DEVELOPMENT OF TITANIA NANOTUBE COATED SURFACES FOR REDUCTION OF AIRBORNE BACTERIA

By

Waqas Latif

(2011-NUST-MSPhD-EnvS-02)

A thesis submitted in partial fulfillment of requirements for the degree of
Master of Science

in

Environmental Science

Institute of Environmental Sciences and Engineering (IESE)
School of Civil and Environmental Engineering (SCEE)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan
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It is certified that the contents and forms of the thesis entitled

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Submitted by

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This thesis is dedicated to my Aunt & Sister

For their endless affection, support and encouragement

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List of Abbreviation

$^{*}OH$	Hydroxyl Radical
BET	Brunauer Edward Teller
CFU/m^3	Colony Forming Unit per Cubic-meter
CFU/ml	Colony Forming Unit per milli-liter
DNA	Deoxy-Ribonucleic Acid
EDS	Energy Dispersive Spectroscopy
eV	Electron Volt
Fe- TNTs	Iron Doped Titania Nanotubes
FL	Fluorescent Light
GPR	General Purpose Reagent
H^+	Hydrogen Ion
HEPA	High Efficiency Particulate Air
HVAC	Heating, Ventilation and Air-Conditioning
IAQ	Indoor Air Quality
ICU	Intensive Care Unit
MRSA	Methicillin Resistant Staphylococcus Aureus
NLVs	Norwalk Like Viruses
NO-NPs	Nitric Oxide producing Nano-Particles
NPs	Nano-Particles
O^-_2	Oxygen Ion
PCI	Plasma Cluster Ion
PCO	Photo-Catalytic Oxidation
RNA	Ribo-Nucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive Organic Species

SARS	Severe Acute Respiratory Syndrome
SEM	Scanning Electron Microscopy
SPIONs	Super Paramagnetic Iron Oxide Nanoparticles
SSI	Surgical Site Infections
TEM	Transmission Electron Microscopy
TiO_2	Titanium Dioxide
TNPs	Titania Nanoparticles
TNTs	Titania Nanotubes
UVGI	Ultra-Violet Germicidal Irradiation
XRD	X-Ray Diffraction

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ABSTRACT

Nosocomial Infections and food spoilage, caused by different airborne bacteria is a major concern. Titania Nano-structure coated surfaces are reliable in reducing pathogenic bacteria present in indoor air. In the present study comparative photo-catalytic disinfection ability of pure Titania Nanotube and 1% Iron doped Titania Nanotube coated wood surface, to disinfect airborne bacteria, has been examined. Prepared Nanotubes (Both pure and Iron doped) were characterized by Scanning Electron Microscopy (SEM); Energy Dispersive Spectroscopy (EDS); Xray Diffraction (XRD); Brunauer-Edward-Teller (BET) surface area measurement; and band gap energy calculations. Nanotubes were coated on wood surfaces using a novel and green method of coating (natural resin as a binding agent). These wood surfaces were found effective in reducing important airborne bacterial species *Pseudomonas aeruginosa* (Gram Negative) and *Staphylococcus aureus* (Gram Positive) present in indoor air. A 70% reduction in population of *P. aeruginosa* was observed when pure Titania Nanotubes coated wood surfaces were exposed to fluorescent light for two hours. In case of 1% Iron doped Titania Nanotubes coated wood surfaces, P. Aeruginosa species were completely degraded just within 75 minutes of exposure to fluorescent light. Similar trends were observed with *Staph. Aureus* with 80% removal in case of pure TNT coated wood surface after two hours and 100% removal within 90 minutes when 1% Iron doped TNT coatings were placed under fluorescent light.

Chapter 1

INTRODUCTION

1.1. Background

Air (a vital environmental factor for survival of human beings) contains significant number of microorganism (especially bacteria) in spite of the fact that it is not a natural environment for them. Consequently, air borne transmission of pathogenic bacteria is one of the important modes for the transmission of infectious disease. Thus, along with chemical pollutants, biological pollutants like bacteria, viruses, spores and fungi also play major role in deteriorating the indoor and outdoor air quality (Fasim, 2003). Bacteria and fungi can contaminate air and water causing diseases of human, plants and animals (Gregory, 1973).

All over the world, 2.7 billion people do not have proper sanitation conditions. 1.7 million people died in 2004 in Pakistan due to lack of these sanitation facilities. Due to poor economic conditions 45 percent of households lack hygienic conditions for proper living. This alarming situation has increased the risk of water and air borne diseases in the country (Rao *et al.*, 2009). According to a survey, major infectious diseases prevalent in Pakistan are bacterial diarrhea, hepatitis A&E and typhoid fever. Among these major airborne diseases caused by bacteria are Lassa fever and Meningococcal meningitis (World Fact Book).

Major bacterial diseases of respiratory systems include strep throat, scarlet fever, diphtheria, pertussis, otitis media, tuberculosis, pneumococcal pneumonia, psittacosis, legionnaires and Q fever (Cliff Notes).

1.2. How Bacterial Contamination Occurs?

People can experience different bacterial diseases living in densely populated and closed places where sun light reaches sparsely. It has been shown that the number of bacterial species is more in closed environments (like auditoriums, class rooms, offices and apartments) due to the presence of more people and poor sanitary conditions compared to empty and sanitized places (Sessa *et al.,* 2002). Patients in hospitals and school going children are more susceptible to indoor pollutants and bacterial attacks, due to compromised immune system. (Karkowska, 2003; Daisey *et al.,* 2009)

In hospital environments microbial contamination (Nosocomial Infections) becomes more important due to presence of immune-depressive patients. Along with nosocomial infections fungal spores, propagules and metabolites are significant for health impacts, not only for patients with compromised immune system but also for the normal visitors or attenders of the patients (Ortiz *et al.,* 2009)

1.3. Methods to Disinfect Bacterial Species

Number of infectious diseases increases with the increase in number of inhalable pathogens in indoor air. Along with biological controls, several kinds of engineering techniques have been employed so that the microbial pathogens can be kept away inside the residential buildings and medical facilities or they are kept at a level that is unable to induce diseases in humans (Bolashikov and Melikov, 2009). Indoor infection control can be achieved by employing various techniques including, air dilution, air filtration, Ultraviolet Germicidal Irradiation (UVGI), disinfectants, plasma-cluster ions and antimicrobial essential oils etc.

The major problem with the engineering technique is that it is very difficult to maintain the air cleaning systems like air-conditioning systems. Further, these kinds of systems are not economically viable and are very expensive. Use of alternative techniques, such as Photo-catalytic Oxidation is, therefore, gaining importance to sanitize the indoor atmosphere.

1.4. Proposed Solution

Sanitation of air, containing pathogenic bacteria, can be achieved by using different methods, but limitation to most of the methods is that, these are either expensive or energy intensive. So keeping in mind all these situations sanitation of air by killing pathogenic forms of bacteria using TiO_2 Nanotube coated surfaces is an effective, energy efficient and relatively cheap method.

Bacterial disinfection through Photo Catalytic Oxidation (PCO) is gaining popularity in recent times. When Ultra Violet light strikes a photo-catalytic substance like Titania, it excites the electron in the valence band to jump into the conduction band of TiO_2 leaving behind a hole. That hole can react with water molecules present in air, to form the Hydroxyl radical (OH^-), while the electron in the conduction band reacts with the surrounding oxygen to form the super oxide radical (O_2^-). These two radicals can react with bacteria, present in the surrounding air to split these into Potassium ion, RNA, Protein and other components leading ultimately to death (Wamer *et al.,* 1997).

Photo-catalytic ability of Titania can be used to disinfect bacteria especially in indoor air by fixing TiO_2 on different substrates. For example Goswami and coworkers, in 1997, prepared Titania coated fiber-glass air conditioning filters to reduce air borne bacteria. Titania coated HEPA filters was demonstrated to be

effective in eliminating 60% - 80% spore forming air borne bacteria and fungi retained on HEPA filters (Chuaybamroong *et al.,* 2010).

1.5. The Present Study

Sanitation of air by killing pathogenic forms of bacteria using TiO_2 Nanotube coated surfaces is an effective, energy efficient and relatively cheap method. Titania Nanotubes also have high surface area to volume ratio; this factor makes Titania Nanotubes more efficient (than simple Titania Nano particles and modified Titania Nano Particles) in photo catalytic disruption of airborne pathogenic bacteria.

This study was structured around three major objectives i.e.

1. Preparation and characterization of pure and doped Titania Nanotubes
2. Immobilization of TNTs on wood surfaces
3. Bacterial disinfection efficiency test of TNT coated wood surfaces

The effectiveness of Titania Nanotube coated wood surfaces, to disinfect airborne bacteria, was thus duly demonstrated. Prepared Nanotubes (Both pure and Iron doped) were characterized by SEM, EDS, XRD, BET and band gap energy calculations. Nanotubes were coated on wood surfaces using a novel and green method of coating (Natural resin as a binding agent). Disinfection efficiency of the prepared coated wood surfaces was tested by two representative bacterial species of indoor microbial flora i.e. *P. aeruginosa* and *Staph. aureus*. Bactericidal properties of prepared wood coatings were also tested by exposing these to general microbial flora of indoor air.

Chapter 2

LITERATURE REVIEW

2.1. Air Quality Issues

Public awareness is ever increasing regarding the risk associated with poor indoor air quality both at workplace and domestic level. Americans of all ages, on average, spend 22 hours a day in indoor environment (either in homes or in offices) which make them susceptible to the risk of chronic health effects arising from low level of contaminants present in indoor air. Most common indoor air pollutants to which inhabitants are exposed include Particulate Matter, Carbon Monoxide, Carbon Dioxide, Ozone, Sulfur Dioxide, Volatile Organic Compounds (VOCs), Passive Smoke, and Microbial Contamination (Bernstein *et al.*, 2008).

Indoor air pollution can cause various chronic health impacts which may be adverse in nature. These impacts include eye, skin, respiratory tract irritation, and different types of cancers (Delfino *et al.*, 2003). When we look at indoor atmosphere from a mycological point of view, the indoor air is a much poorer environment than soil and water. On the other hand, air is our surrounding environment in which we breathe continuously, making it very important that this is as clean as possible (Cabral, 2010).

2.1.1. Microbial Air Quality Problems

Air is an unfavorable environment for microorganisms. It is only a place for microorganisms in which they reside temporarily and use it for movement from one place to another. Various sources can contribute to the presence of microorganisms in

air like wind movement, sneezing, coughing, laughing, talking, and aeration of sewage.

Microbes produce a biological aerosol in atmosphere which spreads like the particulate matter. The bio-aerosols can penetrate into respiratory system of living organisms depending on their size (smaller the size, greater the possibility to enter) (Kolwzan *et al.,* 2006). Airborne transmissions become more important in case of medical environment. So, the control of these transmissions (which are potential cause of infections) also becomes more important in such indoor environments. For example in dental offices, several infectious microbial agents may affect medical staff working in the area and patients visiting the clinic (Cellini *et al.,* 2001).

Indoor Air Quality is an important factor in preventing infections in occupants (medical staff and patients) of hospital facilities. Poor hospital Indoor Air Quality (IAQ) may lead to hospital acquired infections and a variety of occupational and biological hazards (Wan et al., 2011). Microbial infection in an operation room may significantly enhance the risk of Surgical Site Infection (SSI). Threshold limit for airborne bacterial concentration in operation rooms is considered to be 180 (CFU/m^3) during normal surgery and 10 CFU/m^3 during prosthetic replacement and arthroplasty procedures (Gosden *et al.,* 1998).

Inhalation process can expose the upper and lower respiratory tracts of humans to a variety of particulates and vapors. Airborne transmission of microorganism especially pathogenic bacteria and viruses from various sources like surrounding environment, animals and humans, can result in a particular disease. Inhalation process is an important route of exposure to various particles and microorganism as lungs are more prone to infections than the gastro intestinal tract; Ingested microbial population must past through the acidic environment of stomach in order to infect

various body tissues while, inhaled microorganism deposit directly onto the most surface of respiratory tract without any significant hindrance. Inhalation of microbiological aerosols can lead towards adverse health impacts like

- Infection
- Allergic Reaction
- Inflammation
- Respiratory diseases
- Legionnaires' disease
- Tuberculosis
- Nontubercule mycobacterial disease

Viral diseases that may spread due to respiration are:

- Influenza
- Severe Acute Respiratory Syndrome (SARS)
- Norwalk-Like Viruses disease (NLVs)
- Hanta virus disease
- Measles
- Varicella

In general, exposure to some gram negative and gram positive bacteria, endotoxins and actinomycetes, during the process of air dispersal and consequent inhalation, may lead to a variety of diseases. Diseases caused by air dissemination of fungi include:

- Histoplasmosis
- Coccidiomycosis

- Cryptococcosis
- Aspergillosis

Such microbial agents may be used in bioterrorism also (Stetzenbach, 2009).

2.1.2. Important Bacteria in Air Quality

Methicillin Resistant *Staphylococcus aureus* (MRSA) are one of the most important airborne bacteria. Many studies have revealed that routine hand washing, cleaning, and barrier nursing are not sufficient to prevent the outbreak of MRSA, which requires a proper disinfection of the environment (Blythe *et al.*, 1998; Rampling *et al.*, 2001; Singh *et al.*, 2012).

Methicillin Resistant *Staphylococcus aureus* (MRSA) are capable of surviving for many days, even for weeks, on environmental surfaces in healthcare facilities. Environmental surfaces present in the room of patients, infected or colonized with MRSA, are frequently touched by healthcare facility workers. These contaminated hands or gloves may contain pathogenic bacteria like MRSA that can be transmitted to patients with compromised immune system. On the other hand, pathogens may also be directly transferred to susceptible patients from contaminated surfaces. Routine cleaning only is not very effective in removing pathogens from environmental surfaces and disinfection is usually required in hospital environments in order to wipe out pathogens (Boyce, 2007).

The potential for contaminated environmental surfaces to contribute to medical facility acquired infections depends on a number of factors like: (Boyce, 2007)

- Ability of pathogens to remain viable for a longer time period

- Frequency with which the contaminated surfaces are touched by healthcare facility workers and patients
- Level and type of contamination

MRSA can remain viable for up to 14 days on Formica surfaces, and for up to 6 - 9 weeks on cotton blanket material (Duckworth and Jordens, 1990; Beard-Pagler *et al.*, 1988).

Another important airborne bacterium *P. aeruginosa* is abundant in a variety of moist environments. This opportunistic pathogenic bacterium is commonly associated with hospital acquired infections (Nosocomial Infections), especially in patients with compromised immune systems. *P. aeruginosa* is responsible for almost 10% of all hospital acquired infections. Most importantly *P. aeruginosa* is the second most frequently recovered pathogen from Intensive Care Unit (ICU) patients. *P. aeruginosa* can be found in soil and water, fresh water bodies like lakes, streams and rivers, potable waters such as sinks and showers, fruits and vegetables, hot tubs, respiratory therapy equipment and other medical equipment like catheters, dialysis tubing and scissors etc. Another important characteristic of *P. aeruginosa* is that it is normally antibiotic resistant (Lister *et al.*, 2009).

A healthy person can also acquire infection due to *P. aeruginosa* in rare cases due to:

- Trauma that breaks the epithelium giving a chance to bacteria to enter into blood stream
- Presence of large number of bacteria (Special Pathogens Laboratory)

P. aeruginosa is dangerous due to its ability to develop antibiotics resistant speedily even during the treatment process.

Staph. Aureus	Not pathogenic mostly
• Gram positive bacterium • Found in human respiratory tract and skin • Facultative anaerobe • Methicillin resistant	
P. aeruginosa	Opportunistic pathogen
• Gram negative bacterium • Found in soil, water and health care units • Facultative anaerobe • Antibiotic resistant	

Table 2.1: Characteristics of studied bacteria (Boyce, 2007; Special Pathogen Laboratory)

2.2. Need of Disinfection

Surfaces are generally not considered critical in different medical settings as these surfaces come in contact with intact skins only. Such surfaces however, may become contaminated when these are touched by patients infected with certain pathogenic bacteria or medical personnel handling such patients. So it can be concluded that surfaces (which are non-critical in most of the cases) may become critical under specific conditions. As such, there is a dire need to disinfect these surfaces.

It has also been found through various studies that important airborne bacteria like MRSA and *P. aeruginosa* may survive on surfaces, even for months, spreading infections for long periods of time (Rutala and Weber, 2001).

