NOVEL METHOD USING NANOMATERIALS FOR REMOVAL OF *E. COLI* BACTERIA FROM WATER

By

Samer M. Al-Hakami

A Thesis Presented to the
DEANSHIP OF GRADUATE STUDIES

KING FAHD UNIVERSITY OF PETROLUM & MANIRALS

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
Requirements for the Degree of

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This thesis, written by **Samer Mohammed Al-Hakami** under the direction of his thesis advisor and approved by his thesis committee, has been presented to and accepted by the Dean of Graduate Studies, in partial fulfilment of the requirements of the degree of **MASTER OF SCIENCE IN CHEMICAL ENGINEERING.**

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Name: Samer Mohammed Al-Hakami

Title: Novel Method Using Nanomaterials for Removal of *E. Coli* Bacteria from Water

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A novel technology has been presented for the removal of Escherichia Coli (*E. coli*) bacteria from water using Carbon Nanotubes (CNTs), modified CNTs and impregnated CNTs with and without heating effect from microwave radiation. The presented microbiological treatment in this study, which lies under the subject field of bio-nanotechnology has combined between powers of degradation by microwaves and adsorption by Carbon Nanotubes (CNTs). The study demonstrated the removal of Escherichia Coli (*E. coli*) Bacteria and its by-products under various treatment conditions including dosages of CNTs, the effect of functional groups, the effect of the power of microwave source, and treatment time. The final results indicated that different types of the tested nanomaterials (raw CNTs, functionalized CNTs and impregnated CNTs with and without heating effect from microwave radiation with a very small dosage between 0.2 g and 0.007 g of CNTs per 100 ml of water) successfully achieved 90-100% removal of *E. coli* bacteria from water. This significant result can be utilized in many of medical and water treatment applications, such as: disinfection for virus, microbial control, water purification and treatment several types of cancer as part of tumor therapy.

The study was carried out in four phases. The first phase focused on the synthesis and preparation of the functional groups and the impregnation of nano particles (CNTs and Ag). In this phase, the multi-walled carbon nanotubes were functionalized with a carboxylic (COOH) group, phenol (C_5H_5OH) group, dodecylamine group ($C_{12}H_{27}N$), C18 group such as 1-octadecanol ($C_{18}H_{38}O$), and impregnated with silver nanoparticles.

The second phase included the laboratory bench-scale experimental work where several samples under the effect of microwaves were tested under various conditions. In this phase, *E. coli* bacteria numbers evaluated before and after treatment under the individual and combined effects of treatment schemes. The third phase included characterization of nanomaterials and bacterial colonies. The fourth phase included the data analysis in order to identify the treatment conditions that led to complete removal of *E. coli* bacteria from water.

MASTER OF SCIENCE DEGREE

KING FAHD UNIVERSITY OF PETROLEUM AND MINERALS

DHAHRAN, SAUDI ARABIA

ملخص الرسالة

الإسم: سامر محمد الحكمي

عنوان الرسالة: طريقة جديدة لإزالة بكتيريا الإيكولاي من الماء با ستخدام مواد نانوية متناهية الصغر

التخصص: الهندسة الكيميائية

تاريخ التخرج: يونيو 2011

تعتبر إزالة الملوثات البكتيرية من مصادر المياه المختلفة باستخدام تقنية الانابيب الكربونية متناهية الصغر من المواضيع الهامة والتي لاقت مؤخرا الاهتمام من قبل المختصين إما في صناعة معالجة المياه من الملوثات الخطرة او في علاج البكتيريا الضارة والفيروسات المعدية, حيث يلاحظ في الوقت الحاضر الاهتمام الكبير بالتطبيقات الحيوية لتقنية النانو كأحد المجلات العلمية الهامة في هذا الشأن.

لذا قد تم في هذه الدراسة تطوير طريقة جديدة للتخلص من بكتيريا الإيكولاي في عينة ملوثة من الماء وذلك بإستخدام أنابيب الكربون متناهية الصغر وأنابيب الكربون المتناهية الصغر المعدلة, أي بإضافة مركبات على أنابيب الكربون مثل دوديسل أمين $C_2H_{27}N$ و 1 –أكتاديكانول $C_{18}H_{38}O$ والفينول C_5H_5OH و ذرات الفضة النانوية Ag. حيث تم إستخدام هذه الانابيب الكربونية المعدلة بوجود مصادر حرارية وإشعاعية مثل الميكروييف في تقييم فعالية هذه التقنية في إزالة وقتل بكتيريا الإيكولاي.

فقد اضهرت الدراسة قدرة هذه الطريقة الجديدة على ازالة بكتيريا الإيكولاي بنسبة تتراوح بين 90 الى 100 في المائة تحت تأثير عدة ظروف تشمل تركيز انابيب الكربون المتناهية الصغر, انواع المركبات المستخدمة لتعديل خواص انابيب الكربون, طاقة المصدر الإشعاعي و أخيرا الفترة الزمنية لكل تجربة.

وقد نفذت هذه الدراسة على أربع مراحل . المرحلة الأولى تم فيها تعديل خواص أنابيب الكربون متناهية الصغر. وفي المرحلة الثانية تم إختبار مدى تأثير هذه المواد في قتل بكتريا الإيكولاي. أما في المرحلة الثالثة فقد تم خلالها دراسة وتحليل خصائص هذه المواد بألاضافة إلى تحليل خصائص البكتيريا بعد المعالجة.

وفي المرحلة الرابعة تم تحليل النتائج والخروج بالتوصيات المناسبة. وقد اظهرت النتائج النهائية ان بعض المواد النانوية التي تم اختبارها قد حققت نسبة عالية في ازالة وقتل البكتيريا (90 الى 100 %). هذه النتائج المهمه يمكن استخدامها في تتطبيقات عديده تشمل تقنية معالجة المياه, التحكم بالمواد الميكروبية, بالاضافة الى بعض المجالات الطبية كعلاج الفيروسات والاورام السرطانية.

درجة الماجستير في العلوم
جامعة الملك فهد للبترول والمعادن
الظهران, المملكة العربية السعودية

CHAPTER ONE

INTRODUCTION

Although, the rapid growth in nanotechnology has spurred significant interest in the medical, biological and environmental applications of nanomaterials, to date, there are no reports describing utilization of nanomaterials under the affect of microwave radiation to treat and remove microorganisms such as: viruses, bacteria and fungus. For instance, neither pure nor modified Multiwalled Carbon Nanotubes (MWCNTs) nor Singlewalled Carbon Nanotubes (SWCNTs) have been studied thoroughly under the effect of microwave radiation to observe changes in physical and chemical properties of nanoparticles. Presence of bacterial pathogens in drinking water and human body is one of the perennial problems. The challenge to achieve appropriate disinfection without forming harmful disinfection by products (DBPs), like some chemical disinfectants, calls for new technologies and initiatives for efficient disinfection for viruses and microbial control.

Nanomaterials are excellent adsorbents, catalysts, and sensors due to their large specific surface area and high reactivity. More recently, several natural and engineered nanomaterials have also been shown to have strong antimicrobial properties, including chitosan, silver nanoparticles (Ag), photocatalytic, fullerol, aqueous fullerene nanoparticles (C60), and carbon nanotubes (CNTs) and titanium dioxide TiO_2 particles. In recent years, great attention was given to the advances in nanoscale science and engineering which provided unprecedented opportunities to develop more cost effective and environmentally acceptable water purification processes. Titanium dioxide (TiO_2) has emerged as promising photocatalysts for water treatment. Photoassisted TiO_2 particles have shown significant efficiency in treating organic-contaminated waters through formation of free radical oxidation mechanism. When irradiated with UV or visible light, TiO_2 will generate very powerful hydroxyl radicals capable of degrading and breaking down organic contaminants in the water.

Over the last twenty years, carbon nanotubes (CNTs) have received considerable attention from many researchers due to their interesting properties and wide applications. In addition to their outstanding mechanical characteristics, CNTs exhibit excellent electrical and thermal properties. These superior properties provide exciting opportunities to produce advanced materials for new applications.

Nanosorbents such as carbon nanotubes (CNTs) have much better adsorption capacity than bulk sorbents in removing inorganic and organic pollutants from contaminated water mainly due to their large surface areas and their ability to be functionalized with various chemical species to increase their affinity towards target pollutants. On the other hand, nanocatalysts and redox active nanoparticles are used as water-purification media due to their large surface areas and their electronic and catalytic properties.

Since, Nanoparticles (NPs) in particular CNTs have promising properties to slip through the immune system or directly into the brain or blood cells, some highly qualified research centers around the world are looking to a point of connecting machines to individual cells to treat, inject drugs, and to do many things related to health issues.

The effect of CNTs on bacteria and viruses has not received particular attention, probably due to the difficulty of dispersing CNTs in water. Few studies available credited SWNTs with antimicrobial activity towards Gram-positive and Gram-negative bacteria, and the damages inflicted will be attributed to either a physical interaction or oxidative stress that compromise cell membrane integrity. Carbon nanotubes may therefore be useful for inhibiting microbial attachment and biofouling formation on surfaces.

Work presented in such search revealed that the removal of *E. coli* Bacteria from contaminated waters based on the combined CNTs, functionalize CNTs and impregnated CNTs with and without the effect of microwave radiation. This study will mark the actual utilization of nanotechnology and visible techniques for the removal of E. Coli Bacteria from contaminated water sources. The importance of the project lies in addressing one of the main strategic environmental priorities that is expected to benefit the Kingdom of Saudi Arabia in technology transfer, research development, and academia. While the innovation part is focused on introducing new and innovative water treatment technologies that will be not tested before in the kingdom.

CHAPTER TWO

STUDY OBJECTIVES

The objectives of the present study are:

1- To functionalize CNTs with phenol (C_5H_5OH), 1-Octadecanol ($C_{18}H_{38}O$) and dodecylamine ($C_{12}H_{27}N$).

2- To impregnate CNTs with silver nanoparticles

3- To study removal efficiency of Escherichia Coli (*E. Coli*) from contaminated water using CNTs, and impregnated/functionalized CNTs with and without heating effect from microwave radiation.

4- To Publish the work

The proposed work was executed mainly through conducting laboratory bench-scale water treatment studies and included different phases.

CHAPTER THREE

LITERATURE REVIEW

3.1 Carbon Nanotubes (CNTs) in Water Treatment

Over the last twenty years, carbon nanotubes (CNTs) have received considerable attention from many researchers due to their interesting properties and wide applications. In addition to their outstanding mechanical characteristics, CNTs exhibit excellent electrical and thermal properties. These superior properties provide exciting opportunities to produce advanced materials for new applications such as water treatment.

Sorbents are widely used as separation media in water purification to remove inorganic and organic pollutants from contaminated water. Nanoparticles have two key properties that make them particularly attractive as sorbents. On a mass basis, they have much larger surface areas than bulk particles. Nanoparticles can also be functionalized with various chemical groups to increase their affinity towards target compounds. Several research groups are exploiting the unique properties of nanoparticles to develop high capacity and selective sorbents for metal ions and anions. Due to their large specific surface area, small size, and hollow and layered structures, CNTs have been proven to possess great potential as superior adsorbents for removing many kinds of organic and inorganic contaminants [2].

3.2 Water Decontamination: Removal of Bacteria from Water

Worldwide, waterborne diseases remain the leading cause of death in many developing nations. According to the 2004 WHO report, at least one-sixth of the world population (1.1 billion people) lack access to safe water [1]. The consequences are daunting: diarrhea kills about 2.2 million people every year, mostly children under the age of 5. The importance of water disinfection and microbial control cannot be overstated. Although disinfection methods currently used in drinking water treatment can effectively control microbial pathogens, research in the past few decades have revealed a dilemma between effective disinfection and formation of harmful disinfection byproducts (DBPs). Chemical disinfectants commonly used by the water industry such as free chlorine, chloramines and ozone can react with various constituents in natural water to form DBPs, many of which are carcinogens [2]. More than 600 DBPs have been reported in the literature [3]. To be specific, chlorine has been widely used for the disinfection of drinking water, resulting in chloroorganic compounds during the treatment which are highly carcinogenic [4]. Considering the mechanisms of DBP formation, it has been predicted that DBPs will be formed any time chemical oxidants are used in water treatment [5].

Furthermore, the resistance of some pathogens, such as Cryptosporidium and Giardia, to conventional chemical disinfectants requires extremely high disinfectant dosage, leading to aggravated DBP formation. Therefore, there is an urgent need to reevaluate conventional disinfection methods and to consider innovative approaches that enhance the reliability and robustness of disinfection while avoiding DBP formation [2].

Nanomaterials are excellent adsorbents, catalysts, and sensors due to their large specific surface area and high reactivity. More recently, several natural and engineered nanomaterials have also been shown to have strong antimicrobial properties, including chitosan, silver nanoparticles (nAg), photocatalytic TiO_2, fullerol, aqueous fullerene nanoparticles (nC60), and carbon nanotubes (CNT) [6-11].

Several natural and engineered nanomaterials have demonstrated strong antimicrobial properties through diverse mechanisms as follows:

1- Photocatalytic production of reactive oxygen species that damage cell components and viruses (e.g. TiO_2, ZnO and fullerol),

2- Compromising the bacterial cell envelope (e.g. peptides, chitosan, carboxyfullerene, carbon nanotubes, ZnO and silver nanoparticles (nAg)),

3- interruption of energy transduction (e.g. nAg and aqueous fullerene nanoparticles (nC60)), and

4- Inhibition of enzyme activity and DNA synthesis (e.g. chitosan).

Although some nanomaterials have been used as antimicrobial agents in consumer products including home purification systems as antimicrobial agents, their potential for disinfection or microbial control in system level water treatment has not been carefully evaluated [2].

3.3 Carbon Nanotubes and their Antimicrobial Effect

Carbon nanotubes (CNTs) are graphene sheets rolled into a tube and possibly capped by half a fullerene. They can be either single-walled (SWNTs), a single pipe with a diameter from 1 to 5 nm, or multi-walled (MWNTs), with several nested tubes, at lengths varying from 100 nm up to several tens of micrometers. Toxicity studies on CNTs indicate that they exert acute pulmonary toxicity and cytotoxicity to mammalian cells, with the toxicity decreasing from SWNTs >MWNTs > quartz > C60 [13-14].

Surprisingly, the effect of CNTs on bacteria and viruses has not received particular attention, probably due to the difficulty of dispersing CNTs in water. Surfactants or polymers such as sodium dodecyl benzenesulfate (SDBS), polyvinylpyrolidone (PVP) or Triton-X are generally used to facilitate the dispersion. The few studies available credited SWNTs with antimicrobial activity towards Gram-positive and Gram-negative bacteria, and the damages inflicted were attributed to either a physical interaction or oxidative stress that compromise cell membrane integrity. Carbon nanotubes may therefore be useful for inhibiting microbial attachment and biofouling formation on surfaces. However, the degree of aggregation, stabilization effects by NOM, and the bioavailability of the nanotubes will have to be considered with attention for these antimicrobial properties to be fully effective[15-19].

Antimicrobial activity of CNTs requires direct contact between CNTs and target microorganisms. Suspension of non-functionalized CNTs in water is extremely difficult and does not provide enough CNT-microbe contact for disinfection. Accordingly, the antibacterial activity of CNTs could be exploited by coating CNTs on a reactor surface in contact with the pathogen laden water. For example, immobilized SWNTs on a membrane filter surface and observed 87% killing of E. coli in 2 hours. Srivastava et al. (2004), showed that CNTs could be incorporated into hollow fibers and achieve effective inactivation of E. coli and poliovirus. Brady-Este´vez et al. (2008) achieved complete retention and effective inactivation of E. coli as well as up to 5–7 log removal of MS2 bacteriophage using a PVDF microporous membrane coated with a thin layer of SWNTs. Bundles of non-aligned single or multi-walled nanotubes, easy to separate with a filter, could also be applied in a packed column/bed filter. Although the rate of bacterial inactivation by CNT is relatively low compared to conventional disinfectants, it may be sufficient to prevent biofilm formation and the subsequent biofouling of surfaces such as water filtration membranes [20-22].

3.4 Antimicrobial Nanomaterials: Microbial Toxicity Mechanisms

The antibacterial nanoparticles mechanisms have been reported in the literature. They fall into three general categories: naturally occurring antibacterial substances, metals and metal oxides, and novel engineered nanomaterials. These nanoparticles interact with microbial cells through a variety of mechanisms. The major antimicrobial mechanisms reported in the literature are summarized in Fig. 1. The nanoparticles can either directly interact with the microbial cells, e.g. interrupting transmembrane electron transfer, disrupting/penetrating the cell envelope, or oxidizing cell components, or produce secondary products (e.g. reactive oxygen species (ROS) or dissolved heavy metal ions) that cause damage [2].

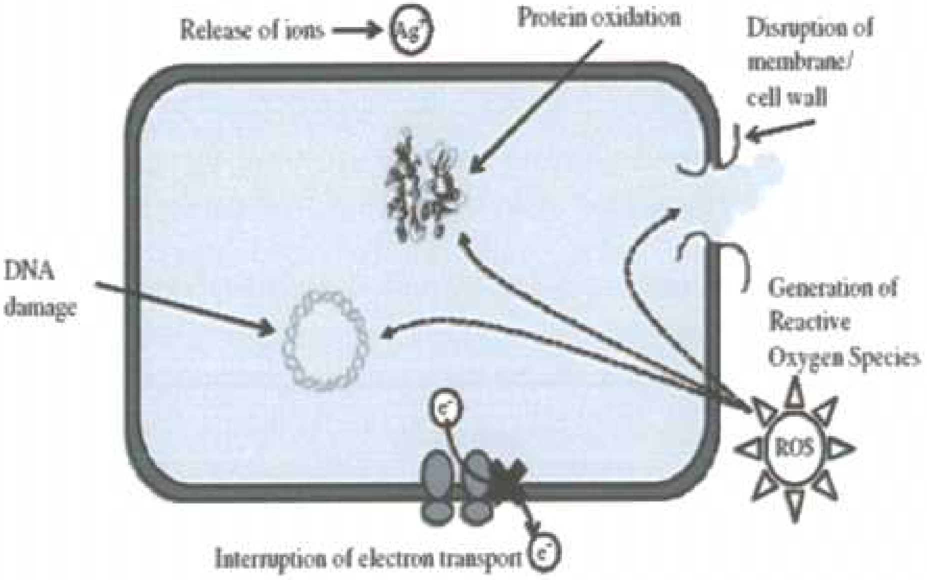


Fig. 1 - Various mechanisms of antimicrobial activities exerted by nanomaterials.

