Effect of Raw-SWCNTs, SWCNTs/ Ag-TiO$_2$ nanoparticles for control the biological contaminates in water Treatment

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Abstract: In this study, hybrid material like Ag-TiO$_2$ coated single-walled carbon nanotubes (Ag–titania hybrid/SWCNTs) was formation by a multistep chemical procedure and evaluated for their application potentials as an antimicrobial agent for slowing down bacterial growth as bacteriostatic and inactivating pathogenic. The structural and morphological properties of Raw-SWCNTs and SWCNTs/TiO$_2$-Ag nanoparticles were investigated by using X-ray diffraction (XRD), Field Emission Scanning Electron Microscope (FESEM) and Raman spectroscopy improving the polycrystalline structure of Ag and TiO$_2$ NPs decorating SWCNTs due to chemical method. As well as, Raman spectroscopy, reveal the formation of nanocomposite by increasing the intensity of D to G and disorder of carbon structure with Ag-TiO$_2$ interaction. In addition, the antimicrobial properties of nanocomposite material (SWCNTs/TiO$_2$-Ag) were tested against bacterial strains, including E. coli as Gram negative by using standard agar dilution (plate count) method to inhibit microbial growth in water treatment.

1. Introduction

Based-carbon nanomaterials have greater bactericidal inactivation efficiency, in addition to improve viral and bacterial spore adsorption capability, as compared to traditional adsorbent substances, because of their larger surface areas [1]. To illustrate, SWCNTs have a specific surface area of around 400m$^2$/g. One more scientific researcher also has demonstrated that SWCNTs show the affinity for fixed groups of pathogens and this removal favorite is essential, since it has the possible ability to remove pathogens selectively over nonpathogenic microorganisms. This property of removing microbial rate for pathogens is a necessary feature for the improvement the filters or membranes that using in water treatment devices, and for the improvement of sensors [2]. In general, increased levels of fecal like negative bacteria E.coli contamination provide a warning of failure in water treatment, a break in the integrity of the distribution system and possible contamination with pathogens, hence nanotechnology is an enormously powerful technology, which holds a huge promise for the design and development of many types of novel products with its potential medical applications on early disease detection, treatment, and prevention [3]. The most common metal/metal oxides used in decorating carbon nanotubes as hybrid materials for water treatment applications include Zn, ZnO, Al, Al$_2$O$_3$, TiO$_2$ and Ag [4-8]. The combination of metal or metal oxides in
hybrid materials has been presented to develop the disinfection and adsorption properties of carbon based nanomaterials [9]. Among metal oxides, titanium dioxide (TiO₂) represented one of the most frequently used semiconductor to prevent bacterial activity due to its chemical and physical stability, high photocatalyst activity, high oxidative power, and harmless for environment [10]. In addition, the deposition SWCNTs with TiO₂ can give functional features, like increasing of the surface area, high mechanical strength, and high conductivity because of their unique electrochemical properties [11]. CNTs have high electron-storage capacity and it may accept the photo-generated electrons from the TiO₂ conduction band. Besides, Ag-NPs have electronic properties, which make it a good choice for utilizing as a dopant into other metals. Therefore, silver can prevent the recombination method of the hole-electron pairs through captured the titanium generated electrons, acting as electron traps. [12]. Herein, we prepared TiO₂-Ag decorating SWCNTs by using sol-gel method and forming nanocomposite. The SWCNTs were functionalized by using mixture of acid treatment to introduce functional groups. Then, the antibacterial activity of nanocomposite was evaluated towards gram negative bacteria which represented the most important pollutant bacteria in waste water.