Number of infectious diseases increases with the increase in number of breathable pathogens in indoor air. Indoor infection control can be achieved by employing various techniques including:

- Administrative techniques (e.g. shuffling in working hours in order to reduce the exposure)
- Engineering techniques (like ventilation, heating and air-conditioning which dilute or completely remove the microbial population from indoor environments)
- Personal Protective Equipment

The major problem with the engineering technique is that it is very difficult to maintain the air cleaning systems like air-conditioning systems. Further, these kinds of systems are not economically viable. Therefore, use of alternative techniques, such as in room air cleaner is encouraged to sanitize the indoor atmosphere.

In short, air disinfection is necessary because indoor air contains various kinds of bacteria, fungi and viruses. Out of these microbial species many are pathogenic capable of inducing infection in humans. Microorganisms can be released into the environment in the form of moisture droplets. These droplets can cause various diseases like:

- Measles
- Varicella or chicken pox
- Tuberculosis
- Herpes zoster (Health Quality Ontario, 2005).

2.3. Methods to Disinfect Bacteria

Disinfection and sterilization involving engineering techniques have now gained paramount importance in controlling nosocomial infection, because hospital environments are very prone to disease outbreaks due to presence of patients with compromised and fragile immune system leading towards increased cost, mortality

and morbidity etc. Along with conventional disinfection methods, several kinds of engineering techniques have been employed all over the world so that the microbial pathogens can be kept away from residential buildings and medical facilities or they are kept at a level that is unable to induce diseases in humans. Some of these methods are (Bolashikov and Melikov, 2009):

- Ultra Violet Germicidal Irradiation
- Filtration
- Dilution
- Ventilation

2.3.1. Disinfectants

Complete disinfection in hospital environment can be achieved by using chemical disinfectants in two ways:

1. Surface Disinfection Method (Like disinfection of surfaces of tables, chairs, trolleys, equipment and floors etc. by applying disinfectants)
2. Solution Disinfection Method (By putting contaminated accessories in disinfection solution)

Hazardous hospital waste like hospital solid waste and pathological or microbiological waste can be treated with chemicals to disinfect it completely. Many factors can affect the disinfection process like:

- Disinfectant pH
- Concentration of solvent
- Temperature
- Concentration of microbial population

- Contact time of disinfectant with contaminants

Keeping in mind these factors, a specific disinfectant should be used for a specific type of setting according to the nature of microbial population present at that place (Chen *et al.*, 2006).

2.3.2. Antimicrobial Agents

Antibacterial activity is defined as the killing bacteria or slowing down their growth locally, without effecting our surrounding environment or tissue. At present most of the antimicrobial agents are chemically modified natural compounds that may include (Von Nussbaum *et al.*, 2006):

- Penicillin
- Cephalosporins
- Carbapenems

In broader terms antimicrobial agents can be classified into:

- Bactericidal that kill bacteria
- Bacteriostatic that stop or slow down the growth

One of the major flaws of using, or abusing, these antimicrobial agents, however, is that different bacterial strains have now become resistant to these agents. Besides the evolutionary process, gene transfer by conjugation, transformation or transduction are also playing a role in developing bacterial resistance against antimicrobial agents (Witte, 2004).

In short, microbial organisms have developed resistance against many antimicrobial agents and infectious diseases are spreading day by day in response of

it. Besides drug resistance, side effects of these antimicrobial compounds should also be taken into consideration as increased resistance forces us to use larger quantities of drugs thus increasing the threat of toxicity of environment. Such limitations has motivated scientists to use alternative methods to deal with bacterial problems (Baker-Austin *et al.*, 2006)

2.3.3. Dilution

Dilution of room air with fresh air, free of bacteria, is one of the most popular and easiest methods to remove pathogenic bacteria or suppress the risk of infectious disease outbreak in indoor environment. Three types of ventilation systems can be used for indoor air dilution:

1. Mechanical Ventilation
2. Natural Ventilation
3. Hybrid Ventilation

Air dilution methods have some limitations like (Hajipour *et al.*, 2012):

- Air distribution pattern
- Thermal comfort of people
- Perfect dilution/mixing requires increase air change rate

2.3.4. Filtration

Air filtration is one of the best methods to reduce or remove pathogenic bacteria from indoor air using HVAC systems. Studies have proved that use of mechanical filtration systems are effective in preventing outside pathogens from entering in the buildings. Kowlaski and Bahnfleth have also showed that 80 - 90% filters can achieve air quality level that presume to be achieved only with HEPA filters at much lower

costs. Enzyme filters are also very effective in reducing indoor bacterial population by destroying their cell membrane when these come close to the filters. Major drawback of filters is that they are not cost effective.

2.3.5. Ultra-Violet Germicidal Irradiation (UVGI)

Low vapor mercury lamps are used to emit Ultra-Violet Germicidal Irradiation with a wavelength of about 250 nm. UVGI works by destroying the RNA or DNA of microbial organisms so that they cannot reproduce themselves even after entering the host cell. Studies have showed that germicidal effect of UV is more prominent on microbes lacking cell wall like adenovirus, Influenza and small pox viruses that are easily killed by UVGI (Jensen, 1964). Spores such as *Bacillus anthracis* contain a protective coating around them making them resistant to UVGI.

There are certain limitations linked with UVGI. For example UV radiations can cause Erythema (reddening of skin) or Photo-kertitis (Painful effect on eyes just like snow-blindness). Due to this reason UVGI lights are installed in deep grooves in ceiling or in walls to avoid humans exposure to the harmful radiation. Such modifications, however, make the technique less effective (Knudson, 1986).

2.3.6. Photo-Catalytic Oxidation

Photo-catalysis is defined as photoreaction accelerated by the presence of a catalyst like TiO_2, WO_3, ZnS etc. The process of photo-catalysis depends upon the ability of light to generate electron hole pairs at the surface of catalysts when light, generally UV, strikes these catalysts. These pairs create free oxidizing radicals having the ability to degrade organic matter that comes in contact with the photo catalyst surface.

Photo-Catalytic Oxidation (PCO) is also useful in reducing airborne bacteria. With PCO only a small portion of bacteria can be degraded at a time (Vohra *et al.*, 2005).

2.3.7. Plasmacluster Ions

Plasma Cluster Ion (PCI) technology is relatively new and this technology can degrade 26 different kinds of airborne substances that may pose threat to human health. Ion generator of PCI uses a plasma discharge between cathode and anode to split the water molecules present in air into Hydrogen ion (H^+) and Oxygen ion (O_2^-). These two reactive species combine with each other through chemical reaction to produce highly reactive Hydroxyl radicals ($^*OH$). These Hydroxyl radicals react with protein or polysaccharide of cell wall of infectious bacteria and viruses, thus killing the pathogens (SHARP, 2008). PCI seems to be an effective method to reduce airborne population of pathogenic bacteria but this technique needs to be investigated further in terms of its impacts on surrounding air quality and human health.

2.3.8. Antimicrobial Oils

Several studies have proved that essential oils being used in cosmetics, beverages and pharmaceutical industries have a strong germicidal effect making them one of the standout candidates for potential application in indoor air disinfection. It has been observed that germicidal effect of essential oils is much higher in air than in solution (Hammer et al., 1999; Inouye et al., 2003). There is a debate going on the usage of essential oils as air purifier as some of the occupants may be hypersensitive to these oils. Furthermore, these oils also show cytotoxicity means they are dangerous to human cells along with microbial cells (Inouye *et al.*, 2003).

2.4. What is Photo-catalysis?

Photo catalysis can be defined as the change in the rate of a chemical reaction in the presence of a substance (Photo-catalyst) that absorbs UV, visible or infrared light (Braslavsky, 2007).

2.4.1. Factors Affecting the Photo-catalysis Reaction

Photo-catalytic reactions generally depend upon several factors which affect the kinetics and performance. Some of these factors are (Rincón and Pulgarin, 2003 and Herrmann, 2005):

- Catalyst loading
- Physical and chemical properties of the photo-catalyst
- Oxygen pressure
- Type of light (UV or visible)
- Concentration and type of organic material
- pH
- Temperature

2.4.2. Ideal Properties of a Photo-catalyst

An ideal photo-catalyst should be (Bhatkhande *et al.*, 2002)

- Photoactive under visible light
- Bearing low band gap energy
- Inert (biologically and chemically)
- Stable under light
- Nontoxic to humans and environment

2.4.3. Examples of Photo-catalysts

Photo-catalysts are usually solid and not consumed or used during a reaction. Photo-catalysts are semiconductor in nature for example CeO_2, Fe_2O_3, TiO_2, WO_3, ZrO_2, and ZnO etc. (Benabbou *et al.*, 2007; Herrmann, 2005).

2.5. TiO_2 Properties as Photo-catalyst

TiO_2 is renowned in the world of photo-catalysis due to properties like (Benabbou *et al.*, 2007; Kumar and Raza, 2009):

- High photo excitation
- Nontoxicity in nature
- Very high stability
- Cost effectiveness

2.5.1. Polymorphs of TiO_2

Three polymorphs of TiO_2 are: 1) Anatase 2) Rutile and 3) Brookite (Carp *et al.*, 2004). Almost all studies have used Anatse and Rutile phase of TiO_2 in photo-catalytic degradation processes. Among these two, Anatase is most preferred polymorph having highest photo-catalytic activity (Beydoun *et al.*, 1999; Carp *et al.*, 2004). Some studies have claimed that mixture of Anatse and Rutile phase give better results in term of photo-catalytic degradation of organic material (Giolli *et al.*, 2007; Sun *et al.*, 2008).

2.5.2. Technical Drawback of TiO_2 and its Solution

TiO_2 is the best among many photo-catalysts but its band gap energy requirements restrict its use on large scale. TiO_2 requires 3.2 eV energy (equivalent to wavelength of 388 nm) in order to act as a photo-catalyst and this energy can only be provided by UV light source making the process of photo-catalysis very expensive (Linsebigler *et al.,* 1995). This difficulty has now been overcome by inducing low energy levels within the band gap of TiO_2 with the help of doping (Gaya and Abdullah, 2008).

2.5.3. TiO_2 Doping

Various elements can be used as dopant for TiO_2, including metal dopants like Iron, Silver, Manganese and Copper or non-metallic dopants such as Carbon, Boron, and Nitrogen etc. (Araña *et al.,* 2004; Wu *et al.,* 2004; Hamal and Klabunde, 2007).

Doping should be done carefully as enhanced dopant concentration can reduce the number of electron hole pairs generated by photo-catalysis because in this case, dopants may act like recombination centers for electron hole pairs (Coleman *et al.,* 2005; Carp *et al.,* 2004)

2.6. Role of Nanotechnology in Bacterial Disinfection

A novel method to reduce airborne microbial population is the emergence of Nano scale materials. Notably a number of classes of Nano-particles are being used in anti-microbial activities and for the treatment of several infectious diseases when these Nano structures are used as drug carriers. Nano materials are gaining importance as disinfectants due to their very high surface area to volume ratio which has resulted in new chemical, mechanical, electrical, optical, magnetic and elctro-

optical properties of these Nano structures that are different from their bulk properties (Whitesides, 2005). *E. coli* in drinking water was successfully disinfected by adding pure and doped TiO_2 Nanoparticles in water under UV and visible light (Younas, 2011).

2.7. The toxicity mechanisms of NPs against bacteria

The proper and complete mechanism of Nanoparticle toxicity against microbial species is yet to be established. In several cases, Nanoparticles have the great ability to adhere themselves to the walls of bacteria by electrostatic interactions and degrade the structure and integrity of membrane (Zeyons *et al.,* 2009). In other cases, when light strikes the Nano species, they produce Reactive Oxidation Species (ROS). These ROS react with bacteria and disrupt their cell wall (Soenen *et al.,* 2011).

Efficiency of Nanoparticle depends on several factors like:

- Composition of Nanoparticles
- Surface modification due to doping
- Intrinsic properties of Nanoparticles
- Type of Nanoparticles

TiO_2 Nanoparticles are toxic towards bacterial species like *E. hire* (gram positive), *Staph. aureus* (gram positive), *P. aeruginosa* (gram negative), *E. coli* (gram negative), and *B. fragilis* (gram negative) under UV light killing all these species within 60 minutes. These Titania NPs don't show any antimicrobial activity under dark conditions. Photo-catalysis of Titania Nanoparticles under UV light disrupts the polyunsaturated phospholipid constituent of lipid membrane leading towards the degradation of cell (Wan *et al.,* 2011).

The antimicrobial effect of Copper Nanoparticles depends on several factors such as (Pramanik *et al.,* 2012):

- pH (Low pH increase the toxicity of NPs)
- Temperature (High temperature help increasing the efficiency of NPs)
- Aeration (High aeration rate is suitable for NPs functioning)
- Nanoparticles Concentration (greater the concentration of NPs, greater will be the efficiency)
- Concentration of Bacteria (Lower the concentration of bacteria, greater will be the efficiency of NPs)

Different Nanoparticles have different efficiencies towards bacterial species like *Staph. aureus, B. subtilis* and *E. coli*. Among NiO, CuO, ZnO, and Sb_2O_3, Nanoparticles of Copper Oxide are more efficient in removing these bacterial species followed by ZnO, NiO and Sb_2O_3 Nanoparticles (Beak and An., 2011). Additionally, Nanoparticles containing Oxide (like ZnO and NiO) do not always disrupt bacteria by entering the cell. In fact, these types of Nanoparticles create microenvironments near their surface and produce Reactive Oxidizing Species (ROS), which disrupt the bacteria (Heinlaan *et al.,* 2008).

Silver Nanoparticles combined with antibiotics is a good combination in killing several species of bacteria. For example, ampicillin (an antibiotic) damages the cell wall thus making it easy for Silver Nanoparticles to enter into the bacterial cell. After entering the cell, these Nanoparticles attack the DNA of cell and inhibiting its unwinding, consequently leading to cell death. Additionally, Silver doped Titania Nanoparticles are efficient in killing *E. coli* and *Staph. aureus*. Silver Nanoparticles bind themselves to the cell membrane of bacteria and disrupt it, then Silver ions bind

themselves with the Oxygen, Sulphur and Nitrogen (components of all major molecules present within cell) thus inhibiting cell growth, ultimately leading towards cell death (Juan *et al.,* 2010).

Selecting suitable Nanoparticles for certain kind of bacterial species in order to kill them is a sound option as this phenomenon will minimize the side effects of Nanoparticles and will enhance the bactericidal effect of NPs (Sondi and Salopek-Sondi, 2004).

Nitric Oxide producing Nanoparticles (NO-NPs) are also toxic towards many bacterial species that are resistive in nature and that are clinically very sensitive. Some of these species include *E. faecalis*, *Str. pyogenes*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*. The working mechanism of these types of Nanoparticles depends on delivery of NO to target bacterial specie. NO released from these NPs has the ability to chemically modify the structure of bacterial membrane. These NPs also produce Reactive Nitrogen Species (RNS) which have the same ability (Friedman *et al.,* 2011).

Zinc Oxide Nanoparticles are also able to kill bacteria like Methicillin Resistant *Staph. aureus* (MRSA). ZnO Nanoparticles have ability to damage and rupture cell membrane that ultimately leads towards the cell death.

2.8. Defense Mechanisms of Tolerant Bacteria against NPs

Different bacterial species have different defense mechanisms against Nanoparticles. For example Copper doped Titania Nanoparticles can easily kill *Mycobacterium smegmatis* but they are not capable enough to inhibit the growth of *Shewanella oneidensis* (Wu *et al.,* 2010). Cu doped Titania Nanoparticles release Cu^{2+}

ions, which act as toxicant in most of the cases. *Shewanella oneidensis*, however, show excellent resistant against Cu doped Titania Nanoparticles in general and Cu^{2+} ions, in particular, by producing extracellular polymeric substances in their presence. The extracellular polymeric substance produced by this bacterium absorbs Nanoparticles present on cell surface and lead towards the decreased amounts of Cu^{2+}. Due to this phenomenon, this bacterial species can be used as a potential agent in reducing metal oxide NPs from environment.

Several bacterial species have also the ability to neutralize the effect of NO produced by Nanoparticles. For example, bacterial species like *P. aeruginosa*, *E. coli* and *Sal. typhimurium* can induce a gene of expression which repairs the DNA being damaged by NO and by changing the metal homeostasis in presence of NO-NPs (Filipa *et al.,* 2009; Bang *et al.,* 2006). Under similar conditions, *K. pneumoniae* produces an enzyme named falvohemoglobin, which neutralizes the stress being exerted by NO-NPs (Frey *et al.,* 2002).