3.5 Current and Potential Applications for Disinfection and Microbial Control

Several nanomaterials, such as nano silver (nAg), chitosan, and TiO_2, have found applications in diverse consumer products and industrial processes, including water treatment, as antimicrobial agents (Table 1) [2].

Among all antimicrobial nanomaterials, nAg is probably the most widely used. It is used as an antimicrobial agent in over 100 consumer products, ranging from nutrition supplements to surface coating of kitchen appliances. Commercial home water purification systems such as Aquapure, Kinetico, and QSI-Nano, which are reported to remove 99.99% pathogens, use membranes impregnated with silver or surfaces coated with silver. Nanoparticulate chitosan is used in cosmetics, food preservation, agriculture, and medical applications. In addition to the antimicrobial function, it also confers hydrophobicity and mechanical strength to the products. Chitosan has been used in full scale water and wastewater treatment systems as a coagulant/flocculant, and was recently used as a disinfectant [23-27].

Because of its whiteness and high refractive index, TiO_2 nanoparticles, particularly in the anatase form, are already used in a large number of consumer products such as sunscreen, toothpaste, paints, and coatings. It is used in home air purifiers, e.g. 3Q_Multi Stage Air Purifier and NanoBreeze_Room Air Purifier, to remove volatile organic compounds and kill bacteria. Industrial scale water purification systems using TiO_2 photocatalysis already exist (e.g. Purifics), although its main application is organic contaminant degradation instead of disinfection. To assess the potential of the antimicrobial nanomaterials as alternative disinfectants in drinking water treatment, the reported antimicrobial effectiveness of selected nanomaterials is compared to conventional water disinfectants in Table 2. It is demonstrated that these nanomaterials have similar or superior antimicrobial activities compared to conventional chemical disinfectants. They can potentially be used as alternative disinfectants or in conjunction with existing technologies, such as UV, to enhance disinfection efficacy. Another potential application is in fouling control of water filtration membranes and other surfaces in reactors and pipelines used in water treatment and distribution. Table 1 summarizes the potential antimicrobial applications of different nanomaterials, the form of which strongly depends on the properties of the specific nanomaterial [2].

Table 1 – Current and potential applications of antimicrobial nanomaterials

Nanomaterial	Antimicrobial mechanism	Current applications	Potential future applications
Chitosan	Membrane damage, chelation of trace metals	Personal care products, microbicide in agriculture and biomedical products, food wraps, biomedical, flocculants in water and wastewater treatment	Immobilizer of bacteria, enzymes and other biological molecules, biosorbents
nAg	Release of Ag^+ ions, disruption of cell membrane and electron transport, DNA damage	Portable water filters, clothing, medical devices, coatings for washing machines, refrigerators, and food containers	Surface coatings, membranes
TiO_2	Production of ROS, cell membrane and cell wall damage.	Air purifiers, water treatment systems for organic contaminant degradation	Solar and UV disinfection of water and wastewater, reactive membranes, biofouling-resistant surfaces
CNT	Physically compromise cell envelope	None	Biofouling-resistant membranes, carbon hollow fibers, packed bed filters
ZnO	Intracellular accumulation of nanoparticles, cell membrane damage, H2O2 production, release of Zn^{2+} ions	Antibacterial creams, lotions and ointment, deodorant, self-cleaning glass and ceramics	Surface coating, mouthwash

Table 2 – Relative effectiveness of various antimicrobial agents

Disinfectant	CT^a (mg-min/L or mJ/cm^2)	References
Chitosan	$7.5–144^b$	Don et al., 2005; Qi et al., 2004
nC_{60}	100	Lyon et al. (2007)
Chloramine	95–180	Hoff (1986)
Silver ion	$0.075^c–26$	Huang et al. (2008b), Kim et al. (2008)
Free chlorine	0.03–0.05	Hoff (1986)
Ozone	0.0007–0.02	MWH (2005), Hunt and Marinas (1997), Sulzer et al. (1959), Farooq and Akhlaque (1982),
UV-C (254 nm)	3.8–4.8	Chang et al. (1985), Oguma et al. (2002)
UV-A (300–400 nm)	16,300–20,000	Choi et al. (2007), Mori et al. (2007)
TiO_2^d + UV (300–400 nm)	700–5000	Benabbou et al. (2007), Liu and Yang (2003), Ibanez et al. (2003)
TiO_2^e + UV (300–400 nm)	3600–8500	Kikuchi et al. (1997), Choi et al. (2007)
UV + H_2O_2 (315–400 nm)	66–89	Mamane et al. (2007)

a All CT values are for 99% disinfection of E. coli unless otherwise specified. Unit for CT values of all UV applications are mJ/cm^2.

b Minimum inhibition concentration multiplied by exposure time.

c CT for 99% disinfection of Pseudomonas aeruginosa.

d TiO_2 slurry in a UV reactor.

e TiO_2 thin film in a UV reactor.

3.6 Nano Sliver (nAg)

The antimicrobial properties of silver compounds and silver ions have been historically recognized and applied in a wide range of applications from disinfecting medical devices and home appliances to water treatment [28].

However, the mechanism of toxicity is still only partially understood.Silver ions interact with thiol groups in proteins, resulting in inactivation of respiratory enzymes and leading to the production of ROS. It was also shown that Ag^+ ions prevent DNA replication and affect the structure and permeability of the cell membrane. Silver ions are also photoactive in the presence of UVA and UV-C irradiation, leading to enhanced UV inactivation of bacteria and viruses. It is hypothesized that complexation of Ag^+ with cysteine accelerates photodimerization of viral DNA contributing to a synergistic effect observed in inactivation of Haemophilus influenzae phage and MS2 phage [29-32].

To date, several mechanisms have been postulated for the antimicrobial property of silver nanoparticles: (1) adhesion of nanoparticles to the surface altering the membrane properties. nAg particles have been reported to degrade lipopolysaccharide molecules, accumulate inside the membrane by forming ''pits'', and cause large increases in membrane permeability; (2) nAg particle penetrating inside bacterial cell, resulting in DNA damage; (3) dissolution of nAg releases antimicrobial Ag^+ ions. Physicochemical properties play an important role in the antimicrobial activity of nAg. In general, particles of less than 10 nm are more toxic to bacteria such as Escherichia coli and Pseudomonas aeruginosa.

Silver nanoparticles ranging from 1 to 10 nm inhibit certain viruses from binding to host cells by preferentially binding to the virus' gp120 glycoproteins. Furthermore, triangular nAg nanoplates containing more reactive <111> planes were found to be more toxic than nAg rods, nAg spheres, or Agþ ions [33-37].

3.7 Limitations of Nanotechnology for Water Treatment

Several challenges exist for efficient application of antimicrobial nanomaterials in drinking water treatment, primarily concerning dispersion and retention of nanomaterials and the sustainability of antimicrobial activity. Although nanoparticles provide very high specific surface area, a primary reason for their high reactivity, aggregation in water negates this benefit [2].

On the other hand, a well-dispersed nanoparticle suspension contains particles of extremely small sizes. If nanomaterials are applied in the form of slurry, an efficient separation process downstream such as membrane filtration is needed to retain and recycle the nanomaterials. Immobilization of nanomaterials on reactor surfaces or membrane filters eliminates the need for separation. However, the effective nanomaterial dose is then limited by the available surface area in the reactor, which in combination with reduced access to light source, lowers the overall disinfection efficiency compared to a slurry reactor. Nanoparticles may also escape from the treatment system and enter the product water. In addition, since all nanoparticles must be retained in the treatment system, no disinfectant residual is provided. Therefore, they must be used in conjunction with a secondary disinfectant that provides residual through the distribution system.

Retention of nanomaterials is critical not only because of the cost associated with loss of nanomaterials, but also, and more importantly, because of the potential impacts of nanomaterials on human health and ecosystems. Our understanding of the potential human health and environmental implication of nanomaterials is improving with the increasing number of nanotoxicity studies in the past few years [2].

Although nano-scale TiO2 was classified recently as a possible carcinogen (if inhaled) by International Agency for Research on Cancer, IARC (2006), its potential ingestion via water is not expected to be a major concern, as reflected by its use in toothpastes and sunscreens. No evidence has been found on toxicity of nAg to human. The only known negative health impact of Ag^+ is darkening of the skin and mucous membrane due to long-term exposure to high silver concentration. However, some nanomaterials such as nC60 have been shown to exhibits toxicity to mammalian cells [38-39].

Nevertheless, available information is insufficient to determine the highest allowable concentration of a particular nanomaterial in drinking water. Until their human toxicity is thoroughly evaluated or technologies developed to retain the nanomaterials in the treatment system, application of nanomaterials in large-scale water treatment systems is unlikely in the near future. Because most studies on antimicrobial activities of nanomaterials were conducted in relatively simple and clean solutions, the sustainability of their antimicrobial activities in natural or waste water, whose constituents may interfere with the nanomaterial–microbe interactions, is unclear [2].

Conventional drinking water disinfection processes, such as chlorination and ozonization, could generate disinfection by-products (DBPs) with carcinogenic and mutagenic potential [40].

Biomedical materials are those materials which can be used to assist or substitute human being's tissue and organs. Due to carbon nanotubes possess fantastic properties such as high adsorption, huge surface ratio etc., it must be widely used in biomedical area. Combining the merits both from silver and carbon nanotubes, it is possible to grow carbon nanotubes on the surface of pyrolytic carbon to enhance its surface area and then coated Ag on it to protect bacteria. Ag coated carbon nanotubes are used in bactericidal application for biomedical materials [41].

Carbon nanotubes (CNTs) have been proposed for use in myriad applications, including electronics, composite materials, fuel cells, sensors, optical devices, and biomedicine. A limited number of studies have explored the use of CNTs for filtration and separation applications. Srivastava et al. constructed a cylindrical membrane filter composed of radially aligned multi-walled carbon nanotubes (MWNTs) that formed a CNT layer several hundreds of micrometers thick. It was shown that the MWNT filter was effective in removing hydrocarbons from petroleum wastes as well as bacteria and viruses [42-43].

The primary separation mechanism of the MWNT filters in the studies discussed above is based on size exclusion or sieving. Such filters often require high pressure for operation and are prone to pore plugging and performance deterioration upon filtration of environmental samples. A novel, highly permeable, single-walled carbon nanotube (SWNT) filter was described by S. Banerjee [42], where the filter was used for the effective removal of bacterial and viral pathogens from water. Anna S. Brady-Este′vez and his group used a filter, which utilizes several key properties of SWNTs: their small diameter and high surface area; their tendency to aggregate and form highly porous structures; and their antibacterial properties, as reported in their work. The work done by S. Banerjee demonstrated that bacteria are completely retained on the SWNT filter and are effectively inactivated upon contact with the SWNTs. It was also show that viruses can be completely removed by a depth-filtration mechanism, that is, capture by nanotube bundles inside the SWNT layer. This work provides a basis for future development of a SWNT-hybrid filter, where the SWNTs are immobilized on a microporous ceramic filter [42].

The SWNT layers were made up of deposited SWNT bundles and displayed highly porous structures. Although the SWNTs were well dispersed for vacuum-assisted deposition onto the PVDF membrane, there were still small-scale heterogeneities in the overall layer depth owing to SWNT aggregation.

The SWNT filter was then tested for its ability to remove microbial pathogens from water. Escherichia coli K12 was selected as a model bacterium. For the bacterial filtration experiments, an equal total number of E. coli cells ($5x10^5$) were filtered through the SWNT filter (0.3 mg cm_2) and through the bare base filter (5 mm pore size) as a control. SEM images and analyses of permeate samples indicate that the E. coli cells were completely retained by the SWNT layer due to size exclusion (Fig. 2b), but passed readily through the base filter (Fig. 2a). Moreover, they found that the SWNT filter exhibited high antibacterial activity. A fluorescence-based viability kit (Live/Dead) was used to determine the percent of E. coli inactivated on the SWNT filter and, for comparison, the finding that the filter was capable of complete retention of E. coli was not surprising, since the bacterial cells (2mm) are much larger than the maximum gap between SWNT bundles (0.3mm) at the top of the SWNT layer [42].

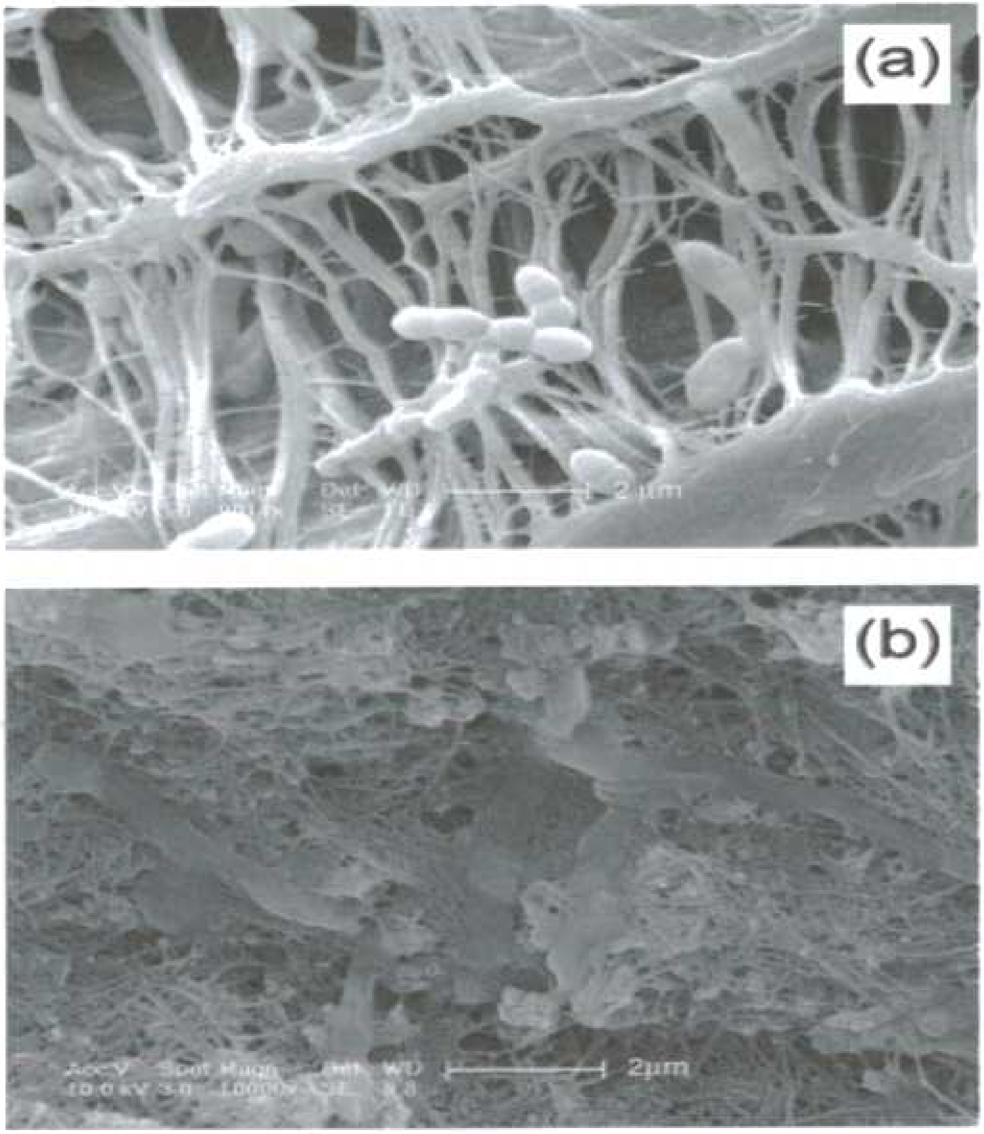


Figure 2: Retention of E. coli by control and SWNT filter. a) SEM image of E. coli cells on the base membrane (5 mm pore PVDF membrane). b) SEM image of E. coli cells retained on SWNT filter. The scale bars in both figures represent 2 mm.

3.8 *Escherichia Coli* (*E. coli*)

Escherichia coli (commonly abbreviated *E. coli*) is a Gram-negative, rod-shaped bacterium that commonly lives in the intestines of humans and animals (Fig. 3, 4 & 5). Although most strains of this bacteria are harmless, several are known to produce toxins that can cause abdominal cramps and diarrhea. One particular *E. coli* strain called 0157:H7 can cause serious food poisoning in humans, severe and life-threatening diarrhea, destroying of red blood cells and kidney damage. Anyone of any age can become infected with *E. coli* 0157:H7, but children and the elderly are more likely to develop serious complications [12].

The symptoms usually appear about three days after exposure, with a range of one to nine days. Most people recover without specific treatment in five to 10 days. Antibiotics should not be used for the treatment of *E. coli* 0157:H7 infection. Studies have shown that some antibiotics may increase the risk of complications. In some people, particularly children under five years of age, the infection can cause a complication called hemolytic uremic syndrome (HUS). This is a serious disease in which red blood cells are destroyed and the kidneys fail [44]. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 and by preventing the establishment of pathogenic bacteria within the intestine.