Carbon based nanomaterials have a highest bactericidal inactivation efficiency, in addition, to improve viral and bacterial spore adsorption capability, as compared to traditional adsorbed substances, because of their larger surface areas [1]. To illustrate, SWCNTs have a specific surface area of around 400m²/g. One more scientific researcher also has demonstrated that SWCNTs show the affinity for fixed groups of pathogens and this removal favorite is essential since it has the possible ability to remove pathogens selectively over nonpathogenic microorganisms. This property of removing the microbial rate for pathogens is a necessary feature for the improvement of the filters or membranes that are used in water treatment system and for the improvement of sensor's device [2]. In general, increased levels of fecal like negative bacteria E.coli contamination provide a warning of failure in water treatment, a break in the integrity of the distribution system and possible contamination with pathogens, hence nanotechnology is an enormously powerful technology, which holds a huge promise for the design and development of many types of novel products with its potential medical applications on early disease detection, treatment, and prevention [3]. The most common metal/metal oxides used in decorating carbon nanotubes as hybrid materials for water treatment applications include Zn, ZnO, Al, Al₂O₃, TiO₂, and Ag [4-8]. The combination of metal or metal oxides in hybrid materials has been presented to develop the disinfection and adsorption properties of carbon based nanomaterials [9]. Among metal oxides, titanium dioxide (TiO₂) represents one of the most frequently used semiconductor to prevent bacterial activity due to its chemical and physical stability, high photocatalyst activity, high oxidative power, and harmless to the environment [10]. In addition, the deposition SWCNTs with TiO₂ can give functional features, such as increasing of the surface area, high mechanical strength, and high conductivity because of their unique electrochemical properties [11]. CNTs have the high electron-storage capacity and it may accept the photo-generated electrons from the TiO₂ conduction band. Besides, Ag-NPs have electronic properties, which make it a good choice for utilizing as a dopant into other metals. Therefore, Ag-NPs can prevent the recombination method of the hole-electron pairs through captured the titanium generated electrons, acting as electron traps. [12]. Herein, we prepared TiO₂-Ag decorating SWCNTs by using the sol-gel method and forming nanocomposite. The SWCNTs were functionalized by using a mixture of acid treatment to introduce functional groups. Then, the antibacterial activity of nanocomposite was evaluated towards gram negative bacteria which represented the most important pollutant bacteria in waste water.

2. Synthesis of SWCNTs/TiO₂-Ag nanocomposite

In this study, the preparation of TiO₂-Ag decorating SWCNTs is produced by sol-gel method. First, 0.1 g of raw-SWCNTs was acid treatment by using mixture of 95% H₂SO₄ and 50% HNO₃ in a ratio (3:1) and ultrasonic in bath for 1h to achieve functionalized SWCNTs with functional groups. Then, the
In this study, the TiO$_2$-Ag decorating SWCNTs was produced by the sol-gel method. First, 0.1 g of raw-SWCNTs were acid treated by using a mixture of 95% H$_2$SO$_4$ and 50% HNO$_3$ in a ratio of 3:1 and sonicated in a bath for 1 h to achieve functionalizing the SWCNTs with functional groups. Then, the mixture was washed by using 400 ml of deionized water (DIW) in vacuum filtered through a 0.22 μm cellulose nitrate membrane. After that, the filtered F-SWCNTs were dried in an oven at 100 °C for 24 h. Preparation hybrid Ag-TiO$_2$ was produced by dispersing 0.02 g of AgNO$_3$ and 0.3 ml of titanium isopropoxide (TTIP) with molar ratio (Ag/TiO$_2$ = 5%) in 100 ml of DIW and stirred the mixture with a high speed for 15 min to obtain homogenous gel solutions at room temperature. Afterward, 200 mg of F-SWCNTs was dispersed in 100 ml of DIW and sonicated for 15 min, then added the mixture of (AgNO$_3$ and TTIP) and stirred for 1 h. Then, 2 ml of 0.5 M nitric acid was added as drops to adjust the solution with pH equal to 4. Finally, the mixture was dried at 100 °C for 24 h and calcined at a temperature of 450°C for 2 h to produce SWCNTs/TiO$_2$-Ag nanocomposite.

2.1 Material characterization

After preparing SWCNTs/TiO$_2$-Ag samples, the XRD (6000, Shimadzu, Japan) is used for determination of phases and crystalline structure of nanocomposite as compared with R-SWCNTs. The Raman analysis was done by using (Renishaw Invia, Germen) set in range (500-3000) cm$^{-1}$. For characterization of prepared nanocomposites morphology, Field Emission Scanning Electron Microscope (FESEM, TESCAN, Mira3, Day Petronic Co., Iran) was done.