2.8.1. Role of the cell wall

Bacterial cell wall is important in providing protection to the cell against external factors like osmotic pressure rupture and Nano-material damage. Cell wall also provides shape and strength to bacterial cell. According to structure and components bacterial cell wall is mainly categorized into 2 types

- Gram Positive
- Gram Negative

Cell wall of Gram positive bacteria is usually comprised of peptidoglycan. By contrast, gram negative cell walls are more complex in chemical nature and structure.

Along with thin Peptidoglycan, cell wall of gram negative bacteria also contains an outer membrane. The outer membrane provides resistant towards hydrophobic compounds like detergents. All these factors also contribute towards the resistance of bacteria against Nano-materials (Singleton, 2004; Ashkarran *et al.*, 2012).

2.8.2. Role of the NP and Bacterial Type

Cell wall alone doesn't play an important role in bacterial resistant or sensitivity both in gram positive and gram negative bacteria (Ashkarran *et al.*, 2012). There are many other factors that can affect the sensitivity of bacterial species against Nanoparticles like type of Nanoparticles being used. On the other hand, *E. coli* (gram negative) is very fragile, while *Staph. aureus* (gram positive) and *B. subtilis* (gram positive) are resistant towards Copper Oxide nanoparticles. On the contrary, *Staph. aureus* and *B. subtilis* are more vulnerable to attack against ZnO, NiO Nanoparticles (Baek and An., 2011).

2.8.3. Role of Growth Rate

Another important factor influencing the resistivity of bacterial species against Nanoparticles of several kinds is bacterial growth rate. For example, fast growing bacteria are more prone towards attack by Nanoparticles and other disinfectants than slow growing bacteria (Mah and O'Toole, 2001). Resistant property of slow growing bacteria can be attributed towards expression of stress gene response (Stewart, 2002). In short, antimicrobial effect of different Nanoparticles depends on the type of bacterial strain.

2.8.4. Role of biofilm formation

One of the major problems with Nanoparticles and antimicrobial agents is their inability to degrade bacteria having capability to produce biofilms (Park *et al.,* 2011). Biofilms are defined as complex microbial communities that are produced by adhesion of these communities with solid surface and by secretion of complex matrix like DNA, proteins and polysaccharides that bind and cover bacterial cell community. Biofilms are of paramount importance as these protect bacterial communities against antibiotics and Nanoparticles that is one of the major causes of the spread of infectious diseases (Landini *et al.,* 2010). Furthermore, it has been studied that ZnO Nanoparticle coating on glass surface can help to degrade biofilms of *E. coli* and *Staph. aurues* (Applerot *et al.,* 2012). It also has been established that Super Paramagnetic Iron Oxide Nanoparticles (SPIONs) have greatest photo-catalytic ability to degrade and reduce bacterial biofilms. SPIONs with doping of Silver and Gold are more preferable as they can penetrate deep into biofilms due to their external magnetic fields (Park *et al.,* 2011).

2.9. Titania Nanotubes

Titania Nanotubes (TNTs) are now gaining importance in the process of photo-catalysis due to their excellent physical and chemical properties like:

- High surface area
- Better photo-catalytic activity

TNTs can be easily produced by very simple yet effective hydrothermal method under moderate conditions of pressure and temperature (Wong *et al.,* 2011).

A number of studies have revealed that Titania Nanotubes have better physical and chemical properties when compared to simple TiO_2 powder. TNTs possess a relatively higher surface area and interfacial charge transfer rate as compared to Titania Nanoparticles. Due to tubular structure and increase in surface area, as a consequence of increase in length, charge carrier transfer takes place along the length of nanotubes which reduces the electron hole pair recombination (Colmenares *et al.,* 2009).

It has been observed that Titania Nanotubes are hollow in structure and are highly efficient in degrading Methyl orange through the process of photo-catalysis when comparison was made with Titania Nano powders (Li *et al.,* 2009). Xu and coworkers (2006) also stated that Titania Nanotubes are excellent photo-catalysts as compared to commercially prepared Titania Nanoparticles (P25). Thus, due to such excellent properties as mentioned above, Titania Nanotubes have raised the expectations for potential application in areas such as dye-synthesized solar cells, organic light emitting diodes, gas sensors and photo-catalysts (Yu *et al.,* 2007). Keeping in mind the enormous benefits of Nanotubes, efforts are now being employed in order to produce well-structured TiO_2 nanotubes (Idakiev *et al.,* 2005).

Titania Nanotubes can be synthesized by a number of approaches. Main methods used in the production of Titania Nanotubes include (Guo *et al.,* 2008; Morgan *et al.,* 2008; Wang *et al.,* 2007):

- Chemical Vapor Deposition
- Anodic Oxidation
- Hydrothermal Synthesis
- Sol-gel method

Out of these methods, the hydrothermal method of Nanotube preparation is generally preferred due to its cost effectiveness, moderate conditions requirements (such as temperature and pressure), low energy consumption and simple equipment. Moreover, a number of variable methods that can alter the morphology of Titania Nanotubes can be easily controlled through hydrothermal method (Guo *et al.,* 2008).

During photo-catalytic reaction in Nanotubes, access to active sites is generally regularized by three main characteristics:

1. Non availability of micro-pores
2. Large surface area to volume ratio
3. Presence of open meso-spores

Semiconducting behavior of Titania is the main cause behind the efficient performance of TNTs as photo-catalyst. Also TNTs are very resistant towards heat treatment, making them more suitable to photo-catalytic degradation (Wong *et al.,* 2011).

2.9.1. Doping and Calcination of TNTs

Pearson *et al.,* (2012) demonstrated that doping of TiO_2 Nanotubes with different dopants like Au, Pt, Ag and Cu can be carried out in the presence of Phosphotungstic acid through the process of chemical anodization. Photo-catalytic activity of these tubes was observed under visible solar light by degrading the organic dye “Congo Red”. Doping with different metals enhanced the photo-catalytic activity of TNTs by 100% under visible light when compared with pristine TNTs.

Grandcolas *et al.,* (2011) successfully deposited Silver Nanoparticles on the surface of Titania Nanotubes and then observed their disinfection efficiency. They

reported that different analysis proved that Nano size Ag Nanoparticles have uniformly distributed on the surface of TNTs. These modified TNTs show excellent antimicrobial results both under visible light and dark conditions. These experiments showed that:

1. TNTs provide a good support for the deposition of Ag NPs on their surface thus enhancing its antimicrobial ability
2. Doping of TNTs with Ag NPs enhance the photo-catalytic activity of modified tubes under visible light and dark conditions by reducing the band gap energy requirements and minimizing the electron hole pair recombination during photo-excitation

Nair *et al.,* (2012) reported that Vanadium doped Titania is a better catalyst than pure forms of Titania when UV and visible light was used for detoxification of phenol and disinfection of bacterial species *E. coli*. All Vanadium doped Titania samples showed visible light activity as compared to pristine Titania samples in terms of phenol detoxification and bacterial disinfection.

Zhao *et al.,* in 2011 showed that pure Titania Nanotubes can be doped with Ag by immersing pure TNTs in solution of Silver Nitrate. Amount of Silver to be deposited on the surface of TNTs depend on two factors:

- Concentration of $AgNO_3$ solution
- Immersion Time

These modified Nanotubes can be used in long term antimicrobial applications especially in places where post operation infection still poses a great threat. These Nanotubes can kill planktonic bacteria present in suspensions during first several days

and can also prevent bacterial adhesion for almost a month thus preventing post operation infections in the hospital environment. Modified Ag-TNTs structures which have long term disinfection ability have potential application in areas like:

- Orthopedics
- Dentistry
- Biomedical devices

Xu *et al.,* (2011) analyzed their synthesized Nano structures by XRD, Raman spectroscopy, SEM and High Resolution TEM. They reported that after the calcination process, the as prepared nanotubes turned into anatase phase Nanotubes different from the original structure. Their results further showed that crystalline Nanotubes exhibit better photo-catalytic activity than the amorphous ones due to improved crystallinity.

2.10. Coating of Titania on surfaces

Coatings can be prepared by embedding synthetic Nano materials in the matrix of coating material. Such type of coating is usually a thin layered composite present on a substrate. Water and ethanol based glass and plastic venetian blinds Ag-TiO_2 coatings were prepared (Khan *et al.,* 2013) to disinfect important indoor bacterial species *P. aeruginosa* and *B. subtilis*. Coatings were efficient enough to kill these pathogenic bacteria just within 2 hours under visible light. Glass beads were also coated by Titania Nanoparticles through the process of calcination in order to remove arsenic from water (Danish, 2012). Most important issue is that whether these coatings are reliable or not. For this purpose, several studies have been performed to check the stability of such coatings that whether they are harmful or not for environment and humans. These tests may include but not limited to abrasion,

scraping, and sanding. Apart from determination of particle size distribution and concentration, chemical and morphological analysis like SEM, TEM, EDS and ICP are also important in order to determine any unintentionally produced particles (which got free from coatings) in Nano range in airborne emission (Kuhlbusch *et al.,* 2011).

Specific arrangements of Titania Nanotubes, thin films and coatings TNTs will have potential applications in near future in areas such as (Tian *et al.,* 2003)

- Filtration
- Catalysis
- Sensing
- Photovoltaic cells
- High surface area electrodes

2.11. Nano-materials against environment and ecosystems

Nano materials are being applied in several fields like biological science, medical science and commercial products thus leading towards the leakage and accumulation of these Nano-materials in our environment mostly in soil and water. It is very important to save our environment and bacteria (beneficial for environment and humans) from hazardous impacts of indiscriminate use of Nano materials. For example use of Silver doped Nano particles causing accumulation of Silver in environment as a toxicant. The leakage of the Nanoparticles in environment is especially hazardous for many beneficial microbes, bacterial populations and public health (Gajjar *et al.,* 2009). Several species of microbes are beneficial to environment and ecosystem, as they play an important role in processes like bioremediation, element cycling and nitrogen fixation (essential for plant growth) (Kumar *et al.,* 2011; Wees *et al.,* 2008; Molina *et al.,* 2006).

During the process of nitrification, Ammonia nitrogen is first converted to nitrite by Ammonia fixing bacteria and then nitrate by nitrite fixing bacteria. The nitrifying bacteria are present in the regions with high concentrations of ammonia. Silver nanoparticles disrupt the membrane of these bacteria which contain ammonia oxidation enzyme by producing ROS. The killing of such bacterial species leads towards the decreased nitrogen removal from environment which disturbs plant growth (Choi and Hu, 2008). Nano sized materials can also help different viruses to penetrate into bacterial cells. For example, Silver and Zinc Nanoparticles disrupt the cell wall of *E. coli* and mediate the internalization of MS2 phage into bacterium by 2 to 6 folds increased magnitude. This is a serious problem, as NPs make internalization easy for MS2 phages carrying drug resistant genes thus facilitating development of multidrug resistant in *E. Coli* (You *et al.,* 2011). Due to these reasons, scientist especially environmentalists should also consider the harmful effects of Nano sized materials on environment along with their beneficial uses.

Nanoparticles antimicrobial activities depend on two major factors

1. Physiochemical properties
2. Type of bacteria being exposed

Result reported by different studies are contradicting regarding bacterial killing rate and functioning of Nanoparticles because most of the researchers used as present NPs and bacterial species rather than using specific bacteria and NPs (Hajipour *et al.,* 2012).

Chapter 3

MATERIALS AND METHODS

3.1. Reagents and Materials

General Purpose Reagent (TiO_2), Sodium Hydroxide Pallets, Hydrochloric Acid, Ferric Oxide, Nutrient Agar and Nutrient Broth were obtained from Sigma Aldrich. Gum Acacia was obtained from the local market. Bacterial Cultures of *P. aeruginosa* (ATCC 27853) and *Staph. Aureus* (ATCC 29213) were grown in laboratory using standard protocols. Distilled water was used during whole exercise and all chemicals of analytical grade, used without further purification.

For photo-catalytic experiments, a fluorescent lamp (commonly used for room illumination) with a wavelength range of 400-700 nm was used as a light source. UV lamp having peak intensity at 365 nm was also used to study photo-catalytic activity in UV region.

3.2. Synthesis

3.2.1. Synthesis of Precursor Material for Nanotubes

Titania Nanoparticles were used as a precursor for the formation of pure Titania Nanotubes. In order to prepare the precursor, General Purpose Reagent (GPR) was mixed in distilled water and stirred for 24 hours on magnetic plate. Then the resulting slurry was placed in an oven for 12 hours at 105 ^{o}C for drying. After drying and crushing, the dried slurry was placed in a muffle furnace for 6 hours at 450 ^{o}C.

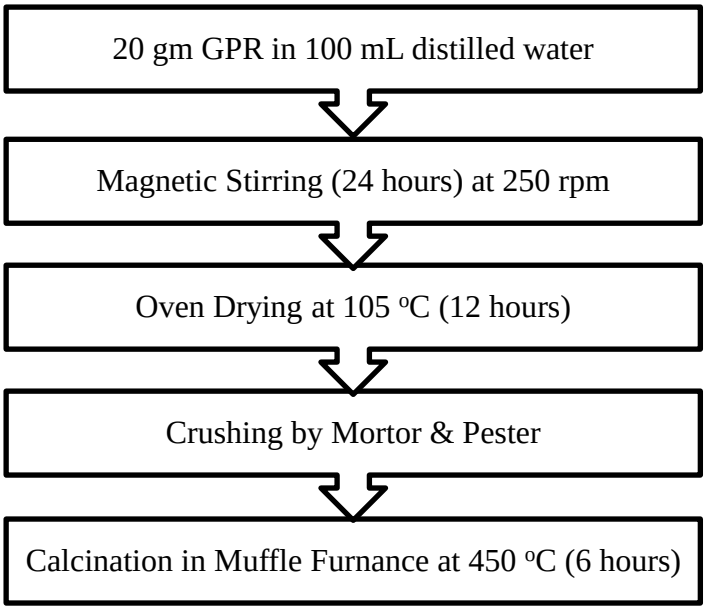


Figure 3.1: Flow sheet for Precursor Material Preparation for TNTs

3.2.2. Synthesis of Pure Titania Nanotubes

Pure Titania Nanotubes were synthesized by hydrothermal method (Asapu *et al.,* 2011) with some modifications. For this purpose, 2.5 gm prepared Titania Nanoparticles were added to 100 ml of 10 Molar NaOH solution with 2 and 1 hour of stirring and sonication respectively. The resulting suspension was placed in a Teflon lined steel autoclave. The autoclave was maintained at a temperature of 135 ^{o}C for 24 hours with continuous stirring at 300 rpm. The treated solution was allowed to cool to room temperature, and washed with 0.1 Molar HCl and distilled water, repeatedly, until the pH was nearly neutral. The sample was dried at 105 ^{o}C for 24 hours. Finally the sample was calcined in a muffle furnace at 500 ^{o}C for 1 hour in order to obtain TNTs in anatse phase.

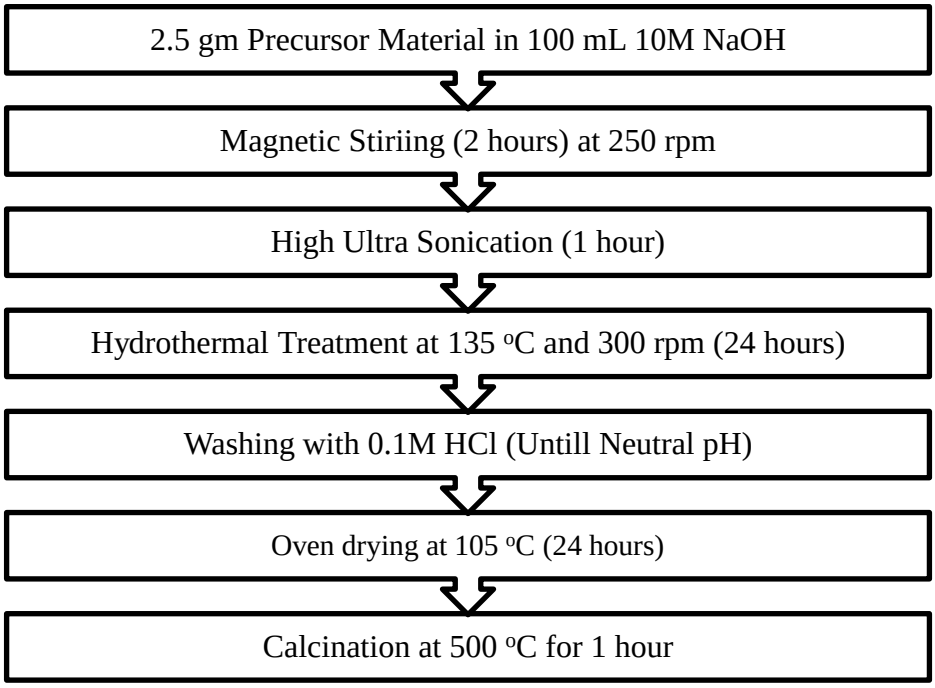


Figure 3.2: Flow sheet for synthesis of Pure TNTs

3.2.3. Synthesis of Iron Doped Titania Nanotubes

Iron Doped Titania Nanotubes were prepared by using the hydrothermal method (Nguyen *et al.,* 2011). 1.5 gm of prepared Titania Nanoparticles and 32 mg of Ferric Oxide was first stirred for 2 hours and then mixed ultrasonically in 100 ml of 10 Molar NaOH solution for 1 hour. The resulting suspension was placed in a Teflon lined autoclave at 135 ^{o}C for 24 hours with continuous stirring at 300 rpm. Follwing this, the solution was allowed to cool at room temperature, and washed with 0.1 Molar HCl and distilled water, repeatedly, until the pH become neutral. The sample was then dried in oven at 105 ^{o}C for 24 hours. Finally, the sample was calcined in a muffle furnace at 500 ^{o}C for 1 hour in order to obtain Nanotubes in anatse phase.

