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Effectiveness of Multi-Walled Carbon Nanotubes and Activated Carbon for Capturing *E. coli* 0157:H7 for Application in Water Filtration

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Abstract: The ability of unmodified and functionalized Carbon Nanotubes (MWCNTs) to capture *Escherichia coli* 0157:H7 from water was tested and compared with Activated Carbon (AC). This study aims to test the effectiveness of these materials to filter out this bacteria strain. Chemical oxidation was used to functionalize MWCNTs to achieve the desired oxygenated functional groups which was analysed with Fourier Transform Infrared spectroscopy (FTIR). Field Emission Scanning Electron Microscopy (FESEM) was used to verify bacteria capture after water filtration on both functionalized and unmodified MWCNTs and AC, respectively. Results showed that the bacteria were captured on functionalized MWCNTs and unmodified AC, however, the bacteria were not detectable on unmodified MWCNTs and functionalized AC. The results indicate that the presence of specific carboxyl functional groups on the surface of functionalized MWCNTs and morphology of the unmodified AC is relevant for *Escherichia coli* 0157:H7 capture.

Key words: *Escherichia coli* 0157:H7, carbon-based nanomaterials, functionalized Multi-walled Carbon Nanotubes (MWCNTs), Activated Carbon (AC), functional groups, capture

INTRODUCTION

Carbon-Based Nanomaterials (CNMs) possess a high degree of carbon purity the size can range from 1-1000 nm. For the last few years, researchers have shown interest in developing a membrane with advanced properties using CNMs (Menawi *et al*., 2016). Carbon Nanotubes (CNTs) are one of the many CNMs, whose properties have been extensively studied for various applications, ranging from biomedical to environmental. A CNT consists of carbon atoms bonded together trigonally in a tube-like structure; multiple layers of this structure are fused together, resulting in a Multi-Walled Carbon NanoTube (MWCNTs). Activated Carbon (AC) is another organic compound material that is commonly used in water treatment. The common AC that has been used for decades is known as charcoal and has been the main component used for bacterial disinfection (Bhatnagar *et al*., 2013). AC has a porosity that is bigger than that of nanomaterials with a different elemental composition it can be easily acquired and produced from organic material by a conventional heating decomposition method (Hesas *et al*., 2013).

Fresh-water scarcity is an urgent environmental problem and global warming contributes towards the scarcity; research shows that by 2030, the population of one-third of the developing countries might live in river basins suffering water stress a condition in which the annual water supply drops below 1700 m^3 per person (Goh *et al*., 2013). The condition worsens when natural water from a forest stream is contaminated owing to logging activities and pollution. Biological contamination from organisms such as bacteria, viruses and algae has polluted some water (Kolangikhah *et al*., 2012), especially as an after effect of flood conditions. Some strains of bacteria such as *Escherichia coli* (*E. coli*) 015:H7 can cause harm to susceptible individuals and even entire communities who consume the contaminated water and temperate climates such as South-East Asia are optimal for bacteria to thrive and proliferate.

AC filters water through the principle of adsorption, and the characteristics of the carbon materials in terms of porosity and surface Chemistry affect the adsorption process these materials are known to be suitable only for filtering microbiologically safe water and for reducing chlorine and organic chemicals (Ann *et al*., 1995). CNTs

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have been shown to have a tubular structure with surface that can be chemically modified with oxygenated groups, these properties are promising for a filter material for bacteria capture. In this study, we evaluated the ability of functionalized and unmodified MWCNTs and AC to filter the bacteria strain *E. coli* 015:H7. Since, both porosity and surface chemistry can affect adsorption, we sought to test these parameters in both materials with regard to bacteria filtration. The functionalization of CNTs is said to be the precondition requirement in CNT-based water treatment and purification (Das *et al.*, 2014a, b). Our goal is to develop a water-filtration system that utilizes MWCNTs not only for filtering organic contaminants but also for filtering bacteria.

MATERIALS AND METHODS

Materials ad reagents: Commercial MWCNTs were obtained from University Sains Malaysia, Nibong Tebal, Malaysia. In Transmission Electron Microscope (TEM) images, these MWCNTs have average lengths of 1-5 μm and an average diameter of 10 nm. ThermoGravimetric Analysis (TGA) shows carbon purity >99% with residuals <10% and amorphous carbon <0.1%. The bacteria strain, *E. coli* O157:H7 was purchased from Essen-Haus, Malaysia. Regenerated Cellulose (RC) membrane filters with diameter of 47 mm and pore size of 0.2 μm were purchased from Sartorius AG (Germany).