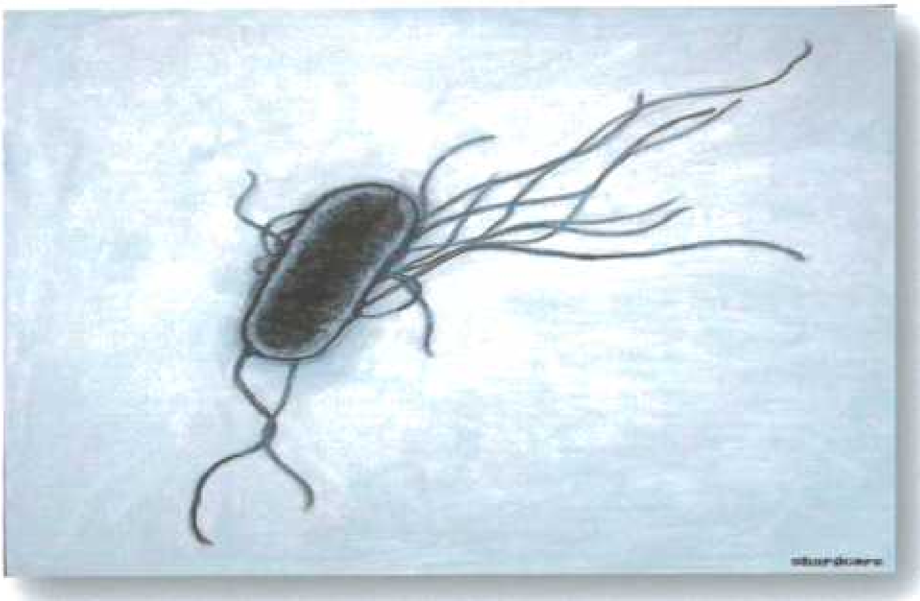


Figure 3: A transmission electron micrograph (TEM) of *Escherichia coli (E.coli)*

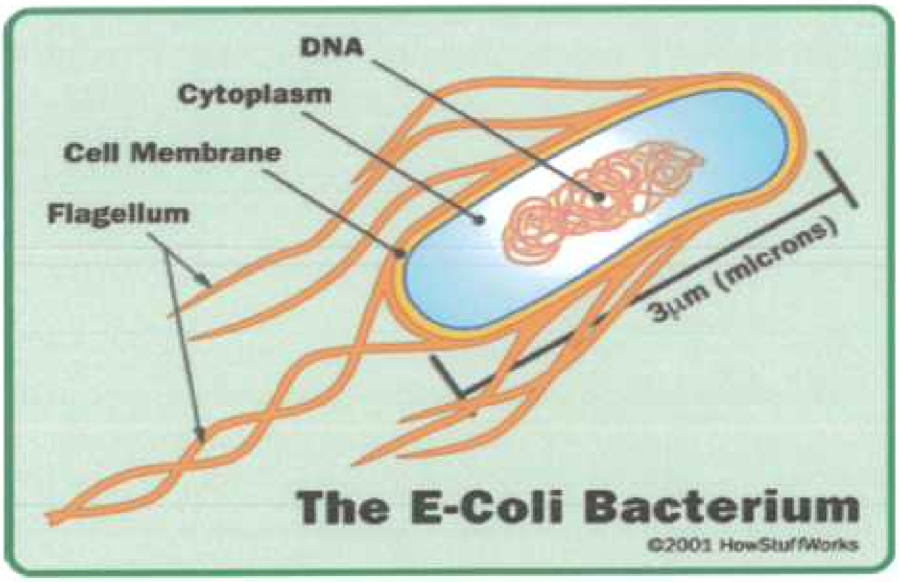


Figure 4: Anatomy of E-Coli Bacterium

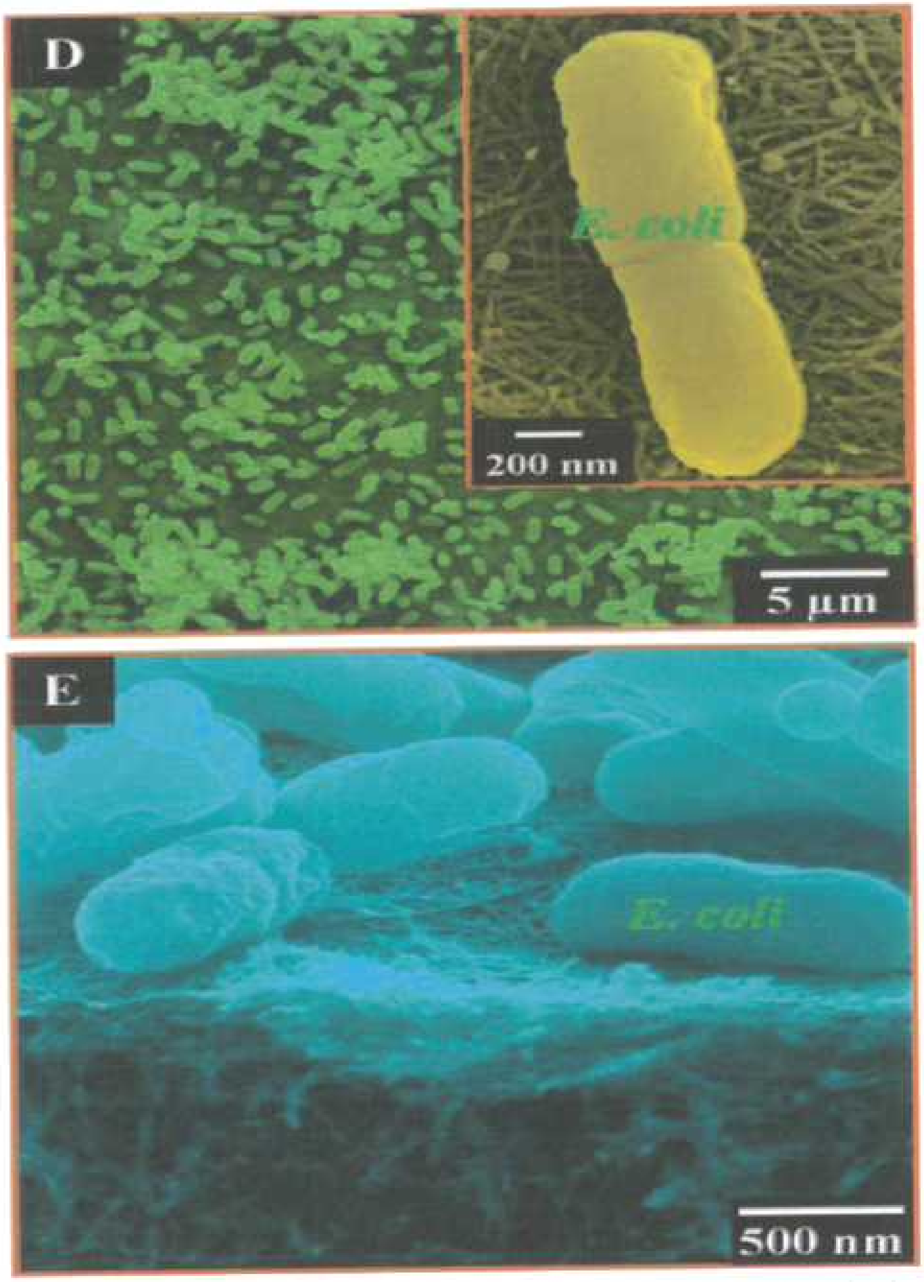


Figure 5: (D) A SEM image of retained *E. coli* and a high magnification SEM image (inset). (E) A cross-section SEM image of *E. coli*.

Certain strains of *E. coli*, such as O157:H7, O121 and O104:H21, produce potentially lethal toxins. Food poisoning caused by *E. coli* is usually caused by eating unwashed vegetables or undercooked meat. O157:H7 is also notorious for causing serious and even life-threatening complications, such as haemolytic-uremic syndrome. This particular strain is linked to the 2006 United States *E. coli* outbreak due to fresh spinach. Severity of the illness varies considerably. It can be fatal, particularly to young children, the elderly, or the immunocompromised, but is more often mild.

If *E. coli* bacteria escape the intestinal tract through a perforation (for example from an ulcer, a ruptured appendix, or due to a surgical error) and enter the abdomen, they usually cause peritonitis that can be fatal without prompt treatment. However, *E. coli* are extremely sensitive to such antibiotics as streptomycin or gentamicin. This, however, could easily change, since *E. coli* quickly acquires drug resistance. Recent research suggests that treatment with antibiotics does not improve the outcome of the disease, and may, in fact, significantly increase the chance of developing haemolytic-uremic syndrome.

Intestinal mucosa-associated *E. coli* are also observed in increased numbers in the inflammatory bowel diseases, Crohn's disease, and ulcerative colitis. Invasive strains of *E. coli* exist in high numbers in the inflamed tissue, and the number of bacteria in the inflamed regions correlates to the severity of the bowel inflammation. Resistance to beta-lactam antibiotics has become a particular problem in recent decades, as strains of bacteria that produce extended-spectrum beta-lactamases have become more common. These beta-lactamase enzymes make many, if not all, of the penicillins and cephalosporins ineffective as therapy. Extended-spectrum beta-lactamase–producing

E. coli are highly resistant to an array of antibiotics, and infections by these strains are difficult to treat. In many instances, only two oral antibiotics and a very limited group of intravenous antibiotics remain effective. In 2009, a gene called New Delhi metallo-beta-lactamase (shortened as NDM-1) that even gives resistance to intravenous antibiotic carbapenem was discovered in India and Pakistan in *E. coli* bacteria.

Due to the severe nature of *E. coli* infection and the potential for lethality, it is necessary to develop alternative treatments for *E. coli* infection and for removal of *E. coli* bacteria from water and food. Thus, a method of removing *Escherichia coli* (*E. coli*) bacteria from an aqueous solution solving the aforementioned problems is desired.

CHAPTER FOUR

RESEARCH METHODOLOGY

4.1 Carboxylation Treatment of Multiwalled Carbon Nanotubes (MWCNT)

Multi-wall carbon nanotubes were purchased from Nanostructured & Amorphous Materials, Inc. USA. The Purity of MWCNT is > 95%, its outside and inside diameters are 10-20 nm and 5-10 nm respectively. The length of these MWCNTs is 10-30 um. Three hundred ml of a concentrated nitric acid of AnalaR (69%) are added to 2g of as-received MWCNT. The mixture is refluxed for 48 h at 120°C. After cooling to room temperature, the reaction mixture is diluted with 500 ml of deionised water and then vacuum-filtered through a filter paper (3 μm porosity).

This washing operation is repeated until the pH become same as deionsed water pH and is followed by drying in a vacuum oven at 100°C. Such conditions lead to remove the catalysts from carbon nanotubes and opening the tube caps as well as the formation of holes in the sidewalls, followed by an oxidative etching along the walls with the concomitant release of carbon dioxide. This less vigorous conditions, minimized the shortening of the tubes and the chemical modification is then limited mostly to the opening of the tube caps and the formation of functional groups at defect sites along the sidewalls.

The final products are nanotube fragments whose ends and sidewalls are decorated with a various oxygen containing groups (mainly carboxyl groups) (Figure 6). Moreover, the percentage of carboxylic functions on the oxidized MWCNT surface does not exceed 4% in the best cases, which corresponds to the percentage of MWCNT structural defects [45-46].

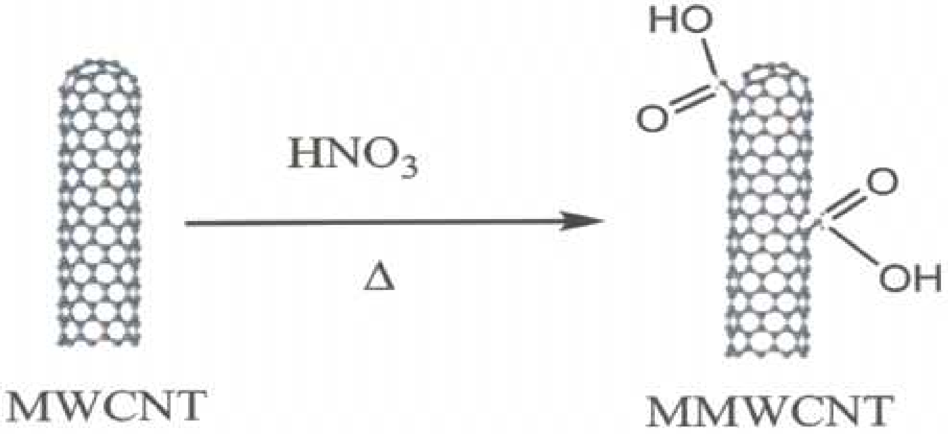


Figure 6: Chemical modification of carbon nanotubes (MWCNT) through thermal oxidation.

4.2 Esterification and Amidation of Carbon Nanotubes

The Fischer esterification is an equilibrium reaction, whereas other esterification routes do not involve equilibrium. To shift the equilibrium to favor the production of esters, it is customary to use an excess of one of the reactants, either the alcohol or the acid. In the present reactions, an excess of the phenol (Aldrich, 98% purity), dodecylamine (Merck, 97% purity), 1-octadecanol (Merck, 97% purity), and polyethylene glycol (Fluka with 98% purity and an average molecular mass of 14000) have been used, because it is cheaper and easier to remove than the carbon nanotubes. Another way to drive a reaction toward its products is to remove one of the products as it forms.

Water formed in this reaction was removed by evaporation during the reaction. The oxidatively introduced carboxyl groups represent useful sites for further modifications [47], as they enable the covalent coupling of molecules through the creation of ester (Figure 7) or amine bonds (Figure 8&9). In a 250 ml beaker, 10 g of the functionalizing reactant were melted on a hotplate at 90°C, 1 g of MMWCNT (modified MWCNT) was added, and the mixture was stirred for 10 minutes before a few drops of sulfuric acid as a catalyst were added. After addition of the catalyst, the mixture was kept on the hotplate and stirred for 2 hours. Upon completion of the reaction, the mixture was poured into 250 ml of benzene and vacuum-filtered through a filter paper (3 μm porosity). This washing operation was repeated five times and followed by washing with petroleum ether three times and THF three times. The product was then washed with deionized water and acetone, and the functionalized MMWCNT material produced was dried in a vacuum oven at 90°C.

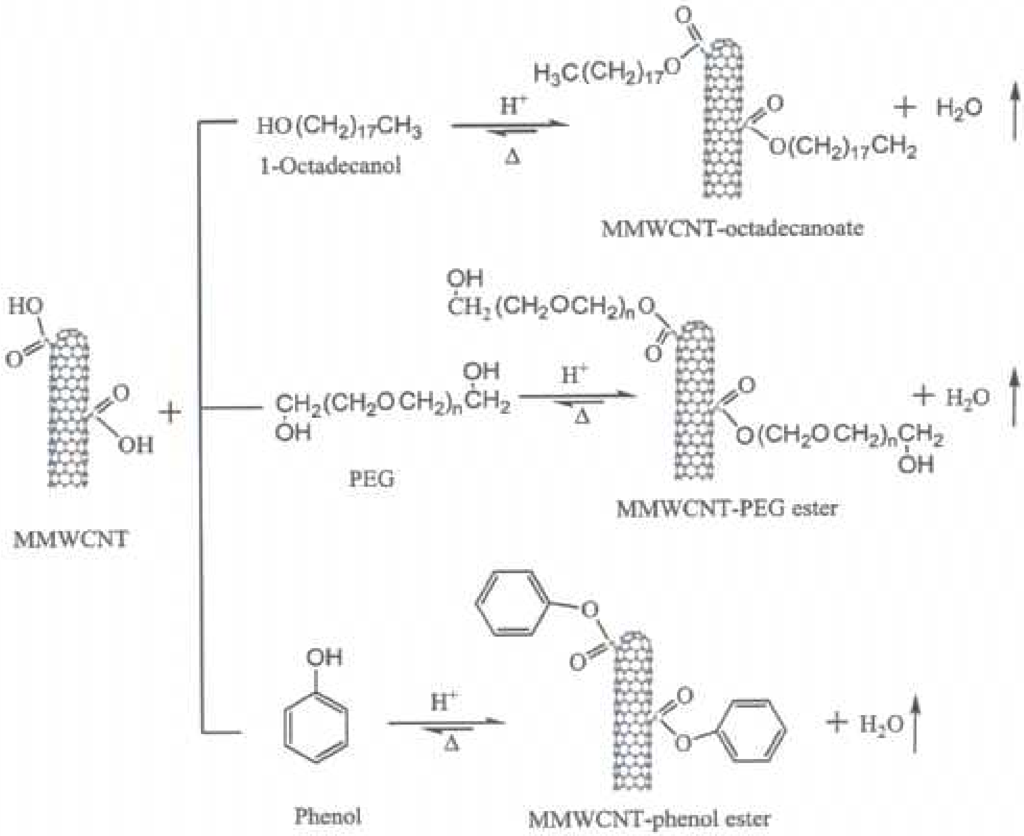


Figure 7: Chemical esterification of modified carbon nanotubes (MMWCNT).
and the functionalized MMWCNT material produced was dried in a vacuum oven at 90°C.

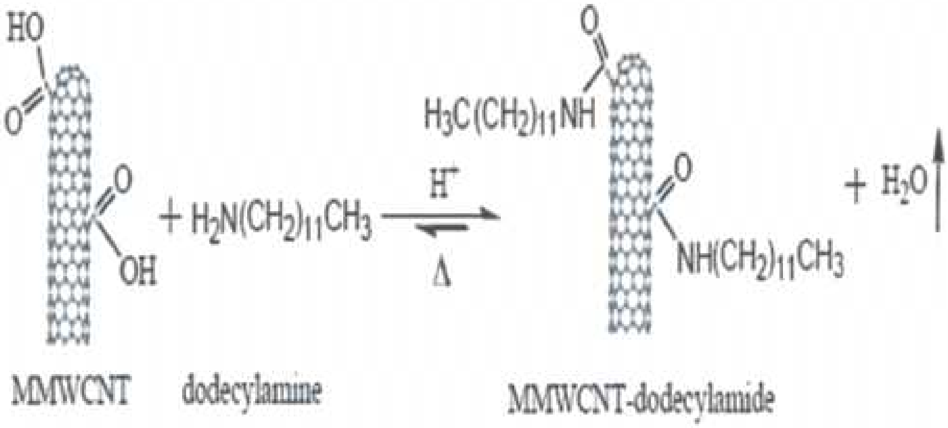


Figure 8: Chemical amidation of modified carbon nanotubes (MMWCNT).