The characterization techniques were all used to characterize both materials (R-SWCNTs and SWCNTs/TiO$_2$-Ag). The x-ray diffraction (XRD / 6000, Shimadzu, Japan) was used to determine the phases and crystalline structure of the nanocomposite (SWCNTs/TiO$_2$-Ag) as compared with R-SWCNTs. The Field Emission Scanning Electron Microscope (FESEM, TESCAN, Mira3, Day Petronic Co., Iran) used to analyze the morphology of both structures. Raman spectroscopy (Renishaw Invia, Germen) was also used in a range (500-3000) cm$^{-1}$ to analyze the structures of the same materials.

2.3 Antibacterial Test

The antibacterial activity of R-SWCNTs and SWCNTs/TiO$_2$-Ag nanocomposite was evaluated by using plate count method against gram negative bacteria (E. coli). The E. coli was supplied by Center of Nanotechnology and Advanced Materials/University of Technology/Iraq. The activated bacterial culture of E. coli was subculture upon nutrient agar at (37°C) for 18 h, after that, bacteria was suspended in the normal saline (0.9% W/V NaCl) to obtain control $10^5$ (CFU/ml) of standard McFarland tube No.5. (2 mg/ml) of each sample were suspended in 9 ml of distal water and sterilization in autoclave for 30 min at 120°C before evaluation. 1 ml of bacterial suspended has been added to R-SWCNTs and SWCNTs/TiO$_2$-Ag nanocomposite and keeping in a shaking incubator at 37°C (170 rpm) for 1 h. One hundred microliter of the bacteria suspension was spread on nutrient agar and incubation at 37°C for 24 h. then, the existing of bacteria colony was determined by the formula [13].

Rate of bacteria killing = \((A-B)/A\) x 100%
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Rate of bacteria killing = ((A-B)/A) x 100%

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3. The Results and Discussion

According to the results of XRD spectrum in Figure 1 (a), the peak phases of the R-SWCNTs displays a strong peak intensity at 20 = 25.40° which is characteristic peak for (002) crystal plane, related to an interlayer distance of 3.5 Å as well as weak peak intensity at 20 = 43.70° which is characteristic peak for (100) crystal plane. Furthermore, in Figure 1 (b), The XRD pattern of SWCNTs/TiO$_2$-Ag nanocomposite displays strong peaks of silver and titanium at 20 = 25.2, 37.4, 44.5, 47.3, 54.2, and 55.6 that related to tetragonal anatase of TiO$_2$ planes TiO$_2$ (101), TiO$_2$ (004), Ag(111),Ag(200), TiO$_2$ (200), TiO$_2$ (105) and TiO$_2$ (211), respectively. These peak positions are in good agreement with the standard (JCPDS card No. 21-1272) of TiO$_2$ and (JCPDS card No. 04-0783) which corresponding to silver metals [13,14]. As well as the peak of SWCNTs at 25.40° is not seen in the XRD pattern Figure 1(b), it suggested due to more presence of titanium and silver in comparison to R-SWCNTs figure 1 (a).

According to the results of XRD spectrum shown in Figure 1 (a), the peaks of the r-SWCNTs displays a strong intensity at 20° = 25.40° with an interlayer distance of 3.5 Å, which is related to the characteristic peak of (002) reflection plane. Another weak peak intensity at 20° = 43.70° which is characteristic of the (100) reflection plane. Furthermore, the XRD pattern shown in Figure 1 (b) displays the strong peaks of silver and titanium of SWCNTs/TiO$_2$-Ag at 20° = 25.2°, 37.4°, 44.5°, 47.3°, 54.2°, and 55.6°, that are related to the tetragonal anatase of TiO$_2$ planes TiO$_2$ (101), TiO$_2$ (004), Ag(111),Ag(200), TiO$_2$ (200), TiO$_2$ (105) and TiO$_2$ (211), respectively. These peak positions are in good agreement with the standard (JCPDS card No. 21-1272) of TiO$_2$ and (JCPDS card No. 04-0783) which corresponding to silver metals [13,14]. As well as the peak of SWCNTs at 25.40° is not seen in the XRD pattern Figure 1(b), it suggested due to more presence of titanium and silver in comparison to r-SWCNTs Figure 1 (a).
Figure 1: XRD analysis of a) R-SWCNTs and b) nanocomposite SWCNTs/TiO$_2$-Ag with peak phases