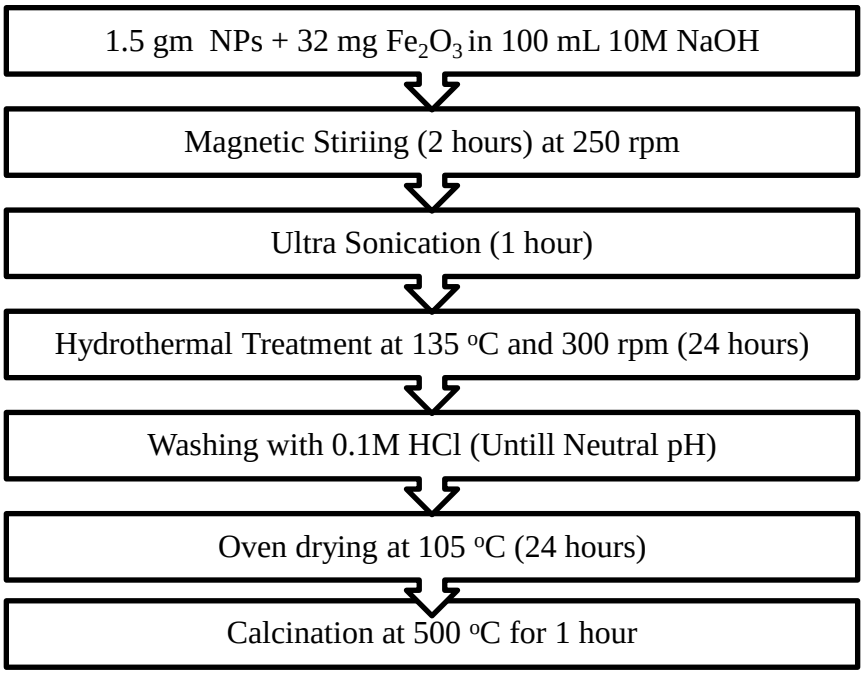


Figure 3.3: Flow sheet for synthesis of 1% Iron doped TNTs

3.3. Characterization of Pure and Doped Nanotubes

3.3.1. Morphology of TNTs (SEM)

Scanning Electron Microscopy (SEM) is a powerful tool, being used now-a-days, in place of optical microscope. An electron beam is produced from an electron gun, and accelerated by high voltage in a vacuum. The electron beam strikes the sample and signals are generated. These signals are detected by electron collector and image of illuminated sample is formed by magnetic lenses.

SEM has a wide range of resolution ranging from 10X to 300,000X and can resolve even few nanometers. In the present study, morphology of the samples was observed by using JEOL JSM-6460 SEM. SEM examined the prepared samples of pure and Iron doped TNTs at an acceleration voltage of 20 kV.

3.3.2. Elemental Analysis of TNTs (EDS)

In order to determine the chemical composition (in percentages) of prepared pure and doped TNTs, Energy Dispersive Spectroscopy (EDS) coupled with SEM

was used. When electron beam strikes the sample, different elements present in the sample produced characteristic X-rays having different energies. The elements are identified by collecting and analyzing these characteristics X-rays. In the present study, Elemental analysis of prepared TNTs was done by using EDS Oxford INCA X-sight 200.

3.3.3. Structure Analysis of TNTs (XRD)

X-Ray Diffraction (XRD) is a standard technique for determining the crystalline phase of any powdered sample. In XRD, a coherent X-ray beam strikes the compact sample. After striking some of the X-rays will diffract at different angles. X-rays diffracting from a specific plane at same angle will reinforce each other giving high peaks indicating the crystallinity of sample.

In the present study, JEOL JDX-II X-ray diffractometer was used to analyze the crystalline phase of the pure and Iron doped Titania Nanotubes.

3.3.4. Surface Areas Measurements (BET)

Brunauer-Edward-Teller (BET) Analyzer is used to measure the surface areas of powders, solids and granules. In BET surface analyzer, Nitrogen (N_2), in liquid form, is adsorbed on the surface of pure solid having no other gas molecules (this is achieved by degassing of sample prior to surface area estimation) in monolayer at very low pressure. Surface area is calculated by measuring the number of molecules present in monolayer.

In the present study, Micromeritics Gemini VII BET surface area Analyzer was used to calculate the specific surface area of pure and Iron doped Titania Nanotubes. For BET analysis TNTs samples were first degassed at 130 ^{o}C for 4 hours under vacuum (Akarsu *et al.*, 2006).

3.3.5. Band Gap Energy Calculation (UV-Vis Spectrophotometer)

UV-Vis spectrophotometer works on the principle of Lambert-Beer Law. UV-Vis spectrophotometer can be used to measure the absorbance or reflectance of visible and adjacent Ultra-Violet light by a solution/suspension. The cut off wavelength determined from obtained spectra (i.e. Absorbance vs. Wavelength) is used to calculate the band gap energy by using Planck's equation (**$E=hc/\lambda$**). Where

E = Energy

h = Planck's Constant i.e. 6.626×10^{-34} Js

c = Speed of Light i.e. 3.0×10^{8} m/s

λ = cut off wavelength obtained from absorbance spectra of pure and doped Nanotubes

In the present study, suspensions of pure and Iron doped TNTs, prepared by adding 0.1 gm of sample in 10 ml ethanol (Reddy *et al.*, 2002) with 1 hour of sonication, were used to find out the band gap.

3.4. Nanotube Coating on Wood Surfaces

3.4.1. Preparation of Coating Agent

Coating/Binding agent was prepared by adding 250 gm crushed and sieved Gum Acacia (a natural resin) in 250 ml of methyl alcohol and heating the mixture at 170 ^{o}C for 10 minutes with constant stirring with the help of a glass rod.

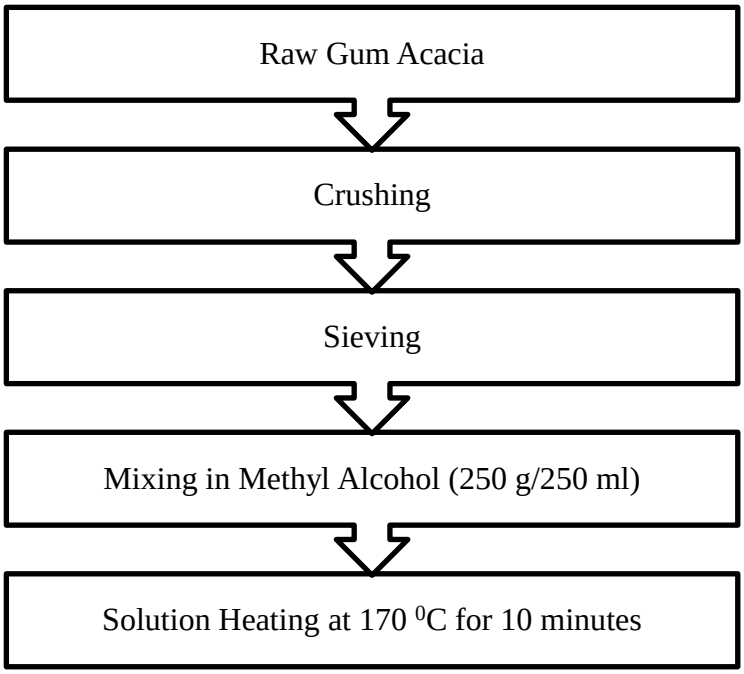


Figure 3.4: Flow sheet for preparation of coating agent

3.4.2. Preparation of Nanotube Coated Surfaces

Nanotubes (pure and iron doped) were dispersed ultrasonically in the binding agent for 15 minutes. Wood pieces of 10 x 10 cm^2 were prepared from a wood plank of *Cedrus* tree commonly known as Deyar (Naturally antibacterial wood used in furniture also) and a single layer of pure binding agent (a common wood furniture polishing material) was coated on these wood pieces. Then the layer of the binding agent, containing pure and doped Titania Nanotubes, was deposited on separate wood pieces and allowed to dry at ambient temperature.

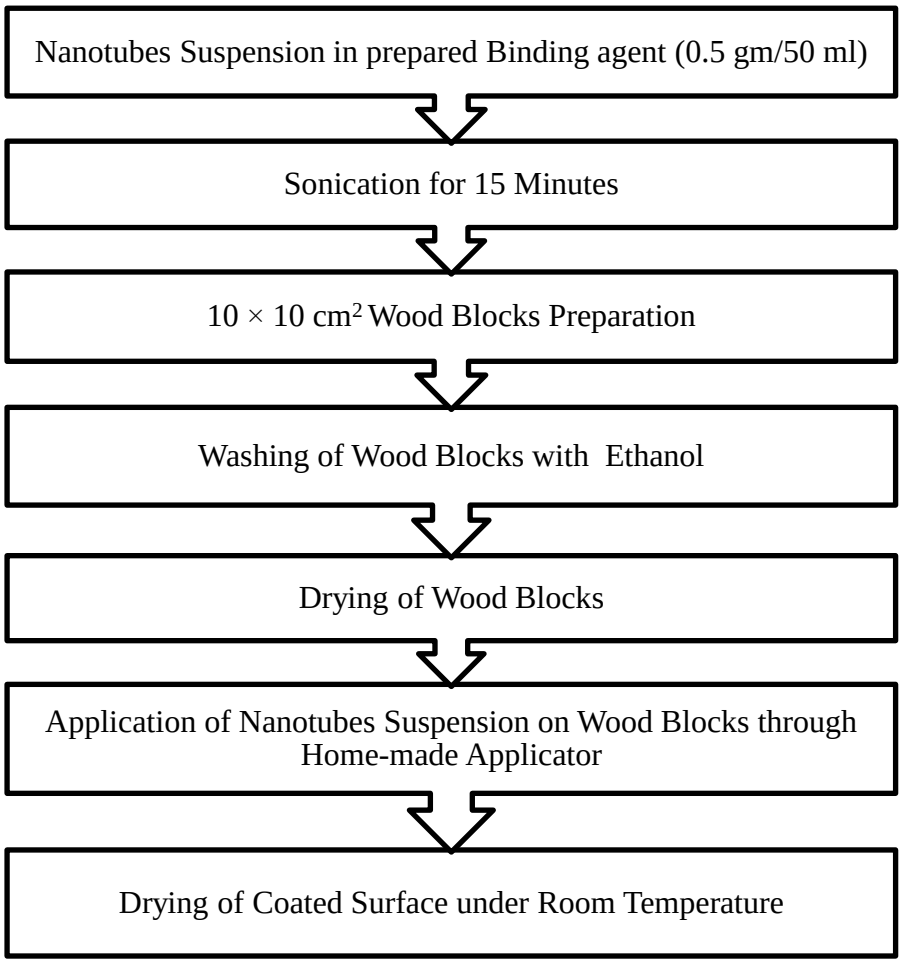


Figure 3.5: Flow sheet for preparation of Nanotubes coated wood surfaces

3.5. Bacterial Culture Preparation

1 ml of pure bacterial culture of *P. aeruginosa* (ATCC: 27853) and *Staph. aureus* (ATCC:23219) were inoculated in 100 ml of Nutrient Broth and Luria Bertani Broth (Sigma Aldrich) respectively and incubated in a shaker incubator at 37 ^{o}C for 24 hours and 16 hours respectively. The density of the bacterial suspensions was estimated using optical density at 600 nm. A range of 0.8-1.0 was chosen as optimal density for subsequent experimental testing. The cell suspension used for bacterial testing was approximately 134 x 10^5 CFU/mL. Standard Plate Count (SPC) technique was used to calculate the number of cells/colonies throughout the experimental

testing. Colony range of 25-250 was taken as a standard during viable count procedure.

3.5.1. Agar Plate Preparation

14 gm of Nutrient Agar (Sigma Aldrich) was mixed in 1 liter warm distilled water with slow mixing by a glass rod. When nutrient agar was completely dissolved, the flask containing the agar solution was transferred to an autoclave for complete sterilization at 121 ^{o}C for 15 minutes. After sterilization, prepared molten agar was poured into petri plates (autoclaved at 121 ^{o}C for 15 minutes) under laminar flow hood cabinet and allowed to cool down. Prepared petri plates, after solidification were transferred into incubator (for 24 hours at 37 ^{o}C) to check their sterility.

3.5.2. Saline Blank preparation

60 test tubes were taken and washed thoroughly first with water containing detergent and then with distilled water. The test tubes were filled with 9 ml solution of 0.85% NaCl (prepared in distilled water). Test tubes were then autoclaved at 121 ^{o}C for 15 minutes for complete sterilization.

3.5.3. Serial Dilution Technique

Serial dilutions of freshly prepared cultures were made by adding 1 ml bacterial culture in 9 ml in 0.85% saline solution in order to obtain a countable range (25-250 CFU/mL) of bacterial colonies. Countable range was achieved after five dilutions. During each dilution, the bacterial suspension was well mixed with the help of a vortex mixer in order to achieve uniform suspension.

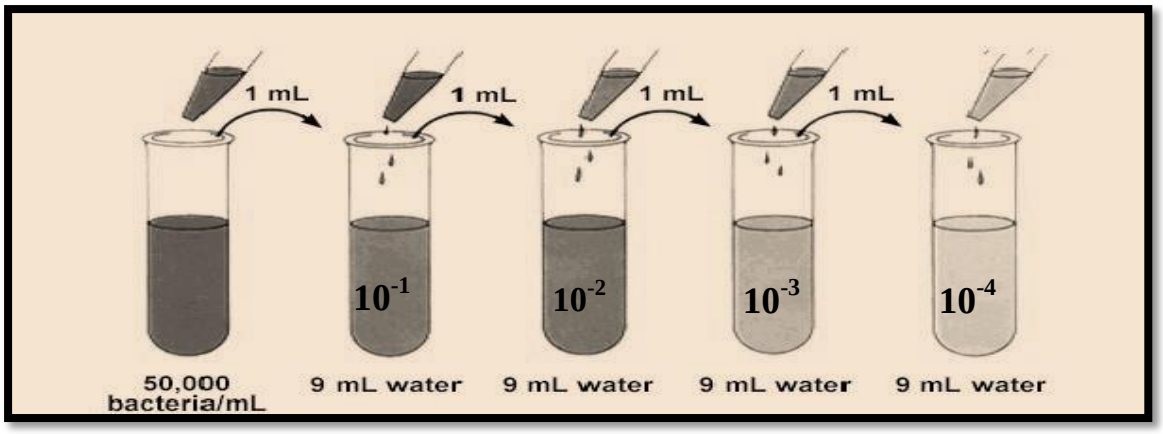


Figure 3.6: Serial Dilution Technique for Bacterial Cultures

3.6. Experimentation

3.6.1. Bactericidal effect of Pure and Iron Doped TNTs on *P. aeruginosa* in Aqueous Phase

0.1 gram Pure Nanotubes and 100 ml of overnight grown fresh culture of *P. aeruginosa* were put together in a quartz cell held on an orbital shaker under laminar flow hood. Another quartz cell only containing 100 ml of pure *P. aeruginosa* liquid culture was used as control. The whole assembly was then exposed to normal fluorescent light. Samples were taken at intervals of 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes, several dilutions were made and spread on prepared agar plates. The agar plates were placed in the incubator at 37 ^{o}C for 24 hours. Colonies were counted after 24 hours with the help of colony counter in order to check the disinfection efficiency of the prepared Nanotubes. Similar procedure was followed for UV light exposure. In case of Iron doped TNTs the procedure was repeated under normal fluorescent light and Ultra-Violet light.