Figure 9: MWCNTs functionalized with carboxylic and dodecylamine groups.

The mechanism for this reaction involves the nucleophilic addition of the alcohol or amine to the carbonyl group of the protonated acid of the carbon nanotubes, followed by elimination of a proton. The tetrahedral intermediate is unstable under the acidic conditions of the reaction and undergoes dehydration to form the ester or amide. The key steps of this mechanism involve activation of the carbonyl group by protonation of the carbonyl oxygen, nucleophilic addition to the protonated carbonyl to form a tetrahedral intermediate, and elimination of water from the tetrahedral intermediate to restore the carbonyl group.

4.3 FTIR Measurements

Fourier Transform Infrared Spectroscopy (FTIR) has shown limited ability to probe the structure of MWCNTs. A factor that has hindered the advancement of FTIR as a tool for MWCNT analysis is the poor infrared transmittance of MWCNTs. A solution to this was the use of KBr preparations of nanotube samples. Because of their black character, the MWCNT strong absorbance and often are unable to be distinguished from background noise makes it necessary to use a very weak concentration of the nanotubes in a KBr powder. However the greater vibrational freedom of attached polymeric species presents much more pronounced peaks and are typically the focus of attention in FTIR results. Despite this, with very careful sample preparation some researchers have managed to elucidate peaks corresponding to surface bound moieties such as carboxylic acid groups at wavenumbers of 1791, 1203 and 1080cm^{-1}.

The spectra of samples were recorded by PERKIN ELMER 16F PC FT-IR instrument. FTIR samples were prepared by grinding dry material into potassium bromide adding of ~0.03 %wt. This very low concentration of MWCNTs is necessary due to the high absorption of the nanotubes.

4.4 Surface Impregnation of Carbon Nanotubes by Silver Nanoparticles

Ag nanoparticles catalysts were prepared by impregnation method. The precursor used was Silver nitrate from Aldrich, AR grade, 99.9% purity. Silver salt was dissolved in deionised water. CNT samples were impregnated with an aqueous solution of silver nitrate in an appropriate concentration to obtain Ag loading with about 1-50 wt%. The impregnation was performed under ultrasonic condition for 30 minutes in a fixed 25°C temperature controlled by the water bath. Every batched was prepared with 0.5gram of CNT in a 32.7ml of glass bottle sample with 3cm in diameter. Ultrasonification instrument was used in this preparation. To maintain at certain temperature circulating water was added to the system from a controlled-temperature water bath. The aim of ultrasonication during impregnation was to have a complete and homogeneous wetting of the whole sample particles during impregnation. And as for experiment where the drying is expected to occur during sonication (open bottle experiment) the aim is also for a homogenous wetting during drying, hence decreasing the possibility of agglomeration due to the formation of clumps of liquid. For the drying step, all samples were dried in conventional oven for 12hours at 60°C. Eventually, the dried samples were calcinated in the tubular furnace at 350 °C for 3-4 hours to produce silver nanoparticles on the surface of carbon nanotubes.

In order to have a homogeneous metal distribution on the CNT support, the solvent have to be ensure to wetted the CNT, and also to ensure the complete exposure of solution of (in this case silver nitrate) throughout the entire batch of preparation. Thus the use of excess impregnation would be appropriate.

4.5 Microwave Radiation

House microwave oven was used to generate the microwave radiation for heating the nanoparticles. It works by passing non-ionizing microwave radiation, usually at a frequency of 2.45 gigahertz (GHz)— Magnetron emitted radiation over a wave length range of 122 millimetres (4.80 in)—through the sample (Figure 10)

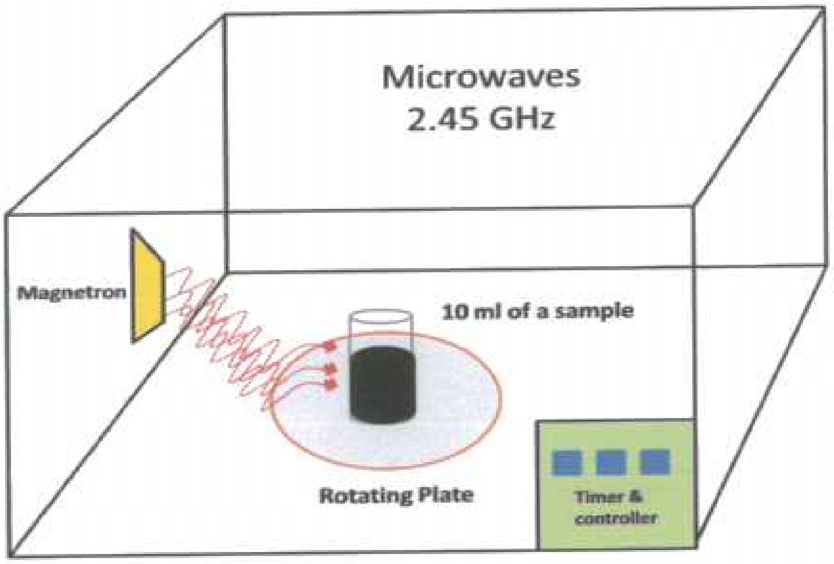


Figure 10: Illustration of experimental setup using Microwaves: The 10ml sample was placed in the middle of the rotating microwave plate.

4.6 Microorganisms and Culture Conditions

Strain *E.coli* ATCC number 8739 (supplied by King Fahd University of Petroleum and Minerals clinic) was used throughout this work. *E. coli* was grown overnight in nutrient broth at 37 ^{0}C on a rotary shaker (160 rpm). Aliquots of the preculture were inoculated into fresh medium and incubated in the same conditions to an absorbance at 600 nm of 0.50. Cells were harvested by centrifugation at 4000g for 10 min at 4 ^{0}C washed twice with a sterile 0.9% NaCl solution at 4 ^{0}C and re-suspended in different carbon nanometeials (MWCNTs, MWCNTs-COOH, MWCNTs-Phenol, MWCNTs-Amine, MWCNTs-C18, MWCNTs-10% Ag, MWCNTs-50%Ag) solution to a concentration of 2 x10^7 Colony Forming Units CFU/ mL.

All carbon nanomaterials were sonicated before mixed with the bacterial solution. For each type of carbon nanomaterial used, the mixture of carbon nanomaterial and bacteria was tested with and without exposure to microwave radiation for 0, 5, and 10 seconds. Cultured bacteria (tested bacteria with different carbon nanomaterials with and without microwave) were analyzed by plating on nutrient agar plates after serial dilution in 0.9% saline. Colonies were counted after 48 h incubation at 37 ^{0}C. Control experiments were carried out in parallel with each experiment done for CNT material tested, i.e. testing of *E. coli* in CNT materials without being subjected to microwave radiation.

4.7 Serial dilution and Bactericidal Rate Calculation

We had seven tested materials of carbon nanometeials (MWCNTs, MWCNTs-COOH, MWCNTs-Phenol, MWCNTs-Amine, MWCNTs-C18, MWCNTs-10% Ag, MWCNTs-50%Ag). An amount of 0.2g of each tested materials was added to 100 ml of sterile 0.9% NaCl solution. Then after 15 minutes of sonication and 20 minutes needed to cool the solution (35 minutes total) we added 1 ml of *E. coli* suspended solution, which contains unknown number of *E. coli* cells. Noting that, *E. coli* cells were harvested and re-suspended in a different sterile 0.9% NaCl solution. After that, 15 ml of that solution was put in a sterilized vial to be exposed to microwave radiation for 0, 5, and 10 seconds, whereas a new sterilized vial having 15 ml of the solution was used for each period of time under microwaves.

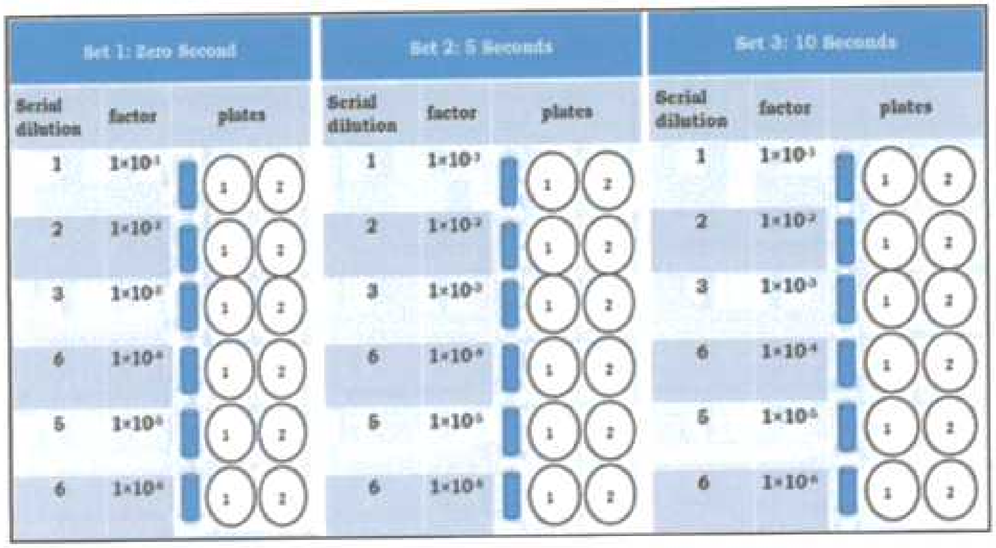
Upon completion, we get two main groups for each tested material; group A: bacterial growth tested with nanomaterials and group B: bacterial growth tested without using nanomaterials. These two groups were studied under the same experimental conditions, i.e with and without microwaves.

The sample with zero seconds in group A, which had not been affected by nanomaterials nor microwaves radiation was considered as the control sample. Figure 12 and 13 below, illustrate the experimental set up.

Using serial dilution, 0.1 ml was taken from each vial and diluted sixth times in 10 ml of sterilized water (10^{-1}, 10^{-2}, 10^{-3}, $10^{-}4$, 10^{-5}, 10^{-6}), so that cell density was decreased and colony forming units (CFU)/ml could be accounted. Counting the original Colony Forming Units (CFU)/ml, i.e. the unknown number of *E. coli* cells in 1 ml added at the beginning can be obtained by multiplying with a dilution factor, which a count stops at.

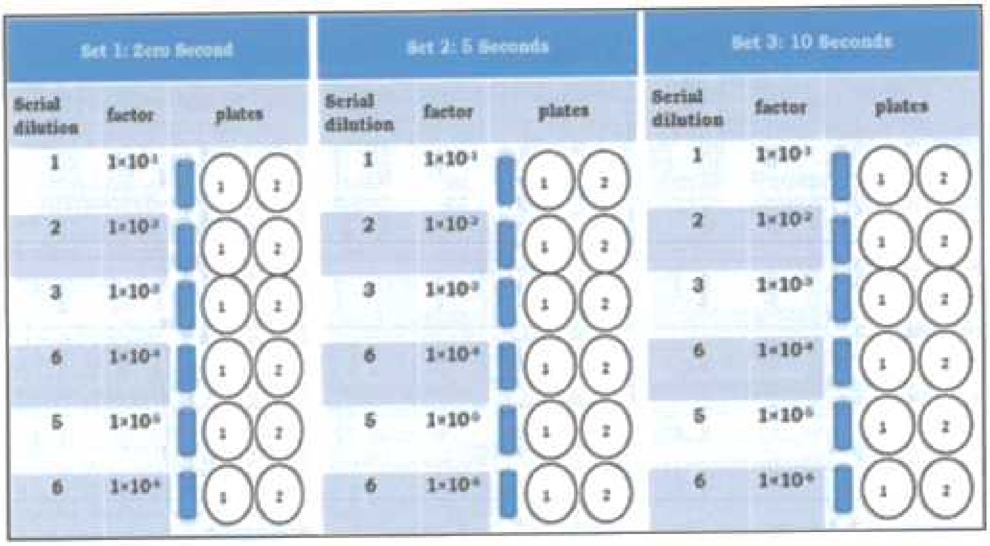
Eventually, Petri dishes containing bacterial cells of *E. coli* were put in an incubator at 37℃ for 48 h (Figure 11) to provide the necessary conditions needed for bacterial growth.

Figure 11: Bacterial Incubator



Set 1: Zero Second			Set 2: 5 Seconds			Set 3: 10 Seconds		
Serial dilution	factor	plates	Serial dilution	factor	plates	Serial dilution	factor	plates
1	1×10^{1}	① ②	1	1×10^{1}	① ②	1	1×10^{1}	① ②
2	1×10^{2}	① ②	2	1×10^{2}	① ②	2	1×10^{2}	① ②
3	1×10^{3}	① ②	3	1×10^{3}	① ②	3	1×10^{3}	① ②
6	1×10^{4}	① ②	6	1×10^{4}	① ②	6	1×10^{4}	① ②
5	1×10^{5}	① ②	5	1×10^{5}	① ②	5	1×10^{5}	① ②
6	1×10^{6}	① ②	6	1×10^{6}	① ②	6	1×10^{6}	① ②

Figure 12: Group A: Petri dishes for bacterial growth without using nanomaterils



Set 1: Zero Second			Set 2: 5 Seconds			Set 3: 10 Seconds		
Serial dilution	factor	plates	Serial dilution	factor	plates	Serial dilution	factor	plates
1	1×10^{1}	① ②	1	1×10^{1}	① ②	1	1×10^{1}	① ②
2	1×10^{2}	① ②	2	1×10^{2}	① ②	2	1×10^{2}	① ②
3	1×10^{3}	① ②	3	1×10^{3}	① ②	3	1×10^{3}	① ②
6	1×10^{4}	① ②	6	1×10^{4}	① ②	6	1×10^{4}	① ②
5	1×10^{5}	① ②	5	1×10^{5}	① ②	5	1×10^{5}	① ②
6	1×10^{6}	① ②	6	1×10^{6}	① ②	6	1×10^{6}	① ②

Figure 13: Group B: Petri dishes for bacterial growth using nanomaterilas

The bactericidal rate **K** can be calculated by following formula:

$$\boxed{K = \frac{(A - B)}{A} \times 100\%}$$

Where,

K: The bactericidal rate

A: colony forming units (CFU)/ml of the control sample

B: colony forming units (CFU)/ml of the tested sample

The optimum bactericidal rate can be accounted in each Petri dish should be in the range of: 30-300 CFU/ml

CHAPTER FIVE

RESULTS AND DISCUSSIONS

The present study reports the results obtained using commercial CNTs and modified CNTs on the removal efficiency of E. Coli bacteria dispersed in water. Further results are given on the additional effect of using microwave radiation on the removal efficiency of the same bacteria.

5.1 Surface Modification of Carbon Nanotubes (Fourier Transform Infrared Spectroscopy)

The as-received MWCNTs show very weak peak at around 1635 cm−1 (Figure 11a) ascribed to the oscillation of carboxylic groups which moves to 1730 cm-1 associated with the stretch mode of carboxylic groups is observed in the IR spectrum of the acid-treated MMWNTs (Figure 11b) [48], indicating that carboxylic groups are formed and C=O liaison of the carboxylic acid function due to the oxidation of some carbon atoms on the surface of the MWNTs by nitric acid [49,50]. The IR spectra of oxidized MWCNTs show four major peaks at 3750, 3450, 2370 and 1562 cm−1. The peak at 3750 is attributed to free hydroxyl groups [51]. The peak at 3445 cm−1 can be assigned to O-H stretch from carboxylic groups (O=C-OH and C-OH) while the peak at 2364 cm−1 can be associated with OH stretch from strongly H-bonded-COOH [52]. The peak at 1565 cm−1 is related to the carboxylate anion stretch mode . It should be noticed that the as-received MWCNTs were purified and a part of the catalytic metallic nanoparticles were possibly eliminated during the purification process cutting the nanotube cap. Thus, the presence of carboxylic groups in these commercial MWCNTs was expectable. Moreover, it should be noted that there is no significant difference between the spectra of the samples before and after the HNO_3 treatment.

The peak at 1635 cm^{-1} can be associated with the stretching of carbon nanotubes backbone . Increased strength of the signal at 1165 cm^{-1} may be associated with C–O stretching in the same functionalities. The peaks around 2877 and 2933 cm^{-1} correspond to the H–C stretch modes of H–C=O in carboxylic group.

In Figure 11c, the MMWCNT−$COO(CH_2)_{17}CH_3$ showed IR absorptions at 2924 cm^{-1} and 2872 cm^{-1} of C−H stretch modes which is indicative of the presence of a long-chain alkyl molecule of octadecanol of the alkyl chain, peak at 1701 cm^{-1} of C=O stretch of the ester. Peak at 1560 cm^{-1} is of C=C stretch of the MMWCNTs and peak at 1461 cm^{-1} of C−H bend of the alkyl chain while peak at 1108 cm^{-1} is for C−O stretch of the ester group, many of these absorptions have been reported previously for functionalized SWNTs. Also spectra MMWCNT-octadecanoate exhibited the typical bands of the $-CH_2$ rocking at 760 cm^{-1}.

Peak at 2281 cm^{-1} is N-C=O asymmetric vibration (Figure 11d), while it observed a new signal peak at 1544 cm^{-1} which is attributed to the overlapping of a signal for N-H, N-C bands and N-C=O group [53]. Moreover, all figures show peaks between 1300 and 1100 cm^{-1}, which are ascribed to the C–C stretch bonds.

As can be seen in Figure 11e, spectra presented the signals, which corresponded to the phenyl structure described as follows: the peaks between 3000 and 3140 cm^{-1} that correspond to the bands of stretching of the group C–H of the aromatic ring; between 1600 and 2000 cm^{-1} the overtones of the phenyl ring substitution; in 685 and 890 cm^{-1} the bands of phenyl ring substitution; in 1565, 1505 and 1460 cm^{-1} the bands of stretching of the group C=C of the aromatic ring .