![XRD analysis](image1)

The Figure 2 (a,b), represented Raman spectra of raw-SWCNTs and SWCNTs/TiO$_2$-Ag, respectively. As shown in Figure 2(a), the SWCNTs presented two bands involving D and G which related to the intensity ratio of D and G of SWCNTs ($I_D/I_G$) and depend on the degree of disorder of the nanotube. Furthermore, the Raman analysis of nanocomposites reveals the presence two bands of D and G. The intensity of ratio of ($I_D/I_G$) was increasing as compared with raw-SWCNTs due to the disorder of carbon structure and interaction with Ag/TiO$_2$ nanoparticles throws the sol-gel method.

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Figure 2: Raman analysis of a) R-SWCNTs and b) nanocomposite SWCNTs/TiO$_2$-Ag with peak phases

In addition, the increasing in the intensity ratio of D and G band of the nanocomposite suggest the presences nanoparticles decorating SWCNTs and producing defect sites by oxidation method which result in interaction with nanoparticles as shown in Figure 2(b). In figure 2(a,b), the Raman bands appears at 1075 cm$^{-1}$ of D-band and 1550 cm$^{-1}$, 2540 cm$^{-1}$ of G-band while in state of nanocomposite the Raman bands mode appears at 280 cm$^{-1}$ and 450 cm$^{-1}$related to presences Ti-O-TO of anatase phase of titanium [15,16].
In addition, the increase in the intensity ratio of D and G band of the nanocomposite suggests the presence of nanoparticles decorating SWCNTs and producing defect sites by oxidation method which results in interaction with nanoparticles as shown in Figure 2(b). In Figure 2(a, b), the Raman bands appear at 1075 cm\(^{-1}\) of D-band and 1550 cm\(^{-1}\), 2540 cm\(^{-1}\) of G-band while in a state of nanocomposite the Raman band mode appear at 280 cm\(^{-1}\) and 450 cm\(^{-1}\) related to presences Ti-O-TO of anatase phase of titanium [15,16].

Figure 3: FESEM images of a) R-SWCNTs and b) nanocomposite SWCNTs/TiO\(_2\)-Ag

Figure 3(a,b) presents FESEM micrographs for R-SWCNTs and SWCNTs/TiO\(_2\)-Ag. From the results, the R-SWCNTs appeared highly tangled tubes with diameter range between (40-60 nm) as shown in Figure 3(a). As well as, the morphology of nanocomposite has revealed the attached quasi-spherical nanoparticles (Ag-TiO\(_2\)) on SWCNTs in Figure 3(b) by using chemical methods as compared to R-SWCNTs. These results indicate the successful attachment of nanoparticles on oxidizing sites by using chemical treatment without destroying the SWCNTs structures with less diameter range between (20-50 nm) during acid treatment.

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In order to confirm the antibacterial activity of SWCNTs before and after decorating with nanoparticles as SWCNTs/TiO$_2$-Ag in static conditions, we measured the viable cell number of these samples against gram negative bacteria (E. coli) as shown in Figure 4(a,b). The results reveal that the antibacterial activity of nanocomposite SWCNTs/TiO$_2$-Ag Figure 4(b) (2mg/ml) becomes higher than R-SWCNTs Figure 4(a) the control 4(c) and less colonies of E. coli have been presented after incubation 24h at temperature 37$^\circ$C. According to the obtained results in Figure (5), we suggest that bacterial cell membrane was disrupt by direct contact of the aggregate SWCNTs and nanoparticles with the membrane which result in oxidation the outer bacterial cell, then reacts and inactivated the inner protein and DNA by adhering to the thiol groups [17]. As well as the SWCNTs in nanocomposite play an effective role which becomes as adsorbent substance for bacterial cell due to the presence reactive oxygen species (ROS) groups that alters the cell membrane of E. coli and finally the bacterial death by nanoparticles conjugated SWCNTs as compared with R-SWCNTs.