3.6.2. Bactericidal effect of Pure and Fe Doped TNTs on *Staph. aureus* in Aqueous Phase

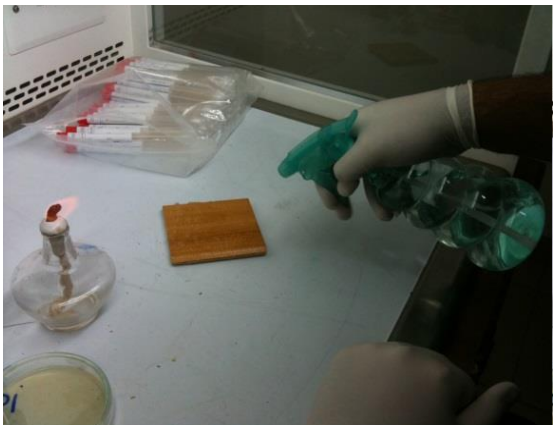
The procedure discussed above was also used for *P. aeruginosa*, first with Pure Titania Nanotubes and then with 1% Iron doped Titania Nanotubes for 2 hours under fluorescent and UV light respectively.

3.6.3. Bacterial Disinfection Efficiency Test of Nanotubes Coated Surfaces on *P. aeruginosa*

The overnight grown bacterial culture of *P. aeruginosa* was transferred into a spray bottle (autoclaved at 121 ^{o}C for 15 minutes) and then sprayed evenly on Nanotubes coated wood surfaces (pure & Iron doped) and simple wood surface only coated with pure binding agent acting as a control surface. The wood surfaces were exposed to fluorescent light for 2 hours under laminar flow hood and swab samples by evenly streaking sterile swab sticks on entire coated surface were taken at intervals of 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes. The bacterial samples collected on swab sticks were then immersed in 0.85% saline solution and serial dilutions were made. Then 1 ml of final dilution was poured on to the surface of agar plates and spread evenly with the help of glass spreader (entire procedure was performed in a laminar flow hood). Following this, the agar plates were placed in an incubator at 37 ^{o}C for 24 hours. Colonies were counted after 24 hours with the help of colony counter (Stuart SC6) in order to check the disinfection efficiency of the prepared surfaces. Same procedure was followed where coated wood surfaces were exposed to UV light.

3.6.4. Bacterial Disinfection Efficiency Test of Nanotube Coated Surfaces on Staph. Aureus

The procedure discussed was also followed for *Staph. Aureus* under normal fluorescent and UV light.



1) Spraying

2) Sampling

3) Dilution

4) Sample Extraction

5) Spreading on Agar Plate

Figure 3. 7: Systematic Approach for Taking Bacterial Sample from Surface

Chapter 4

RESULTS AND DISCUSSION

4.1. Characterization of Nanotubes

4.1.1. SEM Analysis

Scanning Electron Microscopy (SEM) was used in order to observe the morphology and diameters of the Nanotubes. Figures 4.1 and 4.2 show the images of Pure Titania Nanotubes by JEOL JSM-6460 at 10,000 and 30,000 magnification. SEM images confirm that tubular structures have been formed with diameters ranging from 38 nm to 48 nm for pure Titania Nanotubes.

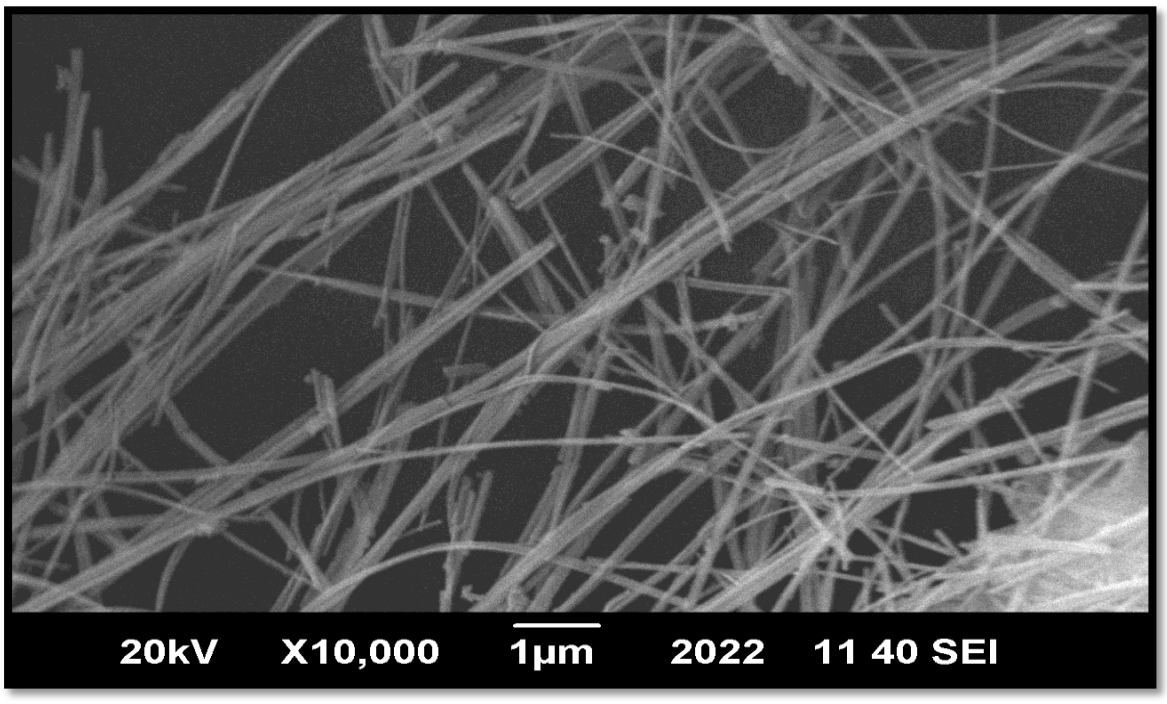


Figure 4.1: SEM image of Pure Titania Nanotubes at X10, 000

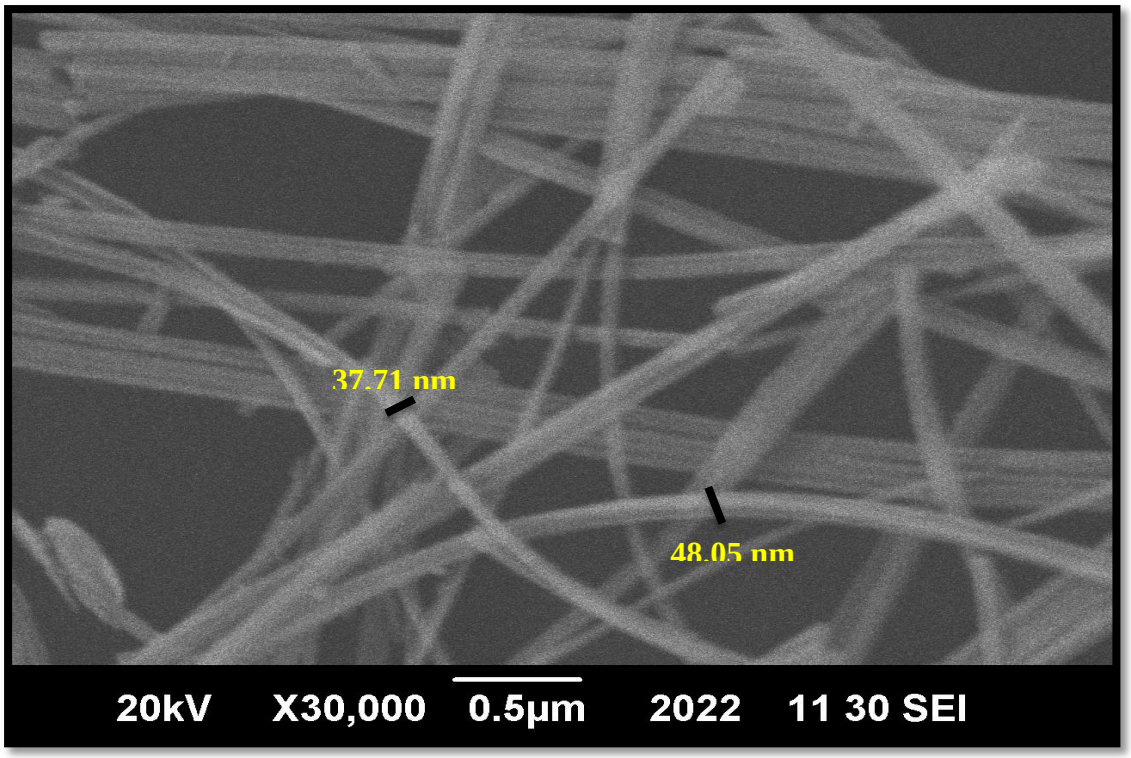


Figure 4.2: SEM image of Pure Titania Nanotubes at X30, 000

Figures 4.3 and 4.4 show the images of Iron doped Titania Nanotubes at 10,000 and 30,000 magnification. SEM images show that very fine tubular structures have been formed with diameters ranging from 18 nm to 42 nm. These tubular structures exhibit high surface area as compared to nanoparticles and are expected to have higher photo-catalytic activity. From images of doped Nanotubes, it can be furthered inferred that Iron doping has helped in reducing diameters of the tubes compared to the pure Titania Nanotubes.

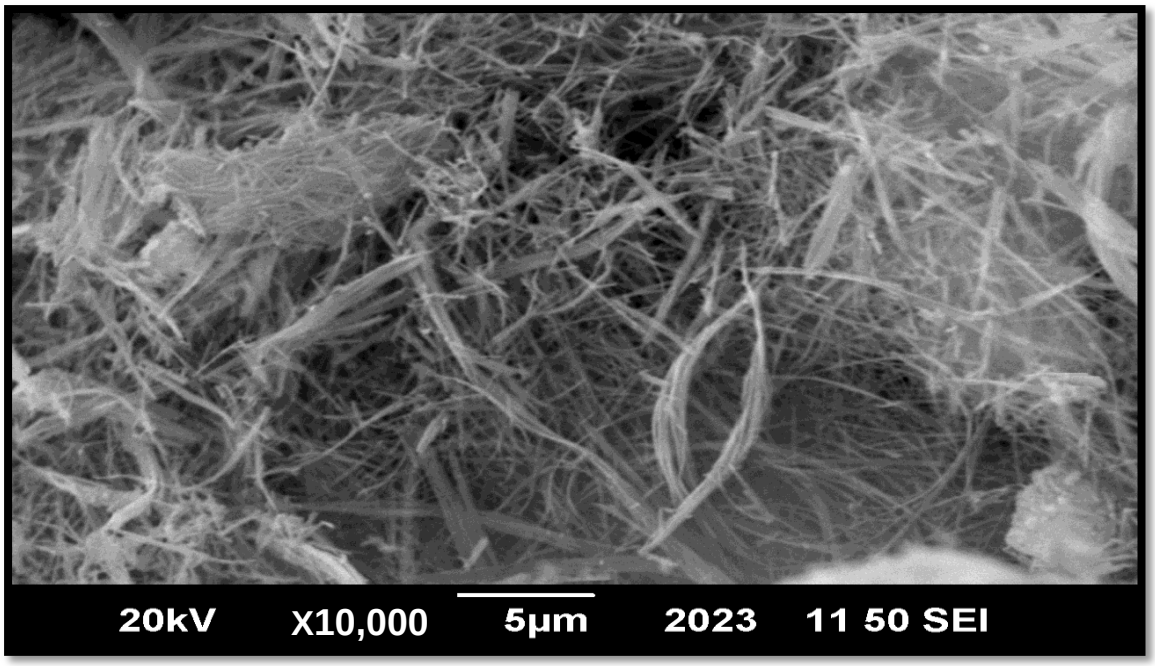


Figure 4.3: SEM images of 1% Iron doped Titania Nanotubes at X5, 000

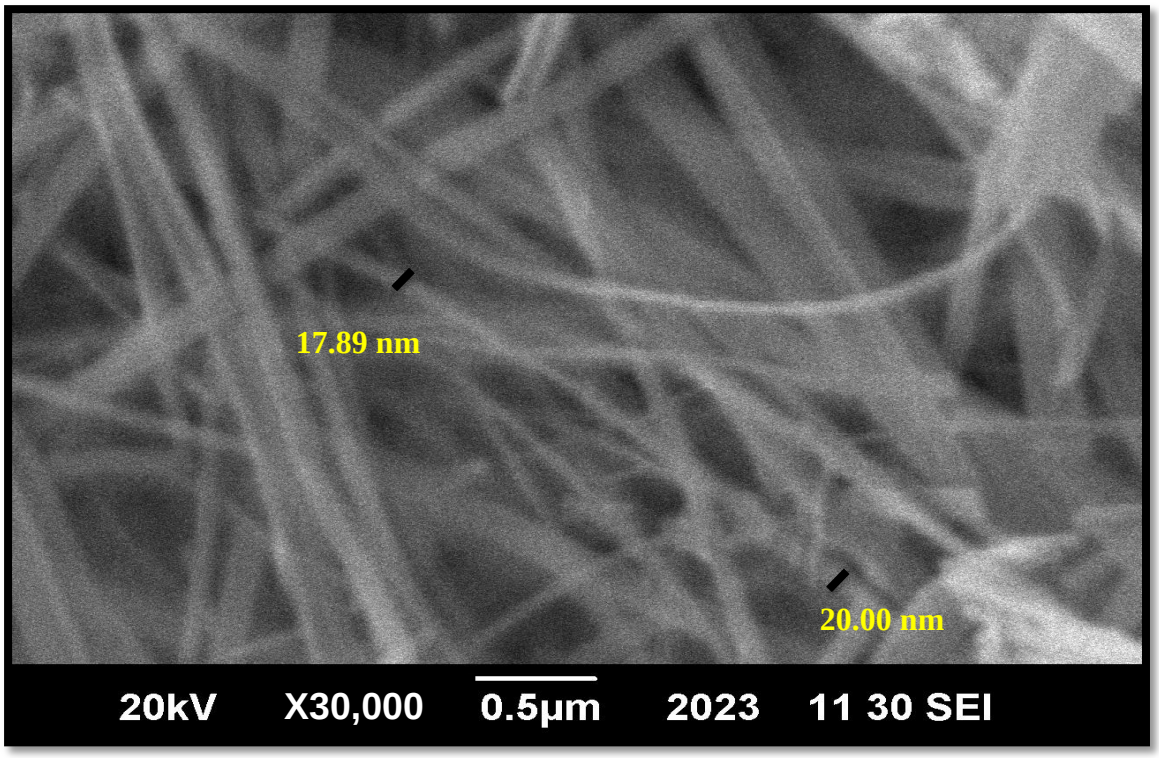


Figure 4.4: SEM images of 1% Iron doped Titania Nanotubes at X50, 000

4.1.2. EDS Analysis

Figure 4.5 shows that the major constituents for pure Titania Nanotubes are Titanium and Oxygen i.e. approximately 61% and 39% respectively (Table 4.1). EDS results of Iron doped Titania Nanotubes in Figure 4.6 and Table 4.2 showed that product is comprised of Oxygen (50.90%), Titanium (46.84%), Iron (1.06%) and Sodium (1.2%). The source of Sodium is NaOH, which was used during the synthesis process; small quantity of Sodium was left over even after several washings. EDS Results confirmed that 1% Iron doping of Nanotubes has been successfully achieved.