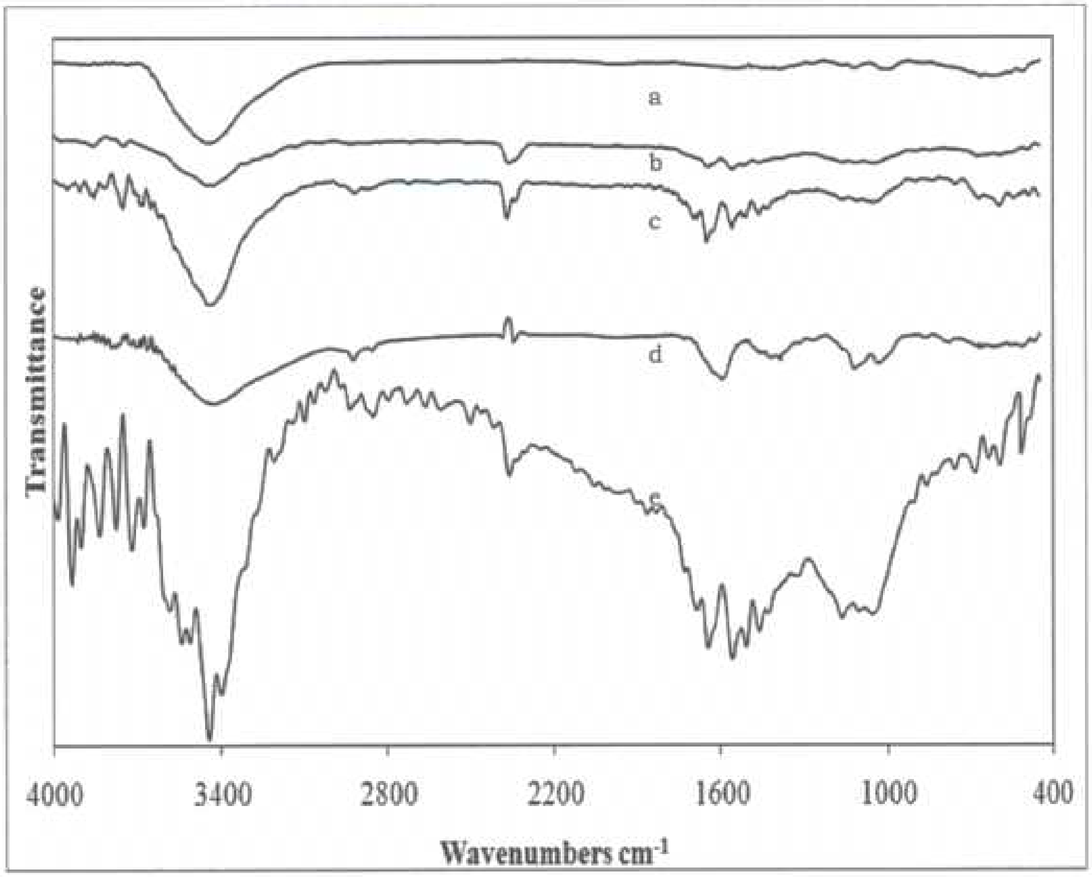


Figure 14: FTIR spectra of (a) as-received MWCNT, (b) MMWCNT, (c) MMWCNT−octadecanoate, (d) MMWCNT−dodecylamide, and (f) MMWCNT−Phenyl ester

5.2 Characterization of Carbon Nanotubes

The carbon nanotubes were observed by using field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM). The diameter of the carbon nanotubes were varied from 20-40 nm with average diameter at 24 nm while the length of the CNTs was up to few microns. Figure 12 (a) shows the SEM image of carbon nanotubes at low magnification. While Figure 12 (b) shows the SEM image of carbon nanotubes at high magnification. From the SEM observation, the product is pure and only carbon nanotubes were observed.

TEM was carried out to characterize the structure of nanotubes (Figure 13). To prepare TEM samples, some alcohol was dropped on the nanotubes film, then, these films were transferred with a pair of tweezers to a carbon-coated copper grid. It is obvious from the images that all the nanotubes are hollow and tubular in shape. In some of the images, catalyst particles can be seen inside the nanotubes. TEM images indicate that the nanotubes are of high purity, with uniform diameter distribution and contain no deformity in the structure. While Figure 13 (b) shows the High Resolution Transmission Electron Microscope (HRTEM) of the carbon nanotubes. It shows that a highly ordered crystalline structure of CNT is present.

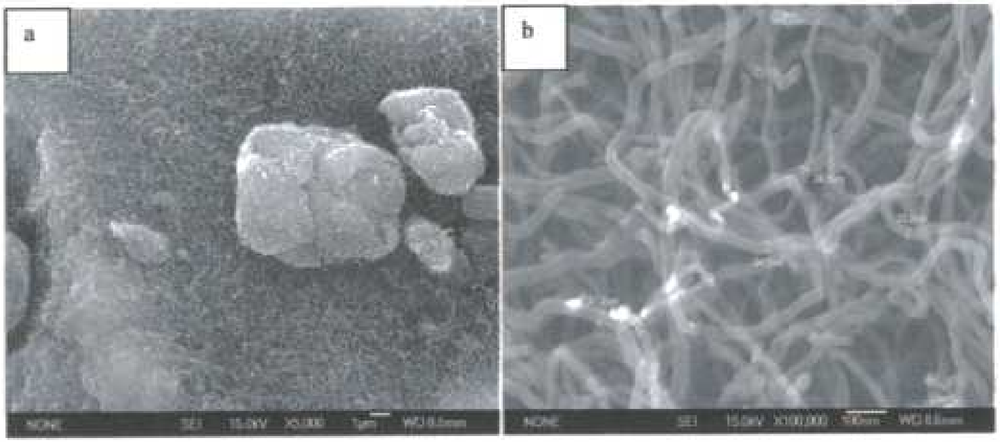


Figure 15: SEM Images of carbon nanotubes at (a) at low resolution (b) at high resolution

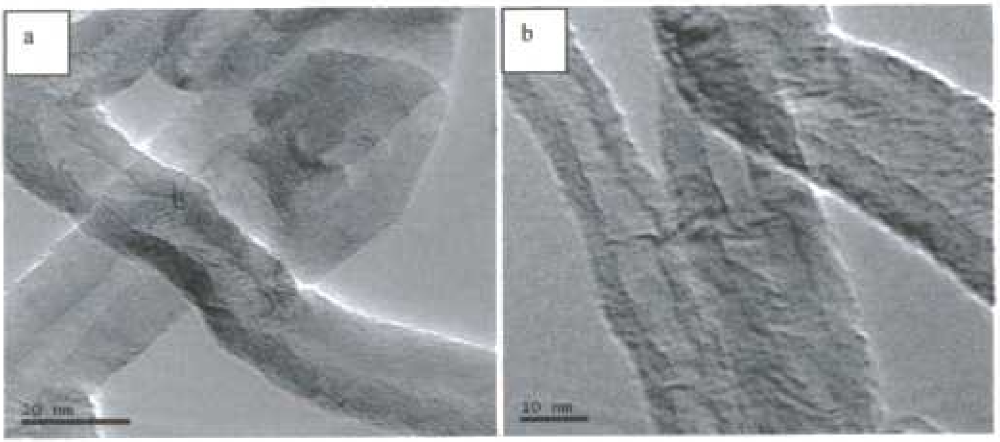


Figure 16: TEM Images of carbon nanotubes (a) at low resolution (b) at high resolution.

5.3 FT-IR Analysis

The FT-IR spectra for the untreated CNT and acid treated CNT are shown in Figure 14. While the untreated CNT show only a few weak peaks in the spectra, especially corresponding to the OH groups and C=O groups due to the absorption of water molecules and oxidation of the carbon chains, acid modified CNT shows that the peak attributed to C=O shows increased intensity in the region 1698cm^{-1} which confirms the oxidation of the CNT surface. The peaks in the region of 3398 and 3797 cm^{-1} are due to the presence of hydroxyl groups [14, 16-19]. Furthermore, there are peaks corresponding to the C-O-C stretching in the 1000-1100 cm^{-1} region.

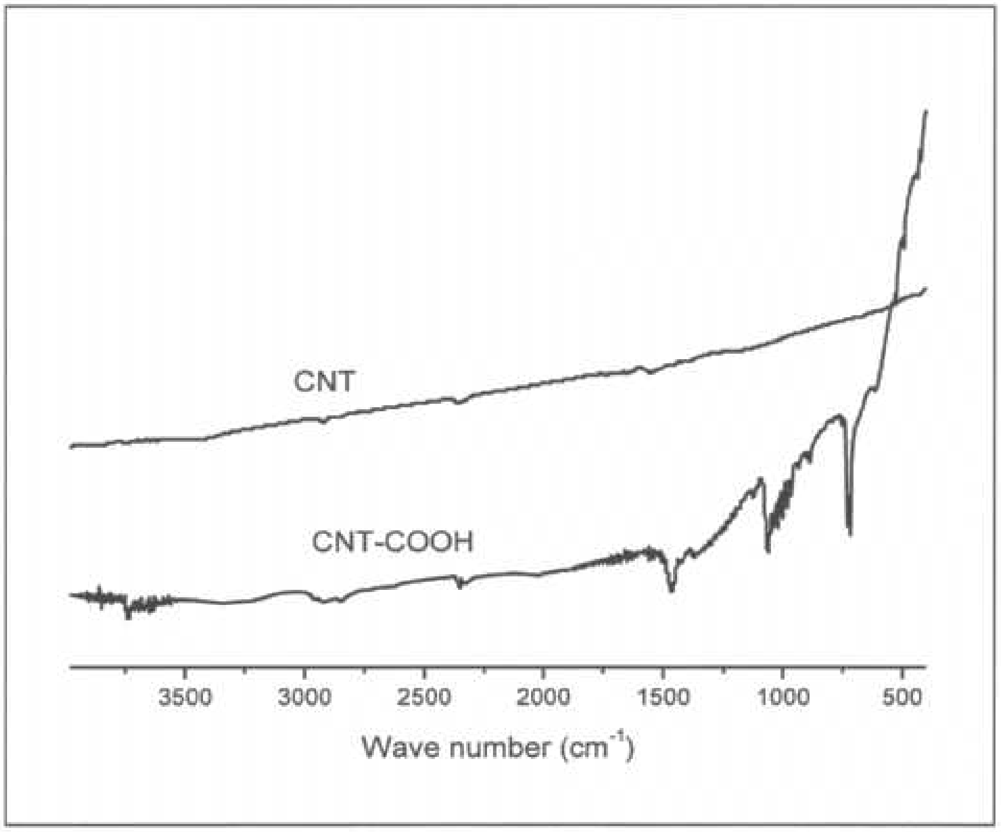


Figure 17: FT-IR spectrum of untreated CNT and CNT-COOH indicating the presence of carbonyl and hydroxyl groups

5.4 Effect of CNTs and Modified CNTs on Removal of *E. Coli*

The effect of Carbon Nanotubes (CNTs), modified CNTs and impregnated CNTs on the removal of *E. coli* Bacteria were investigated in this study. *E. coli*, the most characterized bacterium, has been used as a model bacterial system for various antimicrobial testing programs, where the following seven materials have been tested:

1- Raw CNTs

2- CNTs functionalized with carboxylic (COOH) group (Oxidized CNTs)

3- CNTs functionalized with phenol (C_5H_5OH) group

4- CNTs functionalized with 1-Octadecanol ($C_{18}H_{38}O$) or simply C18 group

5- CNTs functionalized with dodecylamine group ($C_{12}H_{27}N$)

6- CNTs impregnated with 10% of Ag nanoparticles

7- CNTs impregnated with 50% of Ag nanoparticles

The dosing amount of the CNTs during all experiments was fixed; 0.2g of CNTs/100 ml of 0.9 % NaCl autoclaved solution (for bacterial suspension). Table 3 presents the percentage of *E. coli* bacteria removed by adding CNTs, modified CNTs and CNTs impregnated with Ag nanoparticles.

Table 3 below illustrates the percentage of *E. coli* removal in aqueous solution from the addition of unmodified multi-walled carbon nanotubes, multi-walled carbon nanotubes functionalized with a carboxylic (COOH) group, multi-walled carbon nanotubes functionalized with a phenol (C_5H_5OH) group, multi-walled carbon nanotubes functionalized with a C18 group (1-octadecanol ($C_{18}H_{38}O$)), and multi-walled carbon nanotubes impregnated with silver nanoparticles (with silver nanoparticles added at both 10 wt% and 50 wt%).

TABLE 3: E. coli removal without microwave heating

	Number of E. Coli Cells (cfu/ml)		
Type of MWCNT added to control sample	Control sample	After addition of MWCNTs	% of E. coli removal
MWCNT	3.70×10^7	3.50×10^7	5
MWCNT-COOH	3.70×10^7	3.60×10^7	3
MWCNT-C18	3.50×10^7	3.40×10^7	3
MWCNT-Ag (10 wt%)	2.20×10^7	1.90×10^7	14
MWCNT-Ag (50 wt%)	2.20×10^7	1.70×10^7	23
MWCNT-Phenol	2.30×10^7	1.60×10^7	30

As shown in Table 3, the results indicate that the percentage of *E. Coli* bacteria removal for MWCNTs, MWCNTs-COOH and MWCNTs-C18 is relatively small (3-5 wt%) which is relatively minor, with no significant removal. Although MWCNTs and MWCNTs-C18 have similar properties due to their similar C-C bonding, MWCNTs-COOH provide different properties due to the carboxylic group.

From that we can say, there is no significant effect of raw or pure CNTs nor even oxidized CNTs (MWCNTs-COOH) on the removal of *E. coli*. The purpose to oxidize the surface of CNTs is to disperse them in the solution and to homogenous solution of CNTs with water. CNTs are usually in the form of bundles and ropes because of their intrinsic van der Waals force attraction and high aspect ratio nature. Functionalization of MWNTs with COOH can help debundle MWNTs ropes and improve their dispersion in water. Thus, compared with MWNTs-COOH, the increased antimicrobial effect of MWNTs may be attributed partially to their smaller bundle size. The possible mechanism for bacterial growth inhibition by MWCNTs could be due to that gram negative bacteria possessing thin layer of negatively charged liposaccharides on the bacterial cell wall are attracted toward weak positively charged MWCNT leading to the death of bacteria. Another mechanism of inhibition could be due to the interference of MWCNT with the protein synthesis process on the ribosoms of the cell which will lead to cell death.

On the other hand, the percentage of *E. Coli* bacteria removal for MWCNTs-Ag is increased due to the presence of the Ag nanoparticles. Increasing the amount of Ag nanoparticles from 10 wt% to 50 wt% almost doubles the percentage of bacterial removal. The highest removal rate of *E. coli* bacteria was shown with MWCNTs functionalized with phenol functional groups. From Table 3, it is clearly shown that the MWCNTs-phenol removed almost 30% of *E. coli* bacteria from solution, even without the application of microwave radiation.

Table 4 below illustrates the percentage of *E. coli* removal in aqueous solution from the addition of multi-walled carbon nanotubes functionalized with a dodecylamine group ($C_{12}H_{27}N$). The dosing amount of the MWCNTs during all experiments was fixed at 0.2 g of MWCNTs per 100 ml of NaCl autoclaved solution. For MWCNT-amine, the dosage was varied from approximately 0.2 to 0.007 g per 100 ml.

TABLE 4: E. coli removal by MWCNT-CONH(CH_2)$_{11}$$CH_3$

MWCNT-amine	% of E. coli removal
0.2 g/100 ml	100
0.07 g/100 ml	100
0.05 g/100 ml	100
0.03 g/100 ml	100
0.012 g/100 ml	100
0.007 g/100 ml	100

From Tables 4, it can be clearly seen that the total removal of *E. coli* bacteria was achieved using 0.2 g of MWCNTs functionalized with the amine functional group dodecylamine (denoted as MWCNT-amine in Table 4). Since 0.2 g of MWCNT-amine, which contains only 0.02 g of amine on the surface of the MWCNTs (i.e., 10% of the total weight) removed all bacteria from the aqueous solution, a further reduction of the total weight of the sample (0.2 g) has been carried out to study the effect of the dosage on the removal of *E. coli* bacteria.

A sharp decrease in the weight of the sample from 0.2 to 0.007 g showed that this small weight of MWCNTs-amine, which contains 0.0007 g of amine groups at the surface, also removed all bacteria from the water.

By comparing the minimum dosage of CNTs-Amine in our work at 7 mg/100ml which contains only 0.7 mg of dodecylamine to the recent available data in the literature, Charcosset and Périchaud in 2009 [54], which has showed that the minimal amount of dodecylamine needed to remove the bacteria from water is active in concentrations as less as 0.05% to 0.1% i.e. 100 mg/100ml, we can observe that dodecylamine used in Charcosset and Périchaud study is "143 times" higher than our dosage. This important result indicates that 0.7% of dodecylamine on CNTs compared to dodecylamine used by Charcosset and Périchaud was enough to remove the whole bacteria from water.

5.5 Effect of Microwave Heating in Presence of CNTs and Modified CNTs on Removal of *E Coli*

Table 5 below illustrates the percentage of *E. coli* removal in aqueous solution from the addition of unmodified multi-walled carbon nanotubes, multi-walled carbon nanotubes functionalized with a carboxylic (COOH) group, multi-walled carbon nanotubes functionalized with a phenol (C_5H_5OH) group, multi-walled carbon nanotubes functionalized with a C18 group (1-octadecanol ($C_{18}H_{38}O$)), and multi-walled carbon nanotubes impregnated with silver nanoparticles (with silver nanoparticles added at both 10 wt% and 50 wt%) with the added step of microwave treatment, both for a duration of 5 seconds and for a duration of 10 seconds.