Chemical oxidation treatment of MWCNTs and AC: Chemical oxidation of MWCNTs follows closely published methods (Wepasnick *et al.*, 2011; Marshall *et al.*, 2006; Datsyuk *et al.*, 2008) as shown in Fig. 1. For functionalization, 1 g of MWCNTs was poured into a 150 mL round-bottom flask using a glass filter funnel. The 98% concentrated sulfuric acid (H_2SO_4) and 65% concentrated nitric acid (HNO_3) were mixed at a ratio of 3:1 with a final volume of 80 mL. The mixture was refluxed for 8 h at 70°C. The top part of the reflux condenser was sealed during the reaction to prevent dispersion of vapor. After the reaction was completed, the sample was poured into a 500 mL beaker consisting of 250 mL of deionized water (DI H_2O) to be diluted and underwent a final rinse with ethanol before filtered using a vacuum pump to produce a composite membrane filter of RC and f-MWCNTs. Experiment was repeated by replacing MWCNTs with AC for functionalization.

Bacterial dilution: To prepare the bacterial sample, 1 mL solution containing 102 CFU/mL heat-killed *E. coli* O157: H7 was serially diluted into 5 CFU/mL where 0.05 mL of 102 CFU/mL heat-killed *E. coli* was pipetted into a 2 mL microcentrifuge tube. About 0.95 mLof DI H_2O was pipetted into the same microcentrifuge tube to yield a 1/20 dilution using Eq. 1. The 1 mL of the 1/20 diluted sample was then stored at -20°C for further use:

$$\text{Dilution} = \frac{\text{Final vlume}}{\text{Solution volume}} \quad (1)$$

Bacterial filtration: The prepared filter membrane composed of RC and MWCNTs or AC are used as to filter the bacteria. Filtration was done twice in order to optimize the capability of the membrane filter composite to capture the bacteria. For the first filtration cycle, 0.1 mL was taken from the 1 mL of 1/20 dilution stored sample and was pipetted into 99.9 mL tap water and stirred thoroughly. Given the dilution, it was assumed that ~½ colony of 25000 cells was present in the 0.1 mL of sample solution.

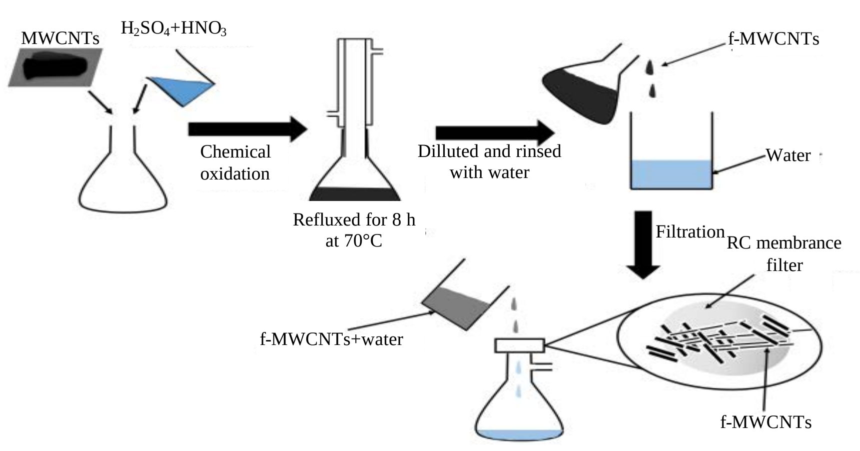

Fig. 1: Experimental setup for chemical oxidation of MWCNTs

The prepared composite membrane filter will be used to filter 100 mL of sample in tap water using the vacuum pump and then dried in a vacuum desiccator at 27°C for 12 h. The composite filter membrane system undergoes second filtration with 50 mL of sample water (49.9 mL tap water +0.1 mL of the dilution sample) by pipetting the sample water slowly onto the membrane filter. The membrane filter was then dried in a vacuum desiccator at 27°C for 12 h. The filtration step was repeated by using unmodified and modified MWCNTS and unmodified and modified AC.

Characterization of CNMs: The functional groups were analyzed using Fourier transform infrared spectroscopy (FTIR, Thermo Scientific model iS50), performed at MIMOS Technology Solutions Sdn. Bhd., Seri Kembangan, Selangor, Malaysia. Sample preparation was not needed because analysis was by Attenuated Total Reflectance (ATR-FTIR). Characterization of the surface features was conducted via. field emission scanning electron microscopy (FESEM, Hitachi model SU8030).

RESULTS AND DISCUSSION

Unmodified Multi-Walled Carbon NanoTubes (MWCNTs) and Activated Carbon (AC): The MWCNTs obtained in powder form with 99% purity were analyzed by FTIR. Figure 2 shows the FTIR results (percent transmittance vs. wavenumbers) for unmodified MWCNTs and AC (Fig. 2a-c). Functionalized MWCNTs (f-MWCNTs) and functionalized AC (f-AC) are shown in Fig. 2b-d. Stretching vibrations was recorded from 1000 to 4000 cm^{-1} with the spectrum transmittance ranging from 0-30%. From Fig. 2a, no functional groups can be found on the surface of the MWCNTs but traces of other elements can be seen as impurities.