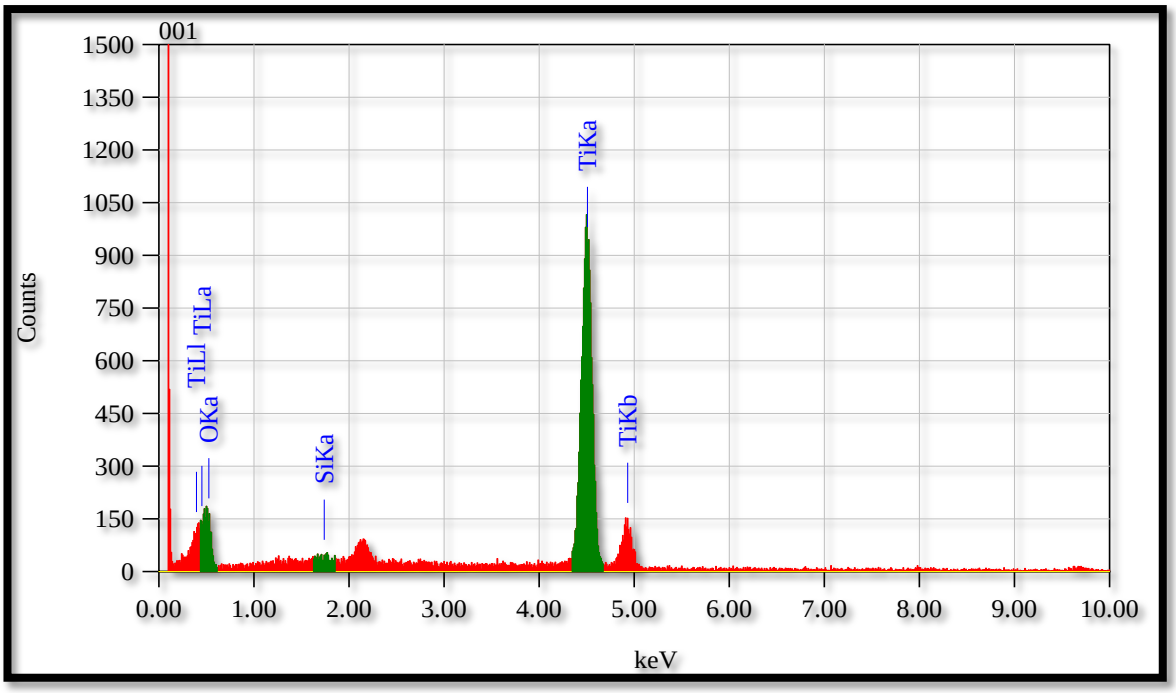


Figure 4.5: Elemental Compositions of Pure Titania Nanotubes

Element	Mass Percent
Ti	61.19
O	38.53
Si	0.28

Table 4.1: Mass% of Elements in Pure Titania Nanotubes

Element	Mass Percent
Ti	46.84
O	50.90
Fe	1.06
Na	1.2

Table 4.2: Mass% of Elements in 1% Iron Doped Titania Nanotubes

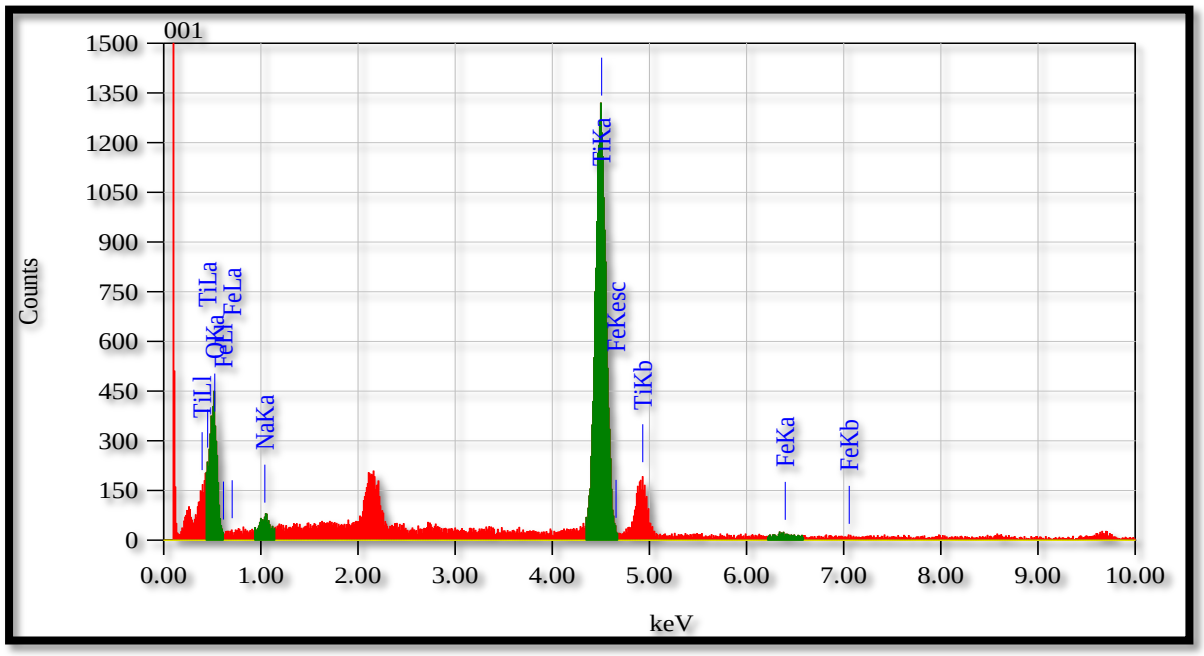


Figure 4.6: Elemental Compositions of 1% Iron Doped Titania Nanotubes

4.1.3. XRD Analysis

X-Ray Diffraction (XRD) characterization for both pure Titania Nanotubes and 1% Iron doped Titania Nanotubes were carried out using Cu-Kα radiations at an angle of 2θ from 10^{o} to 80^{o}. In case of pure TNTs, peaks can be seen at 25^{0}, 38^{0}, 48^{0}, 55^{0}, 56^{0}, 63^{0}, 68^{0}, 71^{0} and 75^{0} in Figure 4.7. These peaks are characteristics of TiO_2 in anatase phase confirming that the synthesized pure Titania Nanotubes are highly crystalline in nature. The main reason behind this crystallinity is calcination of

Nanotubes at 500 ^{0}C for 1 hour. Another cause of highly crystalline structure may be Nanoparticles (used as precursor material for preparation of Nanotubes) that were also highly crystalline when synthesized by the process of calcination.

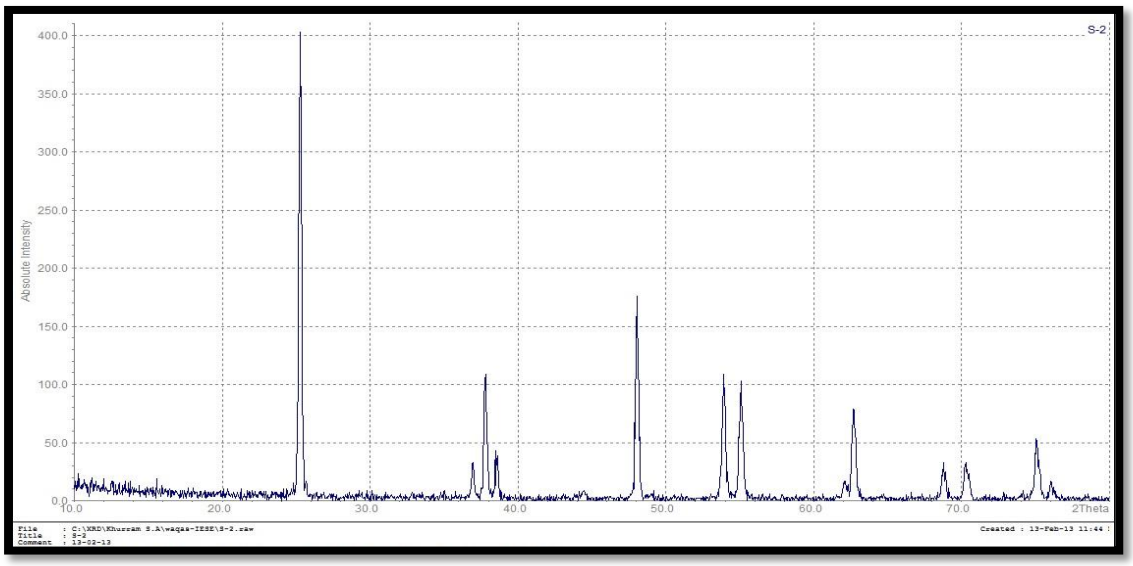


Figure 4.7: XRD Intensity plot for Pure Titania Nanotubes

In case of 1% Iron doped Titania Nanotubes, Figure 4.8 shows peaks at 25^0, 38^0, 48^0, 55^0, 56^0, 63^0, 68^0, 71^0 and 75^0. These peaks strengthen the argument that doped TNTs are also highly crystalline in nature attributing this characteristic towards calcination of prepared Nanotubes in muffle furnace for 1 hour and crystalline structure of Nanoparticles used as a starting material for the synthesis of Iron doped Titania Nanotubes.

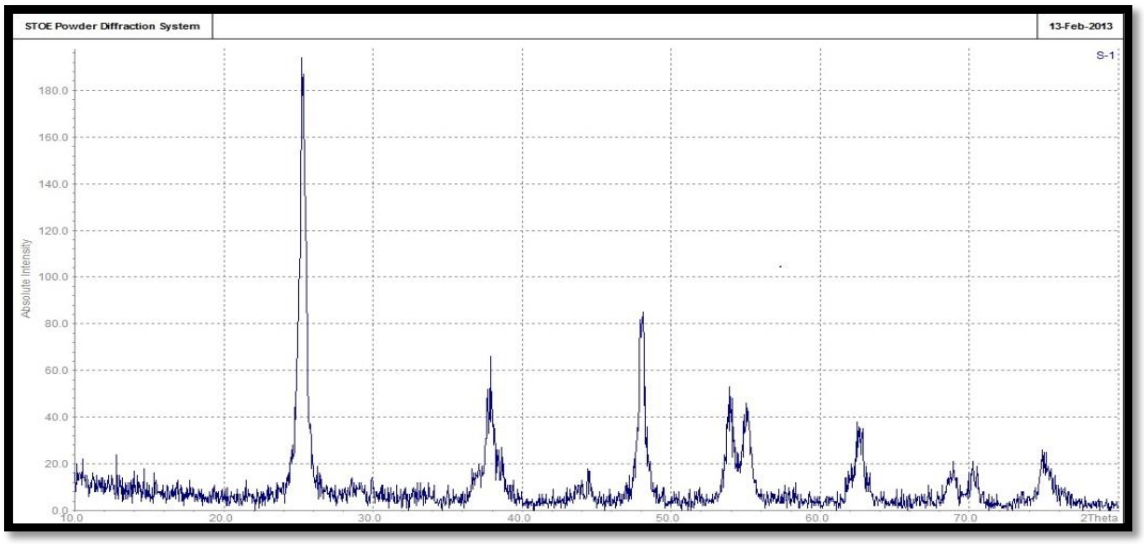


Figure 4.8: XRD Intensity plot for Iron Doped Titania Nanotubes

In short, synthesized Nanotubes are highly crystalline having anatase phase. It has been reported that Nanotubes (pure or doped) when annealed or calcined at high temperature (almost 400 ^{o}C), convert to anatase phase having two to three time more photo-catalytic activity than the commercially prepared Titania Nanoparticles (Sekino, 2010). It has also been shown that Titania in anatse form gives better photo-catalytic results than other forms of Titania i.e. rutile and brookite (Yao *et al.*, 2006). Anatase phase is also a powerful oxidizing agent having other characteristics like non-toxicity and long term photo-catalytic ability (Hoffman *et al.*, 1995).

4.1.4. BET Analysis

BET analysis of pure Titania Nanotubes (Figure 4.9) revealed that at a relative pressure of 0.25 maximum amount of liquid Nitrogen adsorbed on the surface of Nanotubes is about 80 cm^3/g at Standard Temperature and Pressure (STP). This corresponds to almost 320 m^2/g surface area of pure Titania Nanotubes that matches well with the surface area limits (300-350 m^2/g) for pure Nanotubes given by Sekino (2010).

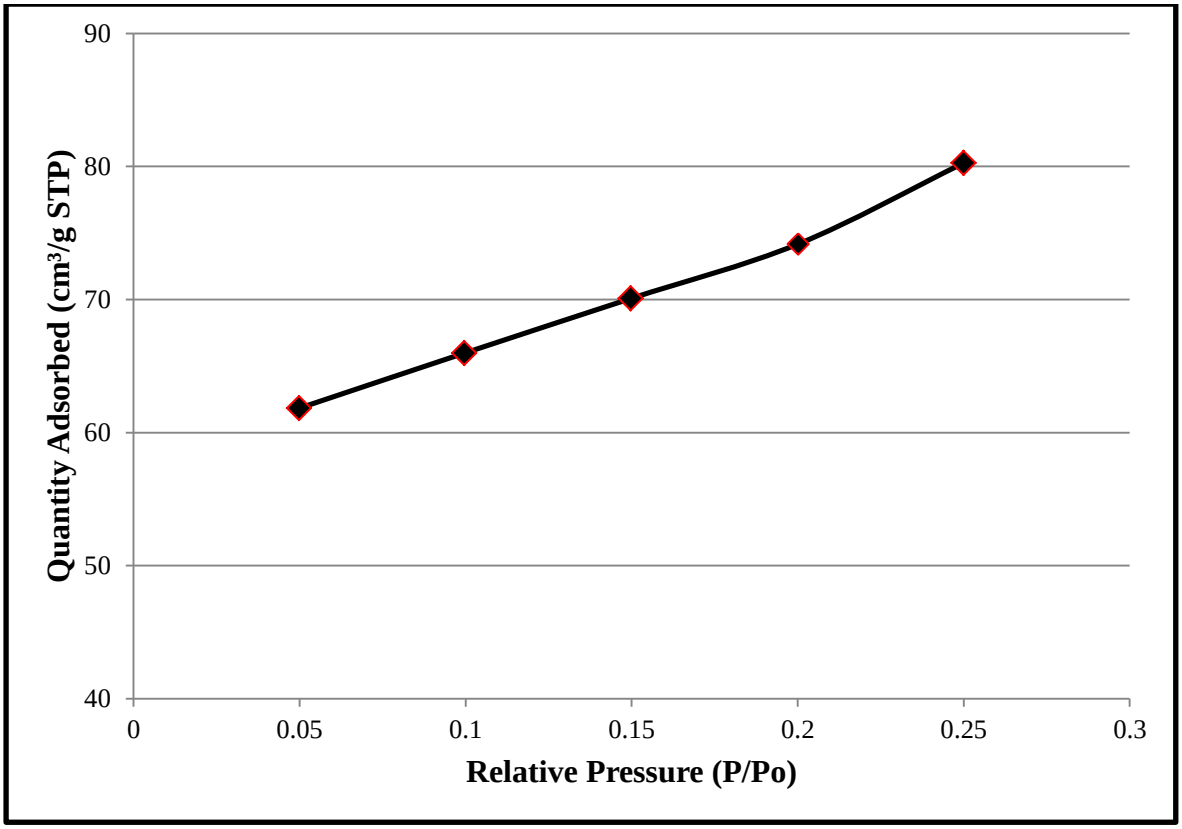


Figure 4.9: Isotherm Linear Plot for N_2 Adsorption (Pure Titania Nanotubes) at 77K

While, on the other hand, BET analysis of 1% Iron doped Titania Nanotubes (Figure 4.10) showed that at a relative pressure of 0.25 the maximum amount of liquid Nitrogen adsorbed on the surface of Nanotubes is about 65 cm^3/g at Standard Temperature and Pressure (STP). This corresponds to almost 230 m^2/g surface area of doped Titania Nanotubes.