TABLE 5: E. coli removal with microwave heating

Type of MWCNT added to control sample	Number of E. coli Cells in control sample (cfu/ml)	% of E. coli removal at 5 sec.	% of E. coli removal at 10 sec.
No MWCNTs added	3.70×10^7	8.1	59.5
MWCNT	3.70×10^7	40.5	77.0
MWCNT-COOH	3.70×10^7	18.9	64.9
MWCNT-C18	3.50×10^7	37.1	97.1
MWCNT-Ag (10 wt%)	2.20×10^7	59.1	100
MWCNT-Ag (50 wt%)	2.20×10^7	94.1	100
MWCNT-Phenol	2.30×10^7	32.6	76.1

As shown in Table 5, the heat generated from the microwave treatment removed 8% of *E. coli* from water after 5 seconds of exposure and up to 60% after 10 seconds. The percentage removal of *E. coli* bacteria after 5 seconds of microwave heating increased from 8% to 40% through the addition of a relatively small amount of MWCNTs to the water (0.2 g of MWCNTs/100 ml). More than 30 % of the *E. coli* bacteria were removed compare to the effect of heating source by microwave which indicated that the CNTs works as a local heating agents that kill the microorganism from water. A higher removal rate of *E. coli* bacteria (77%) was achieved after 10 seconds of microwave treatment by adding same amount of CNTs compare to 60 % of the removal without CNTs.

On the other hand, the results indicate that the percentage of *E. coli* bacteria removal using MWCNTs modified with a carboxylic group, MWCNTs-COOH, (i.e. oxidized CNTs) in water decreased drastically from 40% to about 19% after 5 seconds of microwave exposure. The reason for this behavior is that the COOH group changed the thermal properties of the MWCNTs by forming (on the surfaces of the MWCNTs) chains of C-O-O-H, which are not thermally conductive. Increasing the exposure time to 10 seconds gave the same result in term of the reduction of E. Coli bacteria but still higher than the microwave heating source without the CNTs.

The behavior of MWCNTs modified with phenol, in terms of removal efficiency of the bacteria, after 5 seconds and 10 seconds of microwave exposure is similar to that of unmodified MWCNTs. Similar results were obtained using MWCNTs modified with a C18 group after 5 seconds, while a high removal rate of *E. coli* bacteria was achieved after 10 seconds due to multiple chains of C18 (C-C bonds), which increased the absorption rate of the microwave heat.

For the MWCNTs impregnated with Ag nanoparticles, the percentage of *E. coli* bacteria removal after 5 seconds of microwave exposure was the highest, compared to the above mentioned materials, indicating that the absorption of microwave heat is the highest due to the presence of Ag nanoparticles. Increasing the amount of impregnated Ag nanoparticles from 10 wt% to 50 wt% drastically enhanced the percentage of bacterial removal from 60% to 94%. For 10 seconds of microwave exposure, all *E. coli* bacteria was removed from water (100% removal rate) for both Ag concentrations.

5.6 Proposed Mechanism of Bacterial Killing

The possible mechanism for bacterial growth inhibition by MWCNTs could be due to that gram negative bacteria possessing thin layer of negatively charged lipopolysaccharides (LPS) on the bacterial cell wall are attracted toward weak positively charged MWCNT leading to the death of bacteria.

Interference of MWCNT with the protein synthesis process on the ribosome of the cell which will lead to cell death could be another mechanism of inhibition. Another possible mechanism is the intercalating of positively charged MWCNT to the negatively charged DNA molecules in the bacterial chromosome, which will resulted in disruption of the DNA synthesis and then death of bacteria (Figure 18).

However, the bacterial killing, which was enhanced after using microwaves might referrers to the effect of polarization over the cell wall. This polarization is expected to exert acute and excessive potential and heat leading to burst the cell-wall. In addition to that, the excellent thermal properties of CNTs in absorption of heat emitted from microwaves then transferring it to a different form of highly localized intensive heat inside the cytoplasm of the bacterial cell is another mechanism (combined effect of CNTs and microwave heat).

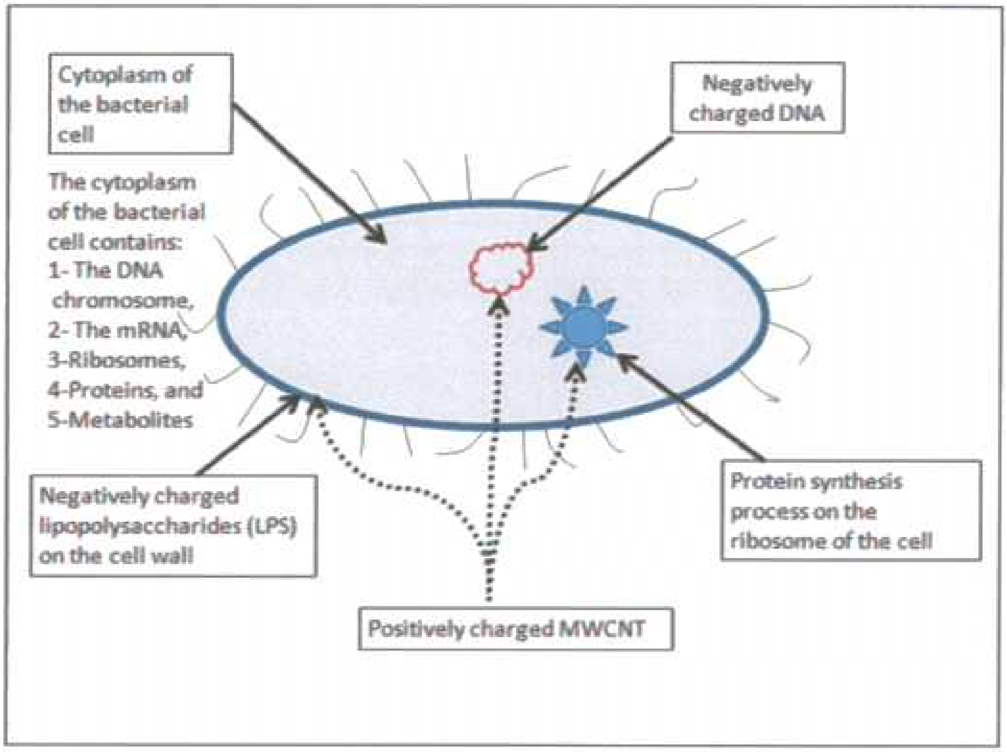


Figure 18: The three possible mechanisims of antimicrobial activities exerted by MWCNTs and modified MWCNTs

CHAPTER SIX

6.1 RECOMMENDATIONS

This presented research, which lies under the subjected field of bio-nanotechnology could be attractive to some medical applications for removal of pathogens and other unwanted biological constituents with sizes greater than 50nm from highly complex medium. Application of CNTs in waste water treatment is not limited to filtration and sorbent; several researchers observed strong antimicrobial properties of CNTs.

Such behaviour allows CNTs to replace chemical disinfectants as a new effective way to control microbial pathogens [2, 42].

Some highly qualified research centres around the world are conducting exploratory studies to a point of connecting machines to individual cells to carry a treatment, inject drugs, cut and remove, and perform many things related to health issues.

Nanoparticles (NPs) in particular CNTs have promising properties to slip through the immune system or directly into the brain or blood cells, so the significant result presented here to show the antimicrobial properties of several types nanomaterials with and without heating effect of microwave radiation could be applicable also for promising applications in medical side to overcome many of the current limitations in treatment of human cancer cells as part of tumor therapy.

There are thousands of species of dangerous bacteria, unknown illnesses, and new viruses found in the world, so extensive and deeper scientific research studies are indeed needed in this field not only for human beings purposes, but also for environment around us (flora and fauna).

6.2 CONCLUSION

In our research we have presented a new approach for the removal of Escherichia Coli (*E. coli*) bacteria from water using Carbon Nanotubes (CNTs) ,modified CNTs and impregnated CNTs with and without heating effect from microwave radiation.

The results indicate that the CNTs absorb the microwave radiation and converted to localized-intensive heat, which lead to remove 40 % of E. Coli bacteria after 5 seconds and 77 % after 10 seconds. Less removal was observed when the CNTs modified with carboxylic functional group which indicated that, the COOH groups affect the properties of CNTs. Similar results were obtained using CNTs modified with C18 group, CNTS-C18, after 5 seconds, while a high removal of E. Coli bacteria was achieved after 10 sec due to multiple chains of C18 (C-C bonds) which increased the absorption rate of the microwave heat.

The behaviour of modified CNTs with Phenol in terms of removal efficiency of the bacteria after 5 sec and 10 sec microwave exposure looks similar to that of CNTs.

For the CNTs impregnated with Ag nanoparticles, the percentage of *E. coli* bacteria removal after 5 sec microwave exposure is the highest compared to all above mentioned materials indicating that the absorption of microwave heat is the highest due to the presence of Ag nanoparticles. Increasing the amount of impregnated Ag nanoparticles from 10wt% to 50wt% has drastically enhanced the percentage of bacterial removal from 60% to 94%. While, for 10 sec microwave exposure, all *E. coli* bacteria was removed from water (100% removal) for both Ag concentrations.

However, 100 % removal of Escherichia Coli (*E. coli*) from water using Multiwalled Carbon Nanotubes (MWCNTs) functionalized with dodecylamine group ($C_{12}H_{27}N$)

has been achieved not only with a very minor dosage of CNT-Amine (0.007g/100ml) but also without effect of microwaves radiation or any other external radiation.

By comparing the minimum dosage of CNTs-Amine in our work at 7 mg/100ml which contains only 0.7 mg of dodecylamine to the recent available data in literature, we can observe that dodecylamine used in our work is "143 times" less than the dosage in the literature. This indicates that 0.7% of dodecylamine on CNTs was enough to remove the whole bacteria from water without any radiation effect.

Several possible mechanisms for bacterial growth inhibition and killing due to presence of CNTs have been suggested including interference of CNTs with the protein synthesis process on the ribosome of the cell and disruption of the DNA synthesis of the bacteria leading to its death. With the combined use of CNTs and microwave heating, the CNTs absorb the heat emitted from the microwaves and then act as a form of highly localized intensive heat inside the cytoplasm of the bacterial cell.

In conclusion, these significant results presented here to show the antimicrobial properties of several types of novel nanomaterials with and without effect of microwaves radiations would open a new window for further intensive and promising reach in many of medical, microbiological and water treatment applications, such as: disinfection for virus, microbial control, and treatment several types of cancer as part of tumor therapy.