Activated Carbon (AC) was acquired by purchasing a commercial water filter from Tupperware®. The company claims that the organic compounds in the unmodified AC in the filter can remove up to 99.9% of bacteria. An FTIR analysis was done to identify the functional group of the unmodified AC. Figure 2c shows the percent transmittance versus wavenumbers obtained from FTIR analysis. Stretching vibrations was recorded from 1000-4000 cm^{-1} with high spectrum transmittance recorded ranging from 0-65%. As with unmodified MWCNTs, no functional groups can be found on the surface of the AC but the carbon shows traces of other elements.

Functionalized MWCNTs and AC: The data for f-MWCNTs and f-AC are shown in Fig. 2b-D carbonyl (C = O) and Hydroxy (-OH) groups were found on the MWCNT surfaces. The spectrum of transmittance shows a blunt peak indicating C = O groups and a broader range of -OH groups (Ma *et al*., 2010). The existence of carboxyl (COOH) groups confirms successful functionalization of MWCNTs into COOH-MWCNTs. It is obvious that the COOH groups on MWCNTs can be the main functional groups involve in bacteria capture and is renowned for its antimicrobial properties.

Figure 2d shows that multiple functional groups, including COOH groups, were produced on the surface of AC. Several broad ranges of -OH groups can be observed at wavelength of 3354, 2516 and 1979 cm^{-1} while

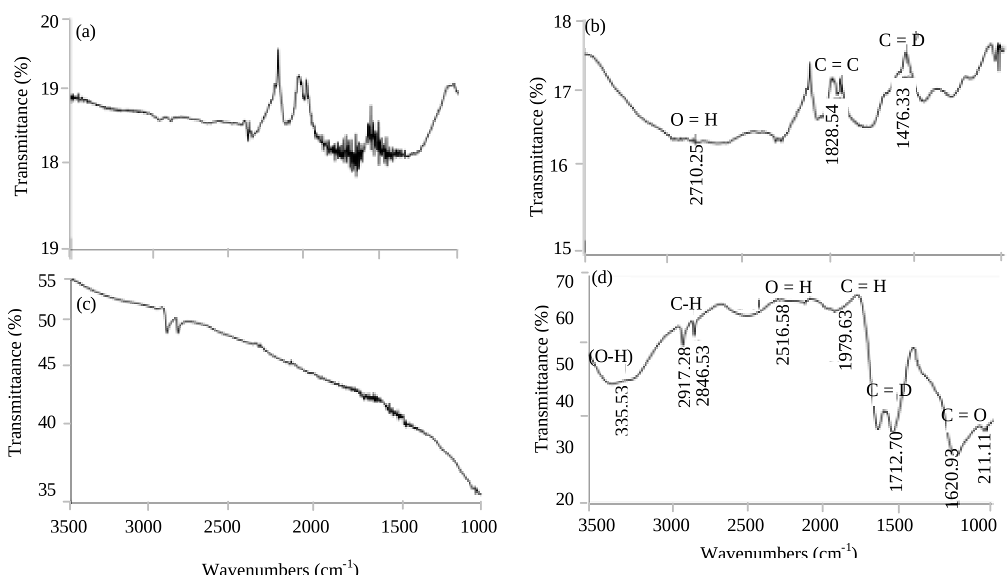

Fig. 2: Comparison of the FTIR results: a) Unmodified MWCNTs; b) f-MWCNTs; c) Unmodified AC and d) f-AC

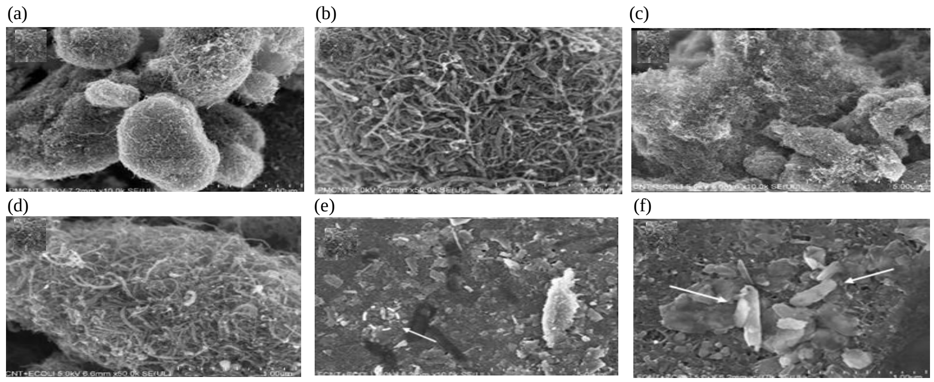

Fig. 3: FESEM micrographs of unmodified MWCNTs before filtration of bacteria (a and b), unmodified MWCNTs after filtration of bacteria (c and d) and f-MWCNTs after filtration of bacteria (e and f) (arrows pointing at bacterial cells)

