above results, the proposal suggestions of antimicrobial action of treated-MWCNTs are represented by (a) highly dispersed MWCNTs deposited on filters had strong ability to adhere and wind to bacterial cells due to the flexibility and fibrous shape of the nanotubes improving the microbial adsorption efficiency,(b) the carboxyl and hydroxyl groups in treated-MWCNTs provide more polycationic which in turn increase the permeability of cell membrane and cause cells death and thus it significantly enhance the antimicrobial activity against the *E. coil* and S. aureus and (c) the role of time of shaker incubator of suspension involves the direct contact of membrane cell with MWCNTs that can cause adsorption of bacteria or membrane cell damage and subsequent cell inactivation. On the other hand, the adsorption in CNTs is based on surface area and thus, high molecular weight portion of bacteria is adsorbed relatively strongly.

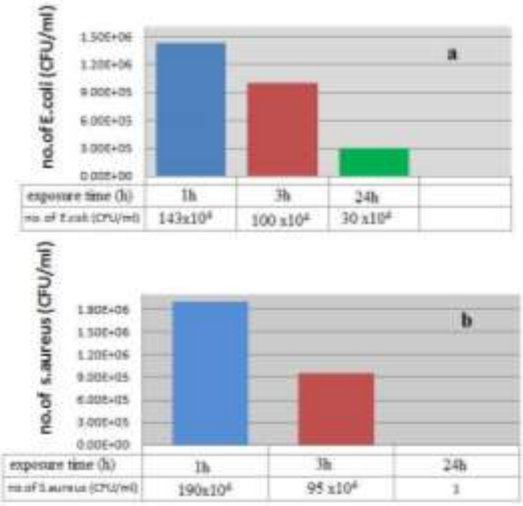
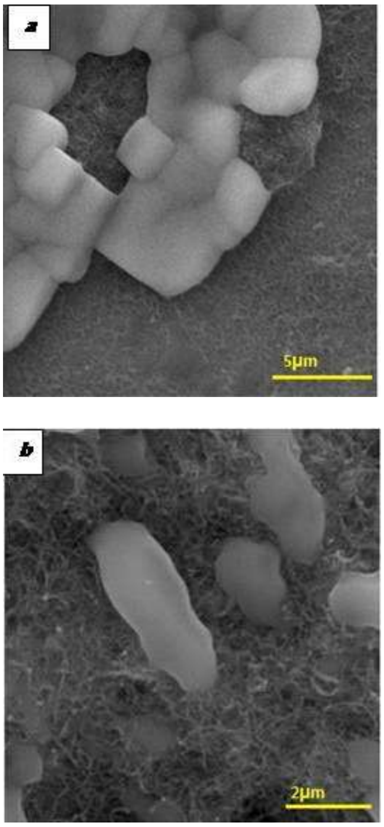


Fig. (5) histogram diagram of antimicrobial activity of treated-MWCNTs against a) gram negative and b) gram positive bacteria after different exposure times



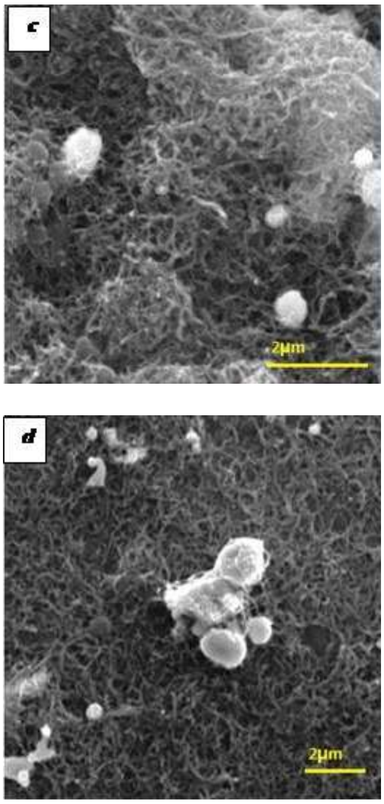


Fig. (6) SEM images of bacteria attachment on the surface of treated MWCNTs after different exposure times with e.coli after a) 3h of exposure time b) 24h of exposure time and *S. aureus* with treated MWCNTs after c) 3h of exposure time and d) after 24h of exposure time

4. Conclusions

The surface modification on the MWCNTs and its antibacterial activity against *E. coli* and *S. aureus* bacteria were investigated. The degree of covalent functionalization was examined by using FTIR spectroscopy. Transmission electron microscopy (TEM) characterized the occurrence of surface modification on the MWCNTs structure due to oxidation during functionalization by acid treatment.

The functionalization of carbon nanotubes induces an increase of the percentage of inhibition from 80-90% for *E. coli* and 100% for *S. aureus* from the initial numbers for *S. aureus* with increasing the exposure time and evaluation of bacterial adhesion on F-MWCNTs and proved to destroy the bacteria successfully.

CNTs registered superior adsorption capacities in the removal of diverse range of biological contaminants including bacteria which are attributed to their fibrous shape with high aspect ratio, provision of large external surface area that can be easily accessed by biological contaminants, and presence of well-developed mesopores.

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