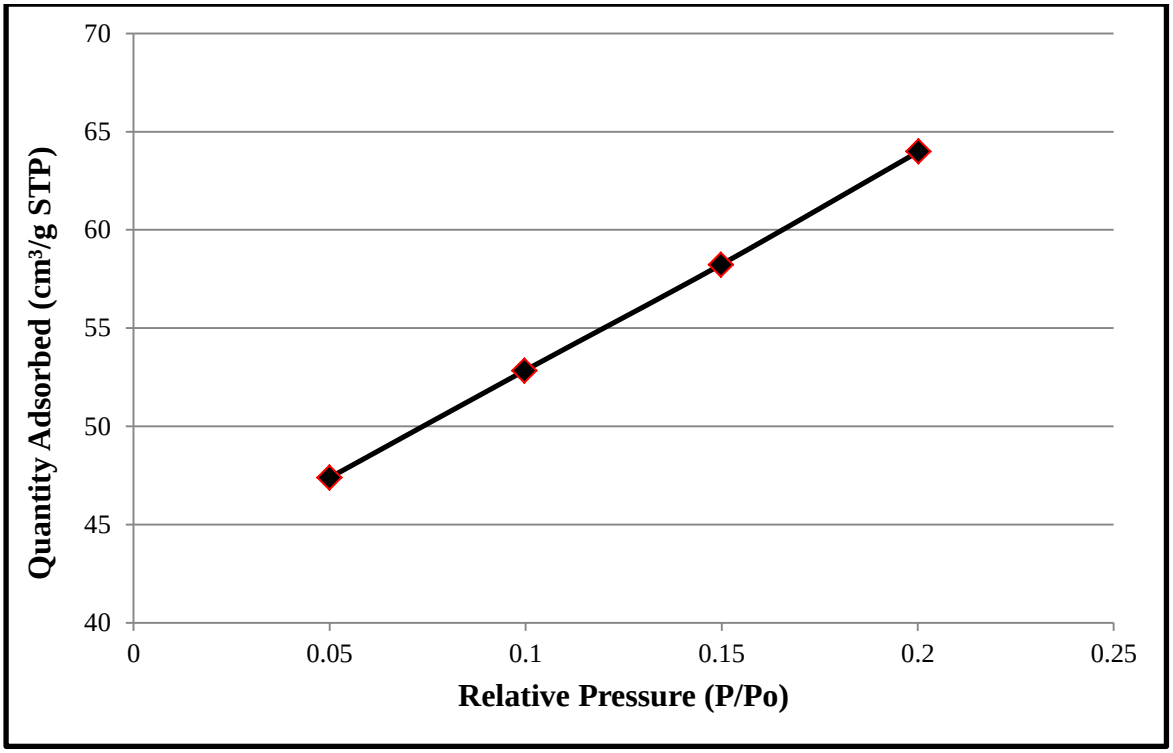


Figure 4.10: Isotherm Linear Plot for N_2 Adsorption (1% Iron Doped Titania Nanotubes) at 77K

4.1.5. Band Gap Energy Calculation

Band gap energy for pure and doped Titania Nanotubes were find out by using Planck's equation (Dharma and Pisal, 2012) **E=hc/λ** where,

E = Energy

h = Planck's Constant i.e. 6.626 x 10-^{34} Js

c = Speed of Light i.e. 3.0 x 10^8 m/s

λ = cut off wavelength obtained from absorbance spectra of pure and doped Nanotubes

In Figure 4.11cut off wavelength for pure Titania Nanotubes is seen to be 378 nm. By putting this value in the above equation

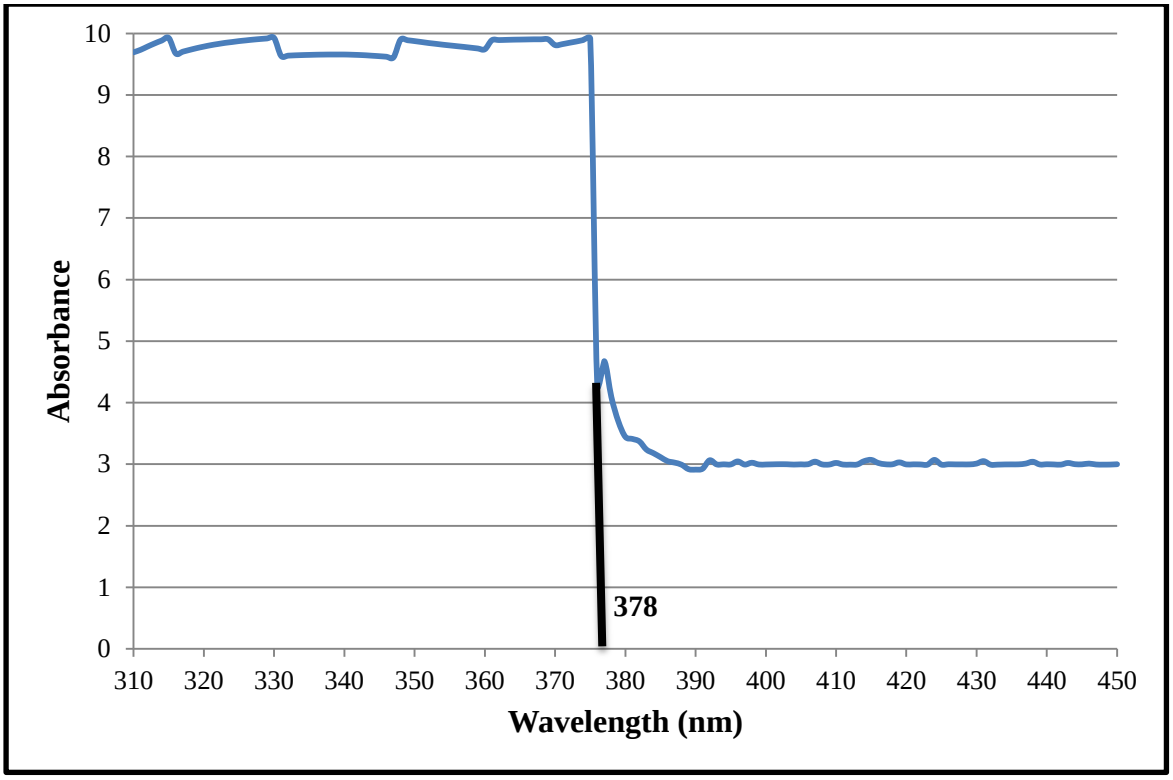


Figure 4.11: Absorption Spectra for Pure TNTs in UV-Vis Spectrophotometer

$E = (6.626 \times 10^{-34}) (3.0 \times 10^{8})/378nm = 5.25 \times 10^{-19}$ J

$E = 5.25 \times 10^{-19}$ J/1.6 X 10^{-19} J = 3.28 eV

So band gap energy for pure TNTs comes out to be 3.28 Electron Volt that is close enough to the cited band gap energy of pure Titania i.e. 3.20 eV (Linsebigler *et al.*, 1995).

In Figure 4.12 cut off wavelength for 1% Iron doped Titania Nanotubes comes out to be 444 nm. By putting this value in the Planck's equation

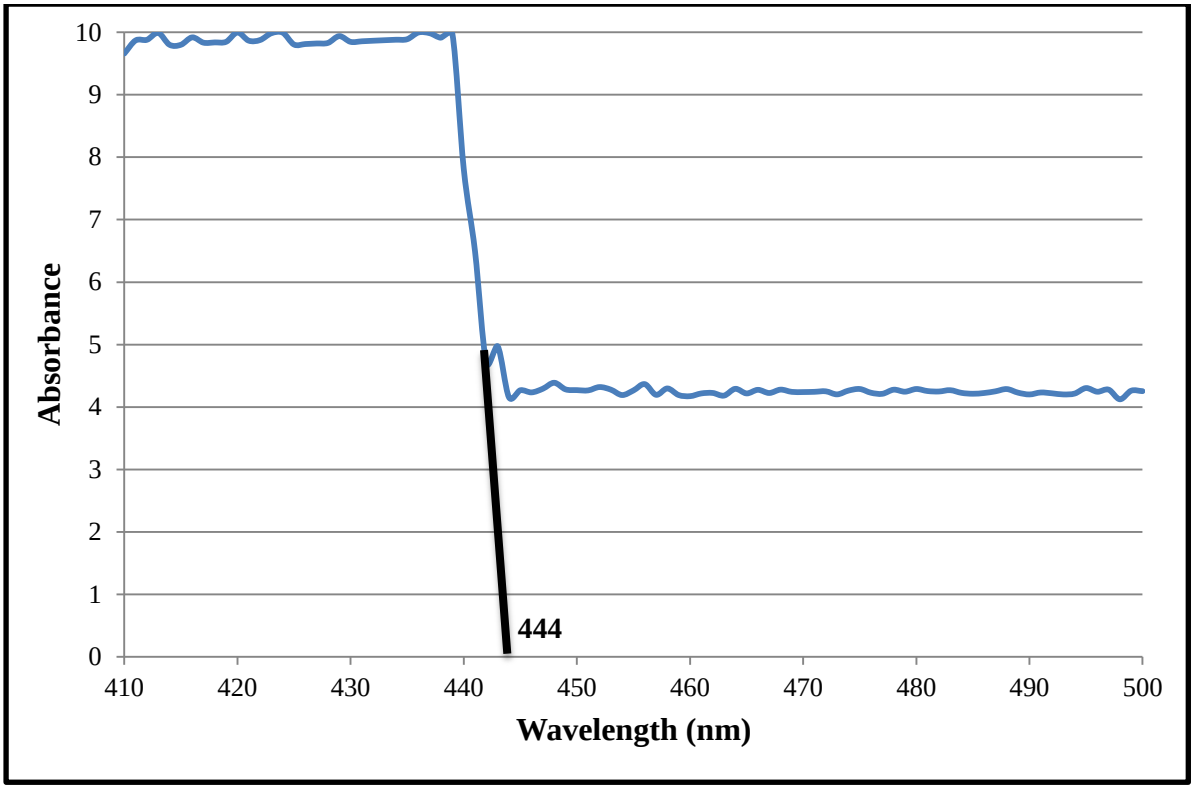


Figure 4.12: Absorption Spectra for Iron Doped TNTs, UV-Vis Spectrophotometer

$E = (6.626 \times 10\text{-}^{34}) (3.0 \times 10^{8})/444nm = 4.477 \times 10\text{-}^{19}$ J

$E = 4.477 \times 10\text{-}^{19}$ J/1.6 X 10^{-19} J = 2.80 eV

The results shows that Iron doping has significantly reduced the band gap energy for Titania Nanotubes meaning that doped Nanotubes can work more efficiently under normal fluorescent light than pure Titania Nanotubes having higher band gap energies (close to 3.20 eV) that work more effectively under UV light.

4.2. Bacterial Disinfection

4.2.1. Bactericidal effect of TNTs in Aqueous Phase

0.1 gm Pure Nanotubes and 100 ml of overnight grown fresh culture of *P. aeruginosa* were put together in a quartz cell and fix it on an orbital shaker. Observations were noted for 2 hours under normal and UV light.

Figure 4.13 represents the degradation of *P. aeroginosa* under visible and UV light. Initially cell count for *P. aeruginosa* was 146×10^7 CFU/mL, however the cell count decrease after exposure to florescent light for 2 hours and is found to be 66×10^7 CFU/mL, showing 50% removal of *P. aeruginosa*, while, complete disinfection of *P. aeruginosa* was achieved after 1 hour, when solution was exposed to UV light with cell count decreased from 146×10^7 CFU/mL to zero. It means UV light triggered the enhanced production of Reactive Oxidation Species (ROS) from pure TNTs thus completely killing the bacterial population. While, on the other hand, fluorescent light manage to generate very ROS from pure TNTs resulting in incomplete inactivation of *P. aeruginosa*. In case of UV, control solution (without Nanotubes), also showed 40% degradation of *P. aeruginosa* after 2 hours due to the bactericidal effect of Ultra-Violet radiations (Hallmich and Gehr, 2010).

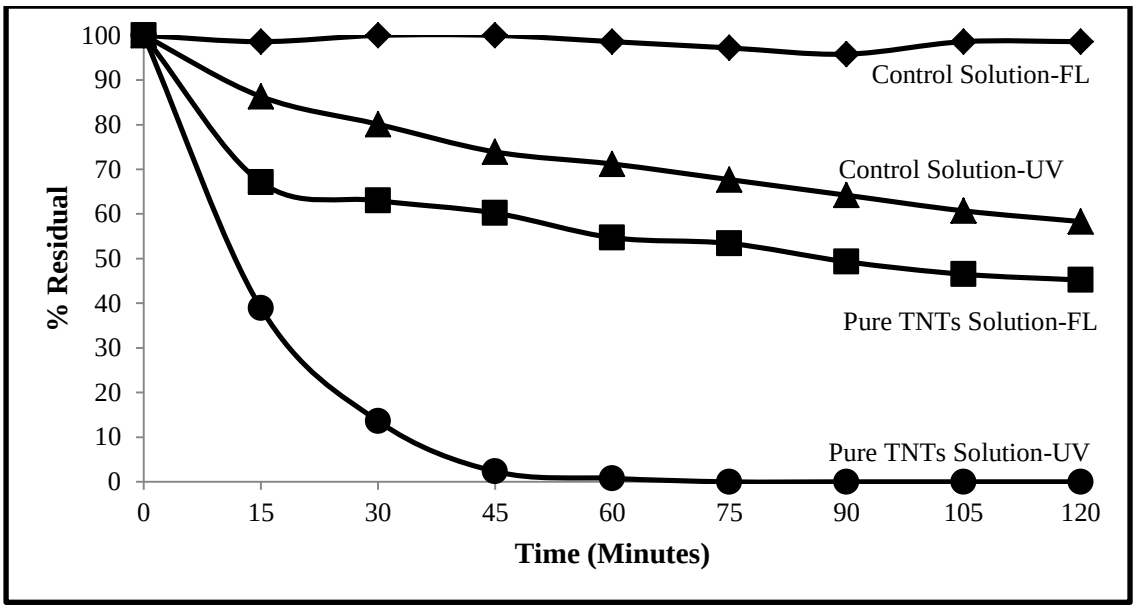


Figure 4.13: *P. aeruginosa* Disinfection by Pure Titania Nanotubes in Aqueous Phase with respect to time

In case of *Staph. aureus*, Figure 4.14 represents that almost 55% bacterial degradation was achieved under Fluorescent light while complete disinfection was achieved after 90 minutes, when solution was exposed to UV light. Control solution here, under UV light showed 30% degradation after 2 hours of exposure. The degradation of bacterial population in control solution may be attributed towards bactericidal effect of UV light even in absence of photo-catalyst (Hallmich and Gehr, 2010).

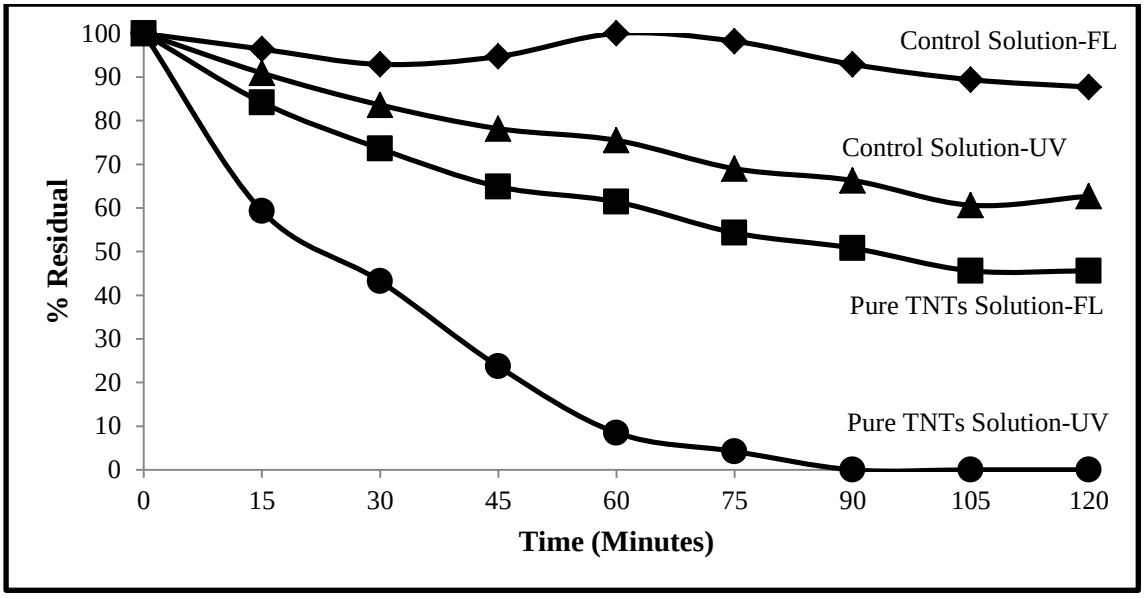


Figure 4.14: *Staph. aureus* Disinfection by Pure Titania Nanotubes in Aqueous Phase with respect to time

When *P. aeruginosa* solution containing Iron doped nanotubes was exposed to Fluorescent light, Figure 4.15 depicts that complete inactivation of bacteria (with decrease in cell count from 214×10^5 CFU/mL to 0) was achieved after 105 minutes. Whereas, for the same solution when exposed to UV, degradation rate was much faster with complete inactivation just within 30 minutes of exposure. These results show (by disinfecting bacterial population completely under normal fluorescent light) that doping has helped lowering the band gap energy requirements for Titania. Control solution degraded up to 47% when exposed to UV due to its bactericidal effect (Hallmich and Gehr, 2010) and cell count was found to be decreased from 196×10^5 CFU/mL to 106×10^5 CFU/mL.