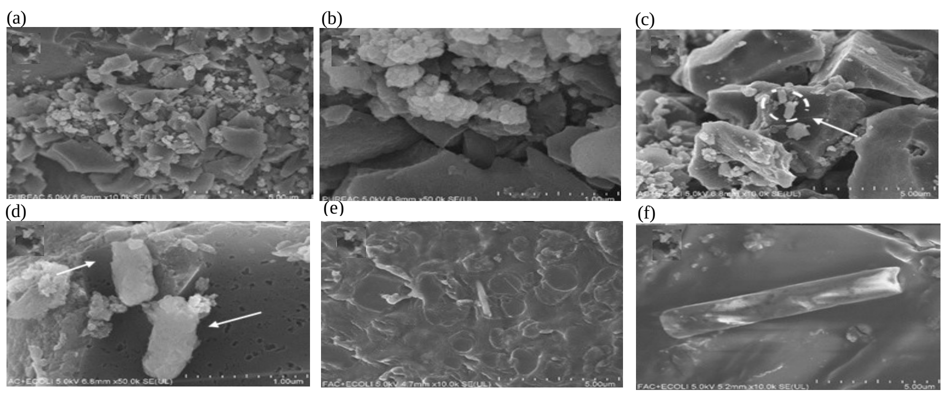

Fig. 4: FESEM images of unmodified AC before filtration of bacteria: a and b) Unmodified AC after filtration of bacteria; c and d) (Arrows pointing at bacterial cells) and e and f) f-AC after filtration of bacteria

the C = O produced sharp peaks at wavelengths of 1712 and 1620 cm^{-1}. Other peaks were produced by alkane (C-H) and ester (C-O) groups. It is interesting to see the effect of multiple functional groups on AC with respect to its effectiveness to capture bacteria.

Characterization of CNMs

Multi-walled carbon nanotubes: The morphology of unmodified MWCNTs is shown in Fig. 3 at different magnifications. MWCNT agglomerates into ball-like structures as seen in Fig. 3a. A closer look at the MWCNT surface at higher magnification is shown in Fig. b showing the interconnected network of tubular MWCNTs. MWCNTs were used as a negative control the material is known to be hydrophobic and no bacteria can attach to its surface. The results shown in Fig. 3c-d confirm this no bacteria were present on the MWCNTs surface.

The morphology of the f-MWCNTs is totally disrupted after functionalizatio as seen in Fig. 3e. The interconnected tubular MWCNT network has disappeared. However, after filtration, rod-like structures can be seen on the f-MWCNT surface (Fig. 3f). From the size, these structures were identified as *E. coli* whose length is known to be ~1-2 μm (Ma *et al.*, 2010). A few tube-like structures can be seen at the side of the image which were similar to unmodified MWCNTs (Fig. 3f). Attachment of bacteria to f-MWCNT surfaces suggests that the material structure with COOH functional groups is suitable for bacteria filtration.

Characterization of Activated Carbon (AC): Before filtration, FESEM images exhibit various structural shapes and sizes as shown in Fig. 4ab. Nevertheless, after filtration (Fig. 4cd), rod-like structures were seen on the AC surface. These structures are ~1 μm in size, indicative of the presence of *E. coli*. The bacteria were surrounded by a bubble like structure (Fig. 4d). A closer look at higher magnification in Fig. 4d shows the ends of the *E. coli* show to burst (Ma *et al.*, 2010). The cell burst could be due to the collision with the sharp edges of the material that sheared the structure of the cell.

Figure 4e and f shows the FESEM images of f-AC after bacteria filtration. The f-AC shows a different morphology from unmodified AC where f-AC is a smooth blackened surface as shown clearly in Fig. 4e. In Fig. 4f, a rod-like shape was assumed to be bacteria, however, owing to its size, this could not be the case. Despite having various functional groups, the performance of f-AC in capturing bacteria was not a success possibly due to its unfavourable morphology.

CONCLUSION

In summary, f-MWCNTs and unmodified AC successfully attach *E. coli* bacteria to their surfaces. Functional groups and morphology of material play an important role in bacteria attachment existing functional groups on f-MWCNTs and unmodified AC indicate the potential of these materials for use as filter material for bacteria.

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