APPENDIX-A: Graphs

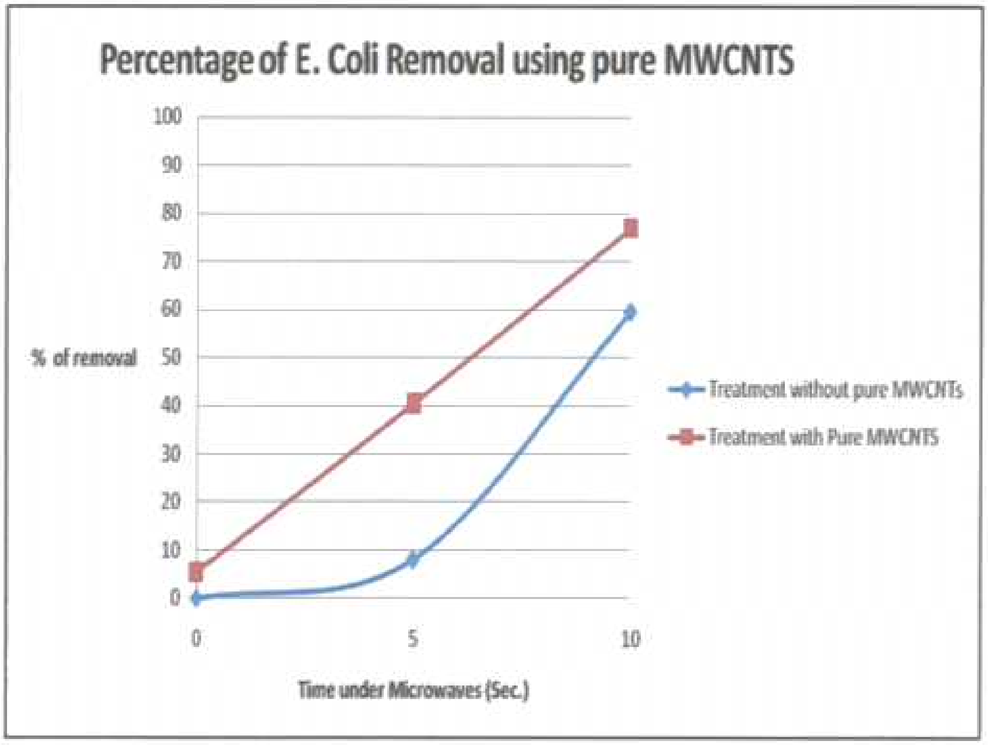


Figure 19: Percentage of *E. Coli* Removal using pure CNTs

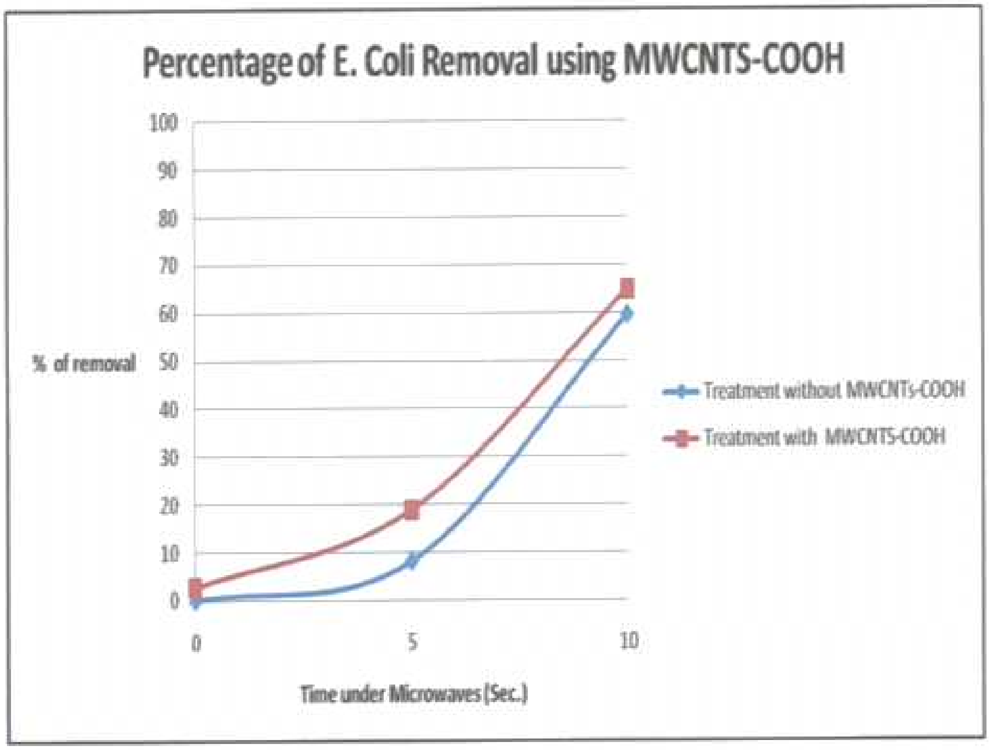


Figure 20: Percentage of *E. Coli* Removal using pure CNTs -COOH

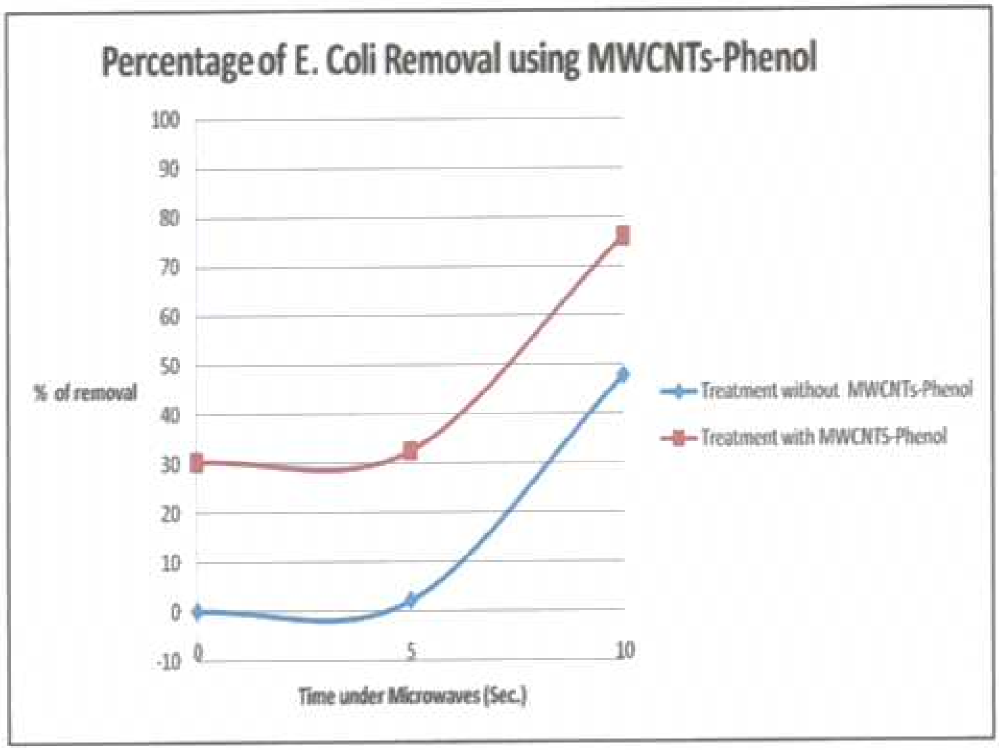


Figure 21: Percentage of *E. Coli* Removal using pure CNTs -Phenol

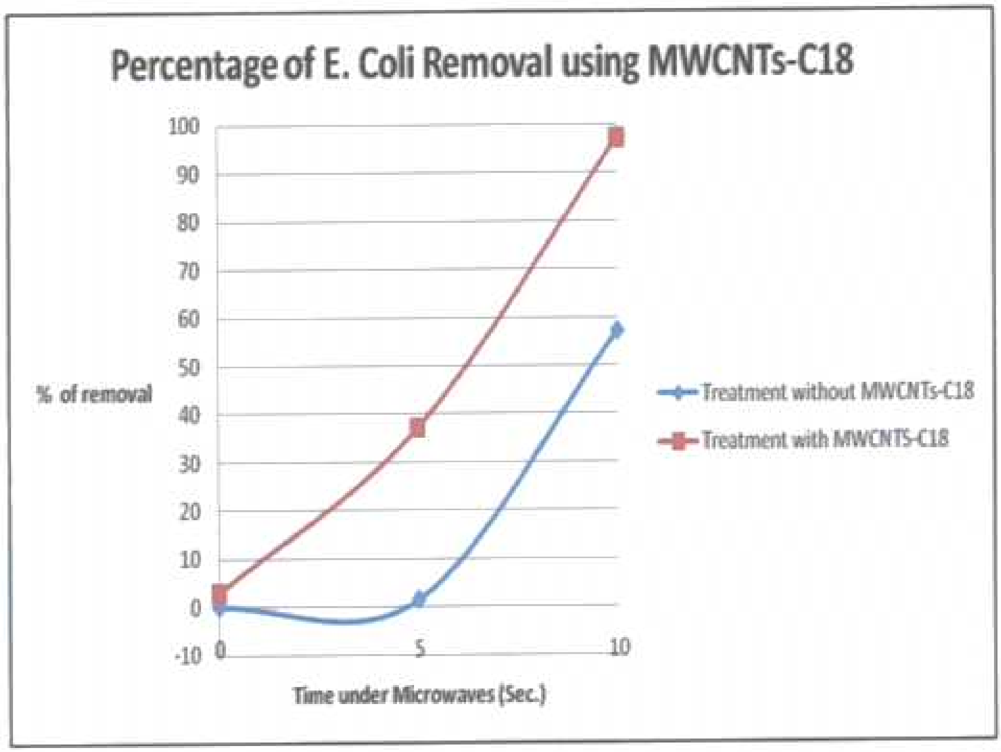


Figure 22: Percentage of *E. Coli* Removal using pure CNTs –C18

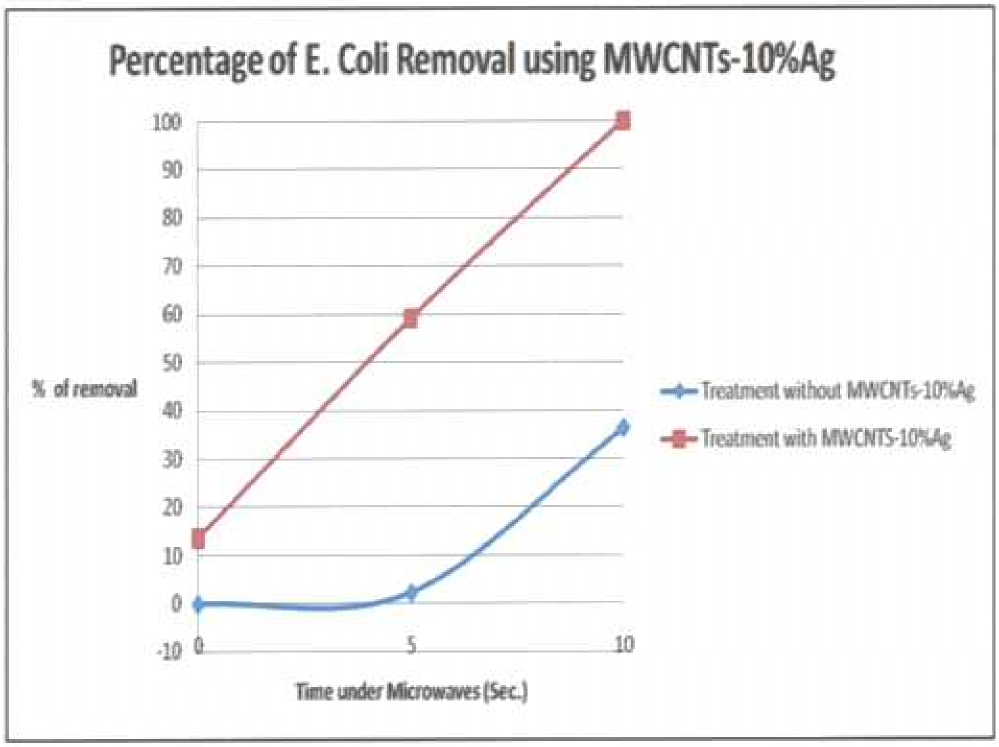


Figure 23: Percentage of *E. Coli* Removal using pure CNTs-10%Ag

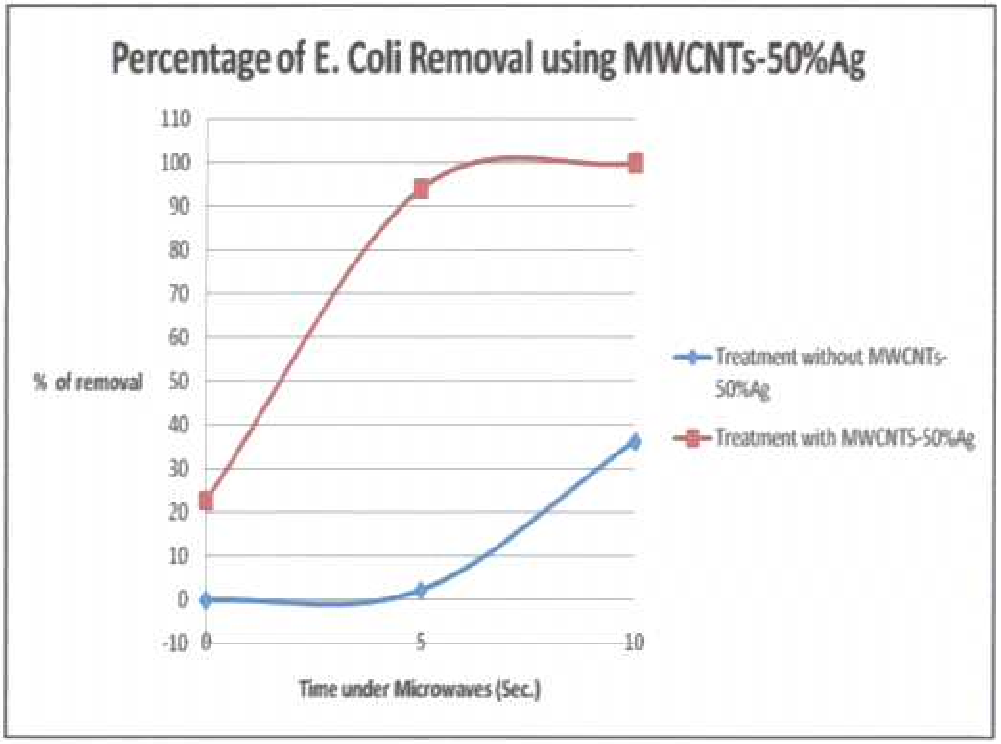


Figure 24: Percentage of *E. Coli* Removal using pure CNTs-50%Ag

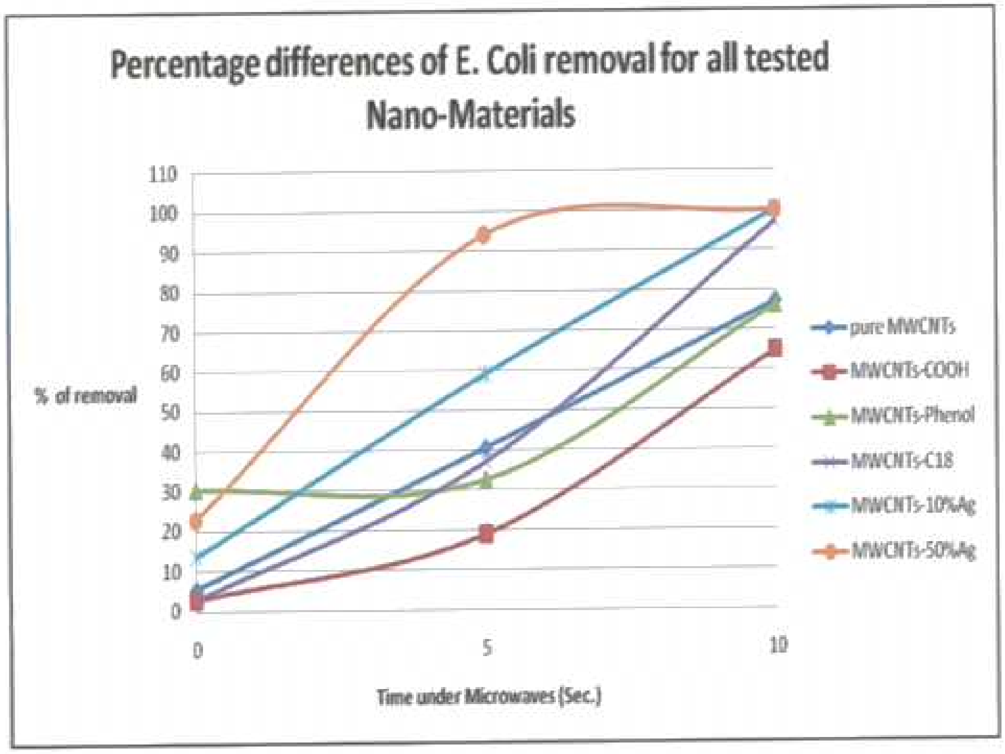


Figure 25: Percentage of *E. Coli* Removal of all tested samples under the effect of microwaves

APPENDIX-B: Photos

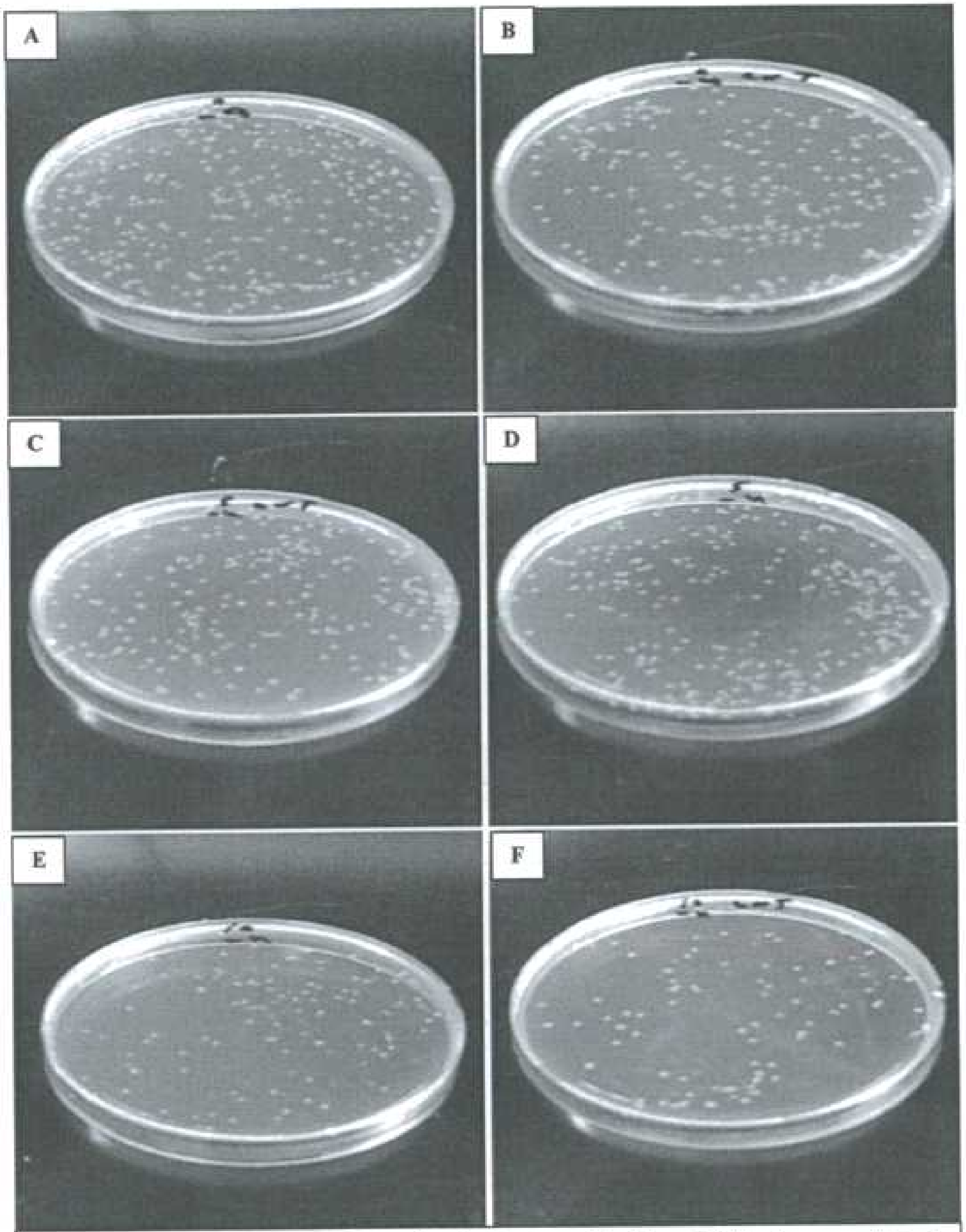


Figure 26: (A) An image of *E. coli* colonies treated without CNTs for zero time in microwaves, i.e. no microwaves radiation (the control sample). (B) An image of *E. coli* colonies treated with CNTs for zero time in microwaves. (C) An image of *E. coli* colonies treated without CNTs for 5 seconds in microwaves. (D) An image of *E. coli* colonies treated with CNTs for 5 seconds in microwaves. (E) An image of *E. coli* colonies treated without CNTs for 10 seconds in microwaves. (F) An image of *E. coli* colonies treated with CNTs for 10 seconds in microwaves.

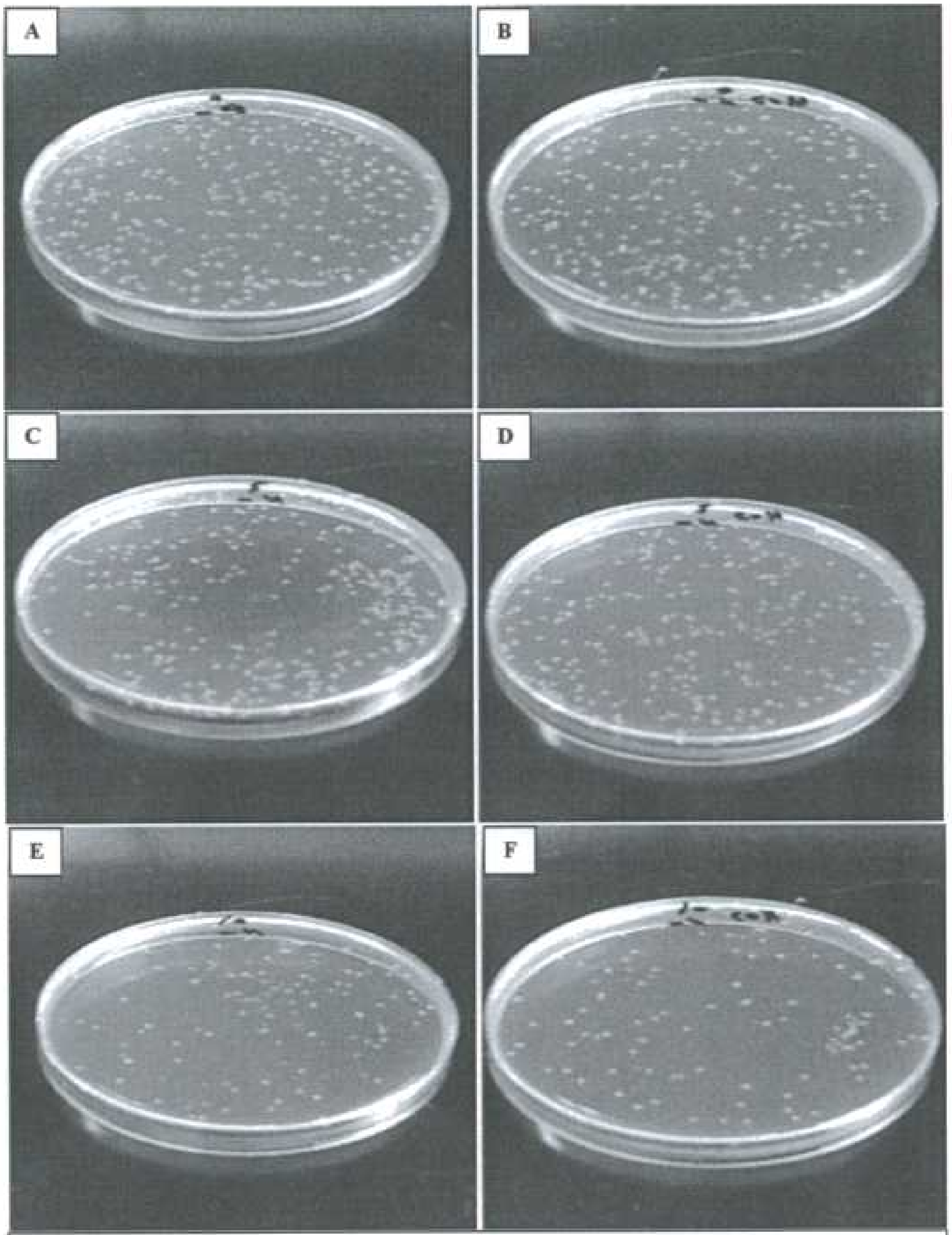


Figure 27: (A) An image of *E. coli* colonies treated without CNTs-COOH for zero time in microwaves, i.e. no microwaves radiation (the control sample). (B) An image of *E. coli* colonies treated with CNTs-COOH for zero time in microwaves. (C) An image of *E. coli* colonies treated without CNTs-COOH for 5 seconds in microwaves. (D) An image of *E. coli* colonies treated with CNTs-COOH for 5 seconds in microwaves. (E) An image of *E. coli* colonies treated without CNTs-COOH for 10 seconds in microwaves. (F) An image of *E. coli* colonies treated with CNTs-COOH for 10 seconds in microwaves.