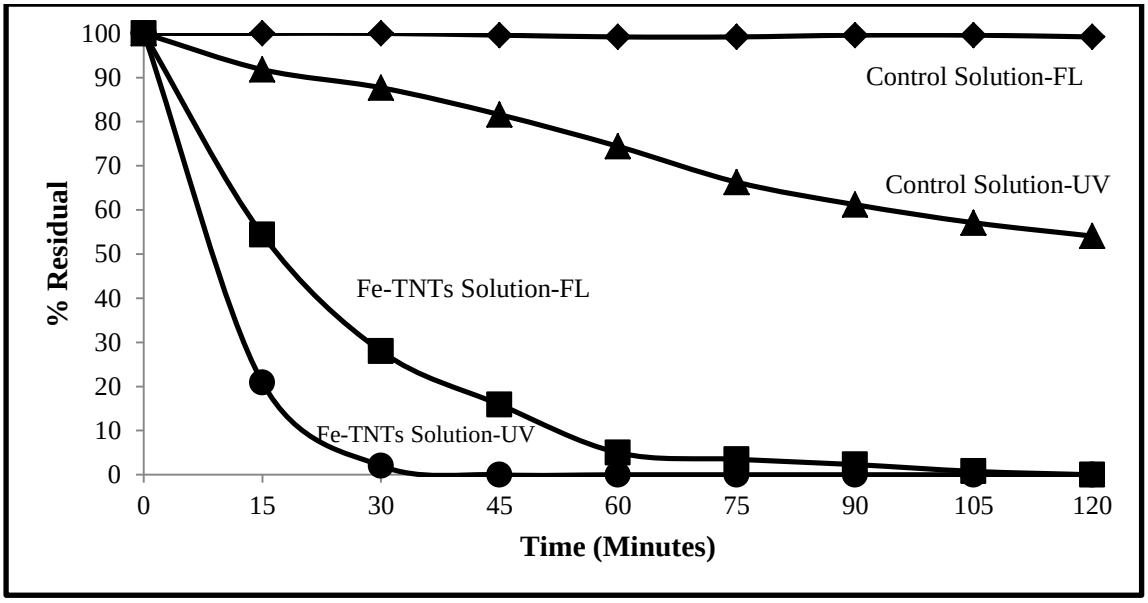


Figure 4.15: *P. aeruginosa* Disinfection by Iron doped Titania Nanotubes in Aqueous Phase with respect to time

In case of *Staph. aureus* and doped Nanotubes solution, Figure 4.16 shows that complete bacterial inactivation (cell count decreasing from 212×10^5 CFU/mL to 0) was achieved after almost 90 minutes of exposure under fluorescent light further solidifying the claim of proper doping of TNTs. When the same solution was exposed to UV light source, complete sterilization was achieved just within 30 minutes. It can be inferred that both Iron doping and UV light enhances the reaction rate (Naira *et al.*, 2012). Control solution showed negligible degradation under fluorescent light while under UV light, almost 30% (with cell count decreasing from 212×10^5 CFU/mL to 142×10^5 CFU/mL) bacterial species of control solution was killed due to its germicidal effect (Hallmich and Gehr, 2010).

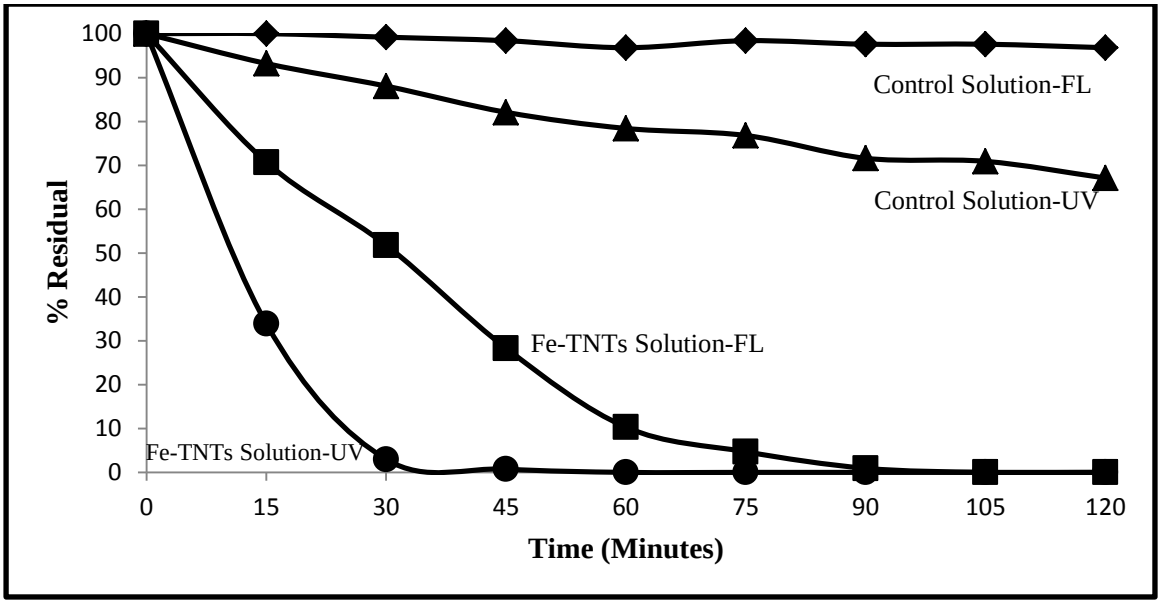


Figure 4.16: *Staph. aureus* Disinfection by Iron doped Titania Nanotubes in Aqueous Phase with respect to time

4.2.2. Bactericidal effect of TNTs Coated Wood Surfaces

Overnight grown fresh cultures of *P. aeruginosa* and *Staph. aureus* were sprayed on wood surfaces i.e. uncoated (as a control surface), pure Titania Nanotubes coated surfaces and 1% Iron doped Titania Nanotubes coated surfaces to observe the inactivation of bacterial species as a function of time under fluorescent as well as UV light.

In the case of wood surface coated with pure Titania Nanotubes, exposed to normal fluorescent light using laminar flow hood, Figure 4.17 shows that population of *P. aeruginosa* was reduced to almost 65% after 60 minutes. While, after 120 minutes of exposure to fluorescent light, almost 70% of the bacterial population was killed (cell count decreasing from 126×10^5 CFU/mL to 38×10^5 CFU/mL) illustrating that even after 120 minutes, almost 30% of the P. Aeruginosa specie survived on pure Titania Nanotube coated wood surface. Incomplete disinfection of bacterial

population is most probably due to presence of 2.5% UV in fluorescent light (NEMA, 1999) as pure Titania Nanotubes work more efficiently in presence of UV light due to large band gap energy requirements. When coated wood surface containing culture of *P. aeruginosa* was exposed to UV light, complete disinfection obtained after 105 minutes emphasizing that UV exposure has enhanced the bacterial disinfection rate as pure Titania Nanotubes became highly functional under UV light.

Control wood surface (without any coating), didn't show significant reduction in bacterial population in case of normal light while almost 40% killing of *P. aeruginosa* was observed under UV light after 2 hours due to its germicidal effect.

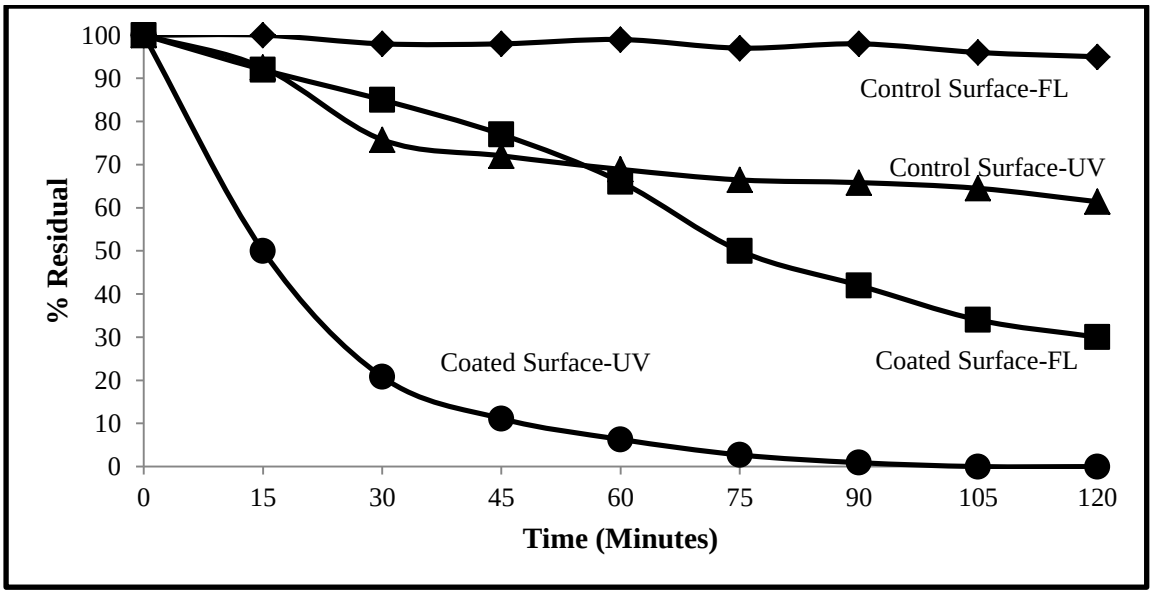


Figure 4.17: P. Aeruginosa Disinfection by Pure Titania Nanotubes Coated Surface with respect to time

Almost similar patterns were observed in the case of *Staph. aureus* when pure Titania Nanotubes coated wood surface was exposed to fluorescent and UV light source. Figure 4.18 shows that *Staph. aureus* cultures exhibited 80% degradation (cell count decreasing from 236×10^5 CFU/mL to 50×10^5 CFU/mL) when coated surface

was exposed to fluorescent light for 2 hours. Incomplete inactivation of *Staph. aureus* can be attributed to its complex wall structure for being a gram negative species (Singleton, 2004) and large band gap energy requirements by pure Titania Nanotubes (Linsebigler *et al.*, 1995). On the other hand, inactivation rate increased under UV exposure with complete disinfection just within 75 minutes as high energy UV rays can easily generate electron hole pairs in pure Titania Nanotubes by satisfying its band gap energy requirements (Linsebigler *et al.*, 1995).

Control surface showed negligible reduction in population of *Staph. aureus* under normal light while 50% degradation (cell count decreasing from 236×10^5 CFU/mL to 124×10^5 CFU/mL) under UV light after 2 hours of exposure.

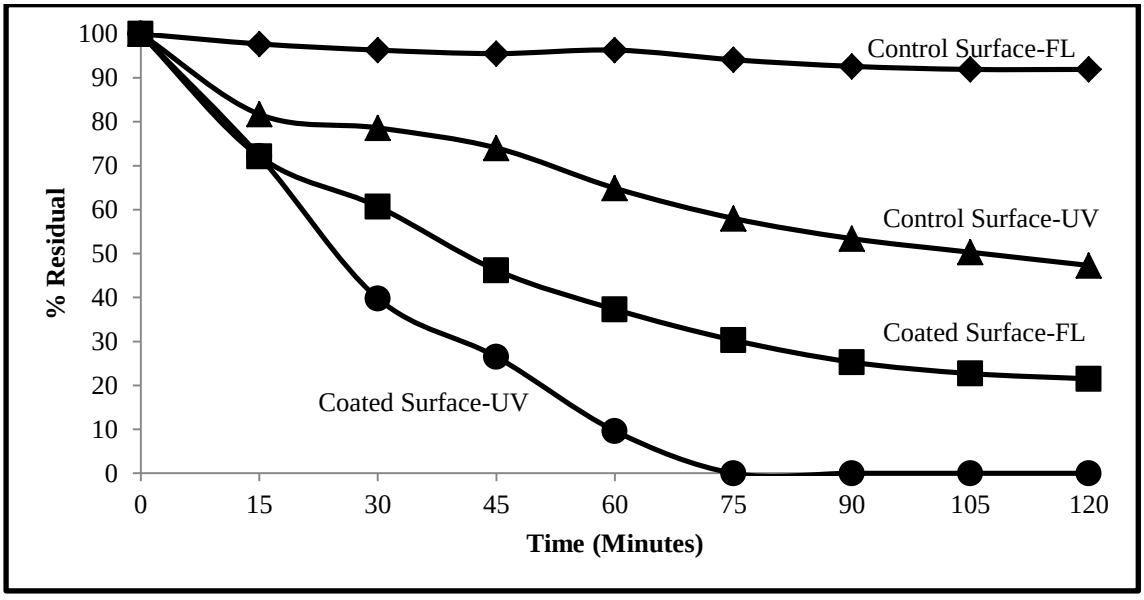


Figure 4.18: *Staph. aureus* Disinfection by Pure Titania Nanotubes Coated Surface with respect to time

Doped Nanotubes coated surface, when exposed to normal fluorescent light under sterile laminar flow hood showed far better disinfection of *P. aeruginosa* than pure Titania Nanotubes coated wood surfaces. Figure 4.19 shows that 70% of the

bacterial population was killed (cell count decreasing from 126 × 10^5 CFU/mL to 38 × 10^5 CFU/mL) just within first 30 minutes of exposure. Almost complete sterilization was achieved just within 1 hour of exposure as compared to pure Titania Nanotubes wood surfaces where complete sterilization was not achieved even after 2 hours. This is due to the fact that Iron doping significantly reduce the band gap energy requirements (Grandcolas and Hanagata, 2011) for Titania. Under UV exposure, bacterial degradation rate enhanced and complete sterilization achieved (cell count decreasing from 216 × 10^5 CFU/mL to 0) within 45 minutes of exposure. When visible light strikes the doped nanotubes, it produces electron hole pairs, which react with water molecules present on the coated surface to form OH^-, O_2^-, H_2O_2 and HO_2 species (Huang *et al.,* 1988). These species have great ability to degrade organic matter like bacteria.

Control wood surface (without any coating), showed a reduction of almost 50% (cell count decreasing from 222×10^5 CFU/mL to 110×10^5 CFU/mL) when exposed to UV light. Under normal fluorescent light, control surface showed negligible reduction in bacterial population.

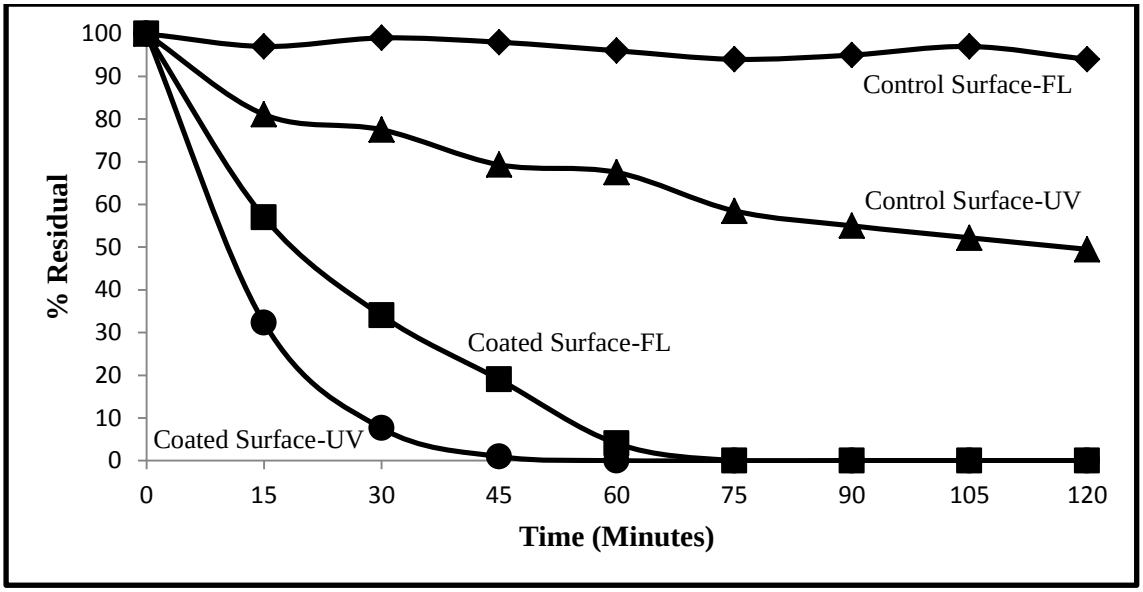


Figure 4.19: *P. aeruginosa* Disinfection by 1% Iron Doped Titania Nanotubes Coated Surface with respect to time

Doped wood surface containing *Staph. aureus* when exposed to light, almost 90% of Staph. Aureus population inactivated (cell count decreasing from 88×10^5 CFU/mL to 12×10^5 CFU/mL) in first 60 minutes (Figure 4.20). While, complete killing of bacteria under fluorescent light were achieved after 105 minutes (showing more resistant than *P. aeruginosa* under similar conditions). When similar bacterial species were sprayed on doped Nanotubes wood surface and exposed to UV light source, complete inactivation was achieved just within 45 minutes emphasizing that UV light enhanced the reaction kinetics.