In order to confirm the antibacterial activity of SWCNTs before and after decorating with nanoparticles as SWCNTs/TiO$_2$-Ag in static conditions, the viable cell number of these samples was measured against gram - negative bacteria (E. coli) as shown in Figure 4(a,b). The results reveal that the antibacterial activity of nanocomposite SWCNTs/TiO$_2$-Ag as shown in Figure 4(b) (2mg/ml) becomes higher than r-SWCNTs as shown in Figure 4(a) the control 4(c) and fewer colonies of E. coli are presented after incubation for 24h at a temperature of 37$^\circ$C. According to the obtained results in Figure (5), we suggested that the bacterial cell membrane was disrupted by direct contact of the aggregate SWCNTs and nanoparticles with the membrane which result in oxidation the outer bacterial cell, then reacts and inactivated the inner protein and DNA by adhering to the thiol groups [17]. As well as the SWCNTs in nanocomposite play an effective role which becomes as an adsorbent substance for bacterial cell due to the presence reactive oxygen species (ROS) groups that alter the cell membrane of E. coli and finally the bacterial death by nanoparticles conjugated SWCNTs as compared with r-SWCNTs.
In addition, the sorption application of SWCNTs/TiO$_2$-Ag nanocomposite to take its role in water treatment have received a considerable attention due to SWCNTs have a huge specific surface area and nanoscale structure, which expose large number of adsorption site. Besides, it has been reported that adsorption capacity of SWCNTs increased with increasing Ag-TiO$_2$ loaded the availably of more binding sites as shown in figure 6 (a,b) which reveal the adhesion of *E.coli* on the R-SWCNTs and nanocomposite, respectively, that improve the role in field of filters in water treatment [18-20].

In addition, the sorption application of SWCNTs/TiO$_2$-Ag nanocomposite has received an attention in water treatment due to the high specific surface area and nanoscale structure SWCNTs, which expose a large number of adsorption site. Besides, it has been reported that the adsorption capacity of SWCNTs increased with increasing Ag-TiO$_2$ loaded the availably of more binding sites as shown in figure 6 (a,b) which reveal the adhesion of *E.coli* on the r-SWCNTs and nanocomposite, respectively. This property could improve the role in the field of filters in water treatment [18-20].
Conclusion

In summary, TiO\textsubscript{2}-Ag/SWCNTs have been successfully prepared by sol-gel method as improved by XRD analyses that reveal a strong peak of Ag and TiO\textsubscript{2} related to tetragonal anatase as well as FESEM which reveal the formation NPs with different sizes decorated the carbon tubes in different region. The Raman spectra represent increasing the intensity of (I\textsubscript{D}/I\textsubscript{G}) ratio due to the disorder of SWCNTs structure and interaction with Ag-TiO2 NPs throw sol-gel method. The viable count method proves that the TiO\textsubscript{2}/Ag/SWCNTs ternary nanohybrid had stronger antibacterial activity than R-SWCNTs against \textit{E. coli} cells.

In summary, the nanocomposite (TiO\textsubscript{2}-Ag/SWCNTs) were successfully prepared by the sol-gel method. This was confirmed by XRD analyses, when that reveals a strong peak of Ag and TiO\textsubscript{2} related to tetragonal anatase as well as FESEM which reveal the formation NPs with different sizes decorated the carbon tubes in a different region. The Raman spectra were represented the increasing the intensity of (I\textsubscript{D}/I\textsubscript{G}) ratio, which could be attributed to the disorder of SWCNTs structure and interaction with Ag-TiO2 NPs through the sol-gel method. The viable count method proves that the TiO\textsubscript{2}/Ag/SWCNTs ternary nanohybrid had a stronger antibacterial activity than the r-SWCNTs against \textit{E. coli} cells.

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