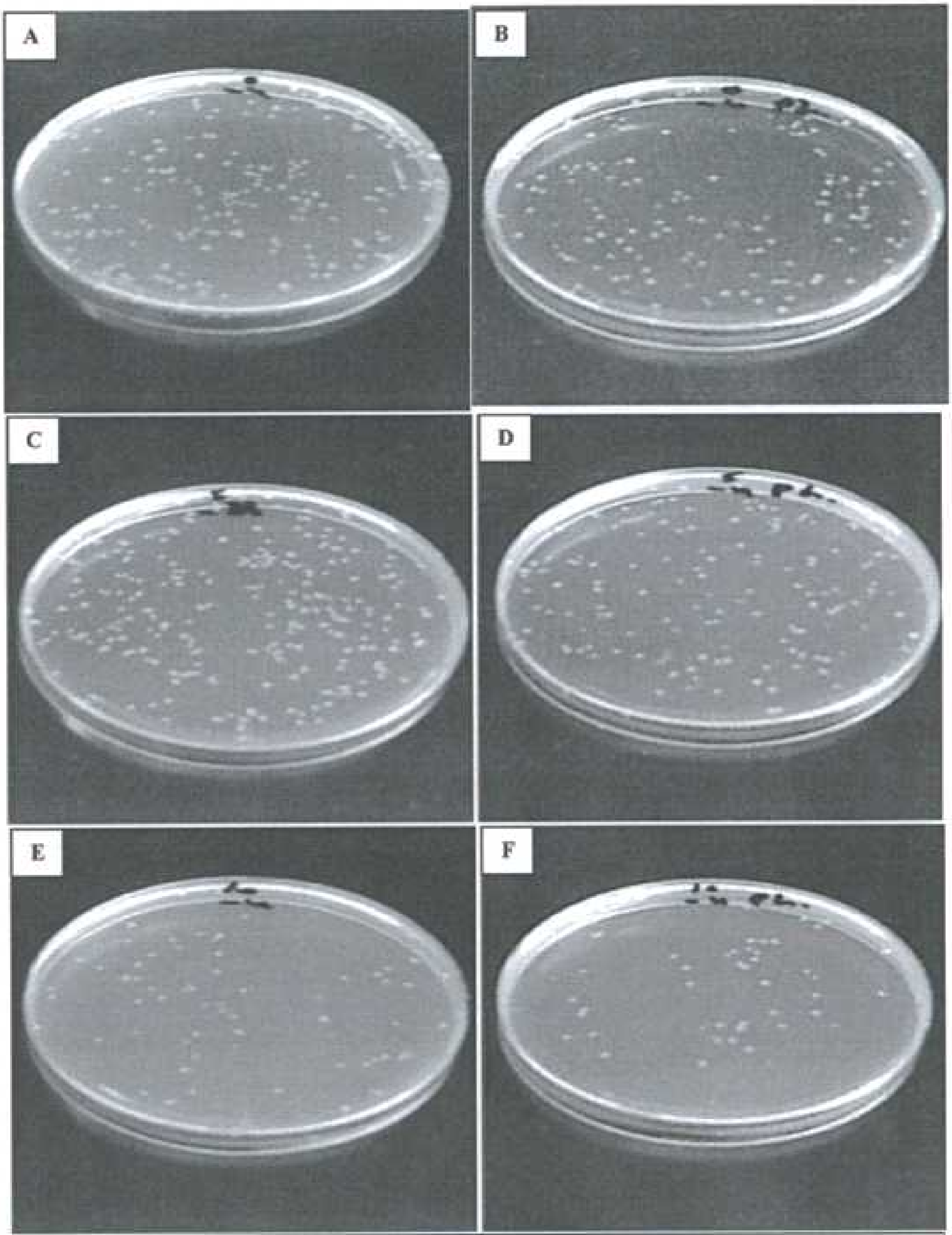


Figure 28: (A) An image of *E. coli* colonies treated without CNTs-Phenol for zero time in microwaves, i.e. no microwaves radiation (the control sample). (B) An image of *E. coli* colonies treated with CNTs-Phenol for zero time in microwaves. (C) An image of *E. coli* colonies treated without CNTs-Phenol for 5 seconds in microwaves. (D) An image of *E. coli* colonies treated with CNTs-Phenol for 5 seconds in microwaves. (E) An image of *E. coli* colonies treated without CNTs-Phenol for 10 seconds in microwaves. (F) An image of *E. coli* colonies treated with CNTs-Phenol for 10 seconds in microwaves.

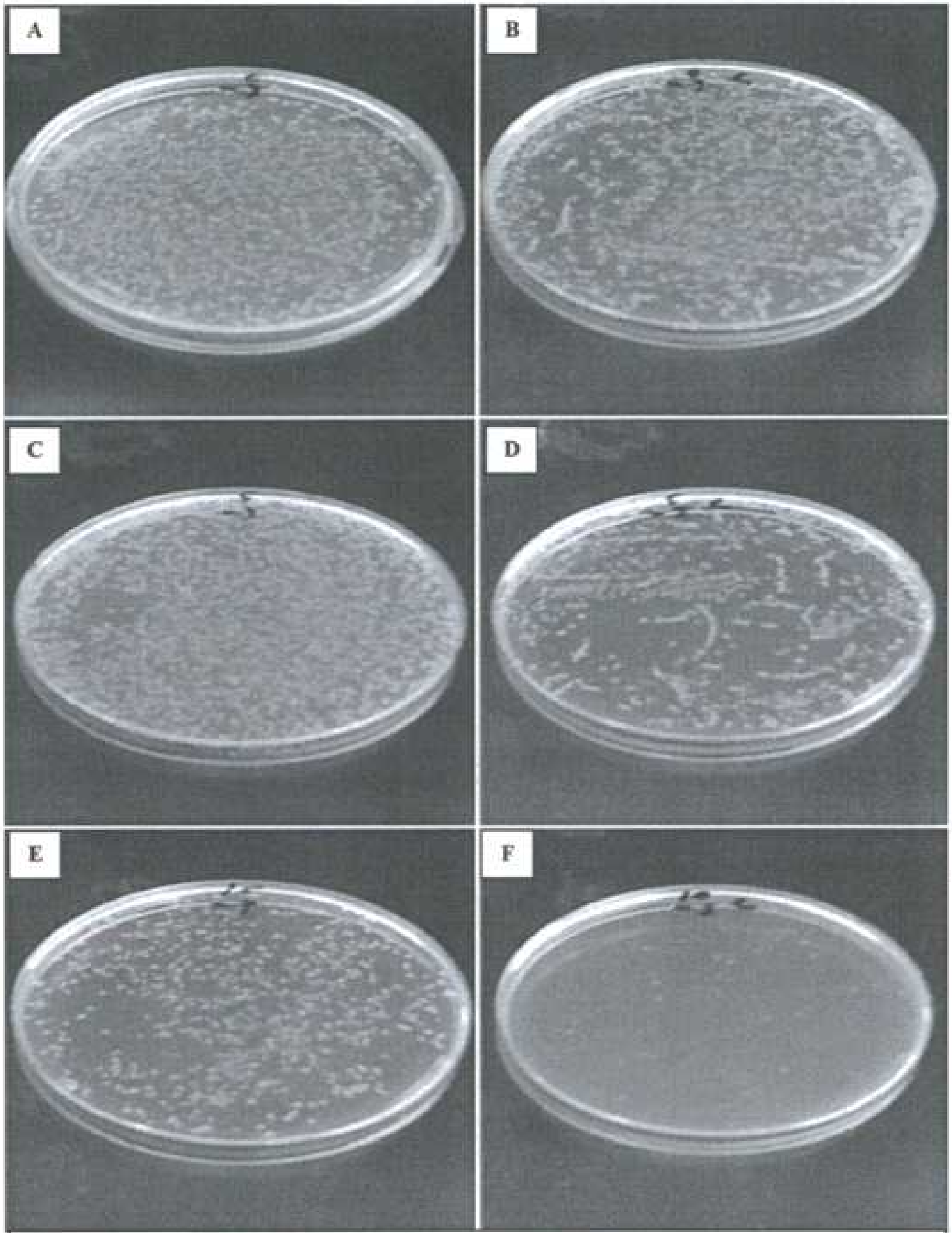


Figure 29: (A) An image of *E. coli* colonies treated without CNTs-C_{18} for zero time in microwaves, i.e. no microwaves radiation (the control sample). (B) An image of *E. coli* colonies treated with CNTs-C_{18} for zero time in microwaves. (C) An image of *E. coli* colonies treated without CNTs-C_{18} for 5 seconds in microwaves. (D) An image of *E. coli* colonies treated with CNTs-C_{18} for 5 seconds in microwaves. (E) An image of *E. coli* colonies treated without CNTs-C_{18} for 10 seconds in microwaves. (F) An image of *E. coli* colonies treated with CNTs-C_{18} for 10 seconds in microwaves.

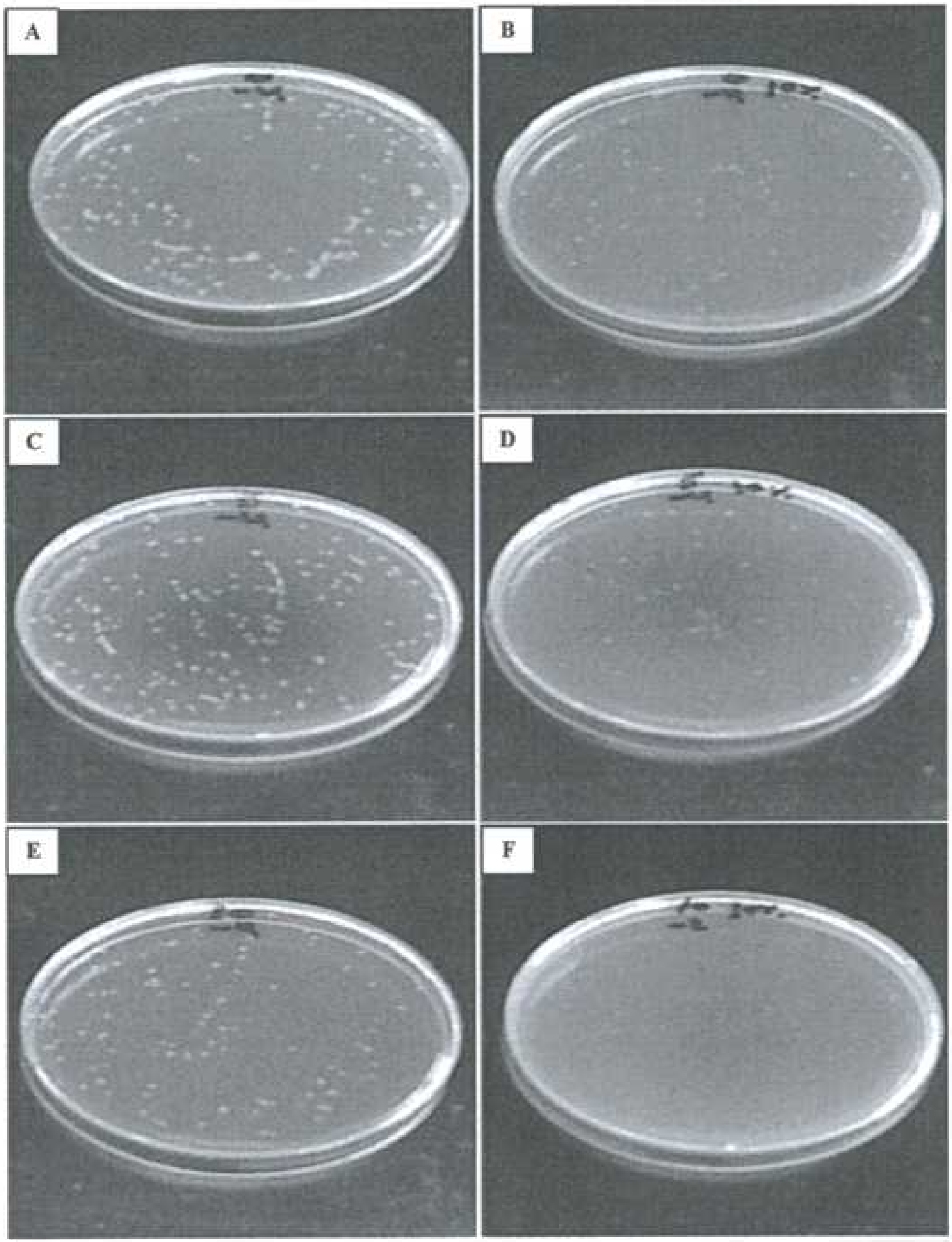


Figure 30: (A) An image of *E. coli* colonies treated without CNTs-10%Ag for zero time in microwaves, i.e. no microwaves radiation (the control sample). (B) An image of *E. coli* colonies treated with CNTs-10%Ag for zero time in microwaves. (C) An image of *E. coli* colonies treated without CNTs-10%Ag for 5 seconds in microwaves. (D) An image of *E. coli* colonies treated with CNTs-10%Ag for 5 seconds in microwaves. (E) An image of *E. coli* colonies treated without CNTs-10%Ag for 10 seconds in microwaves. (F) An image of *E. coli* colonies treated with CNTs-10%Ag for 10 seconds in microwaves.

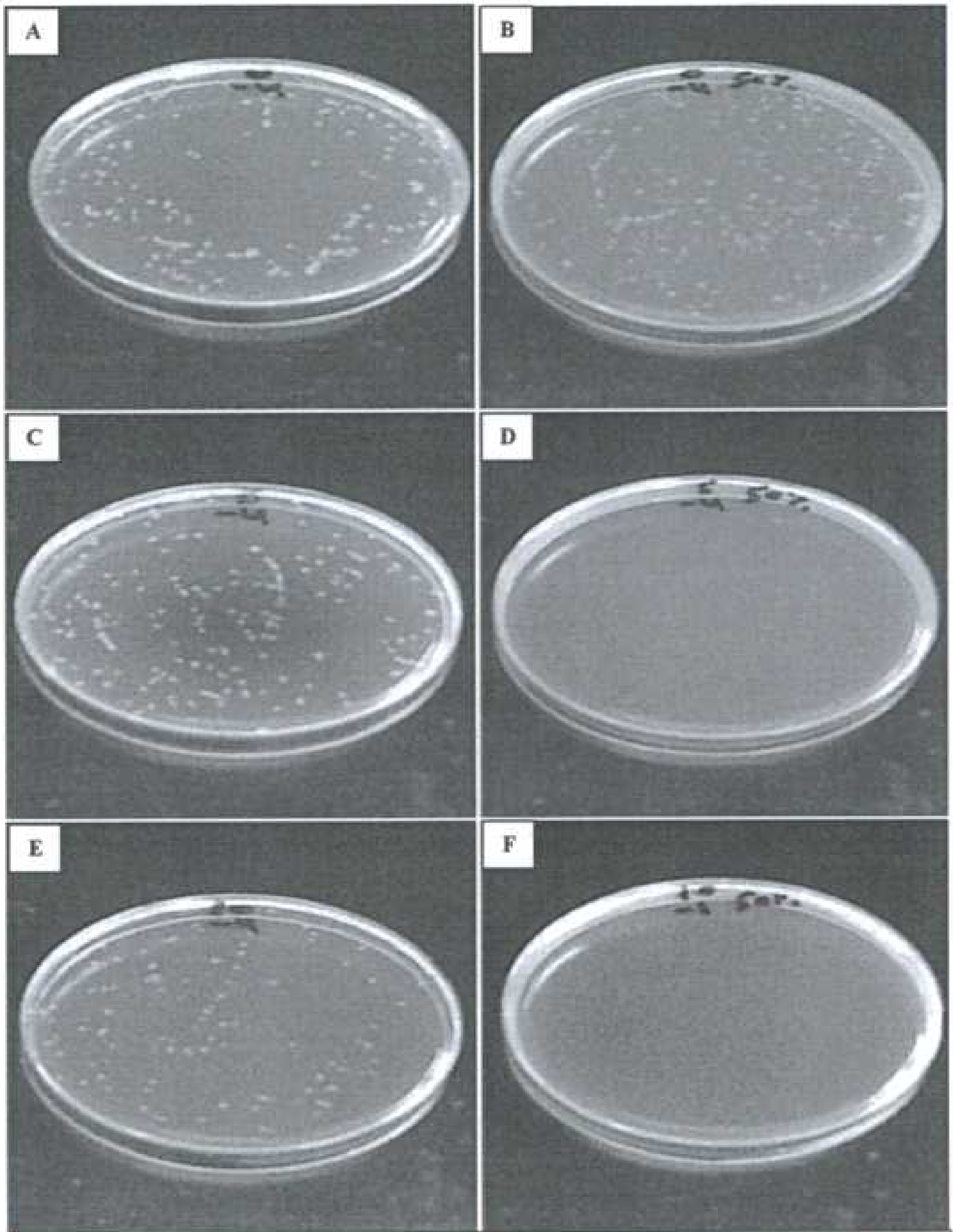


Figure 31: (A) An image of *E. coli* colonies treated without CNTs-50%Ag for zero time in microwaves, i.e. no microwaves radiation (the control sample). (B) An image of *E. coli* colonies treated with CNTs-50%Ag for zero time in microwaves. (C) An image of *E. coli* colonies treated without CNTs-50%Ag for 5 seconds in microwaves. (D) An image of *E. coli* colonies treated with CNTs-50%Ag for 5 seconds in microwaves. (E) An image of *E. coli* colonies treated without CNTs-50%Ag for 10 seconds in microwaves. (F) An image of *E. coli* colonies treated with CNTs-50%Ag for 10 seconds in microwaves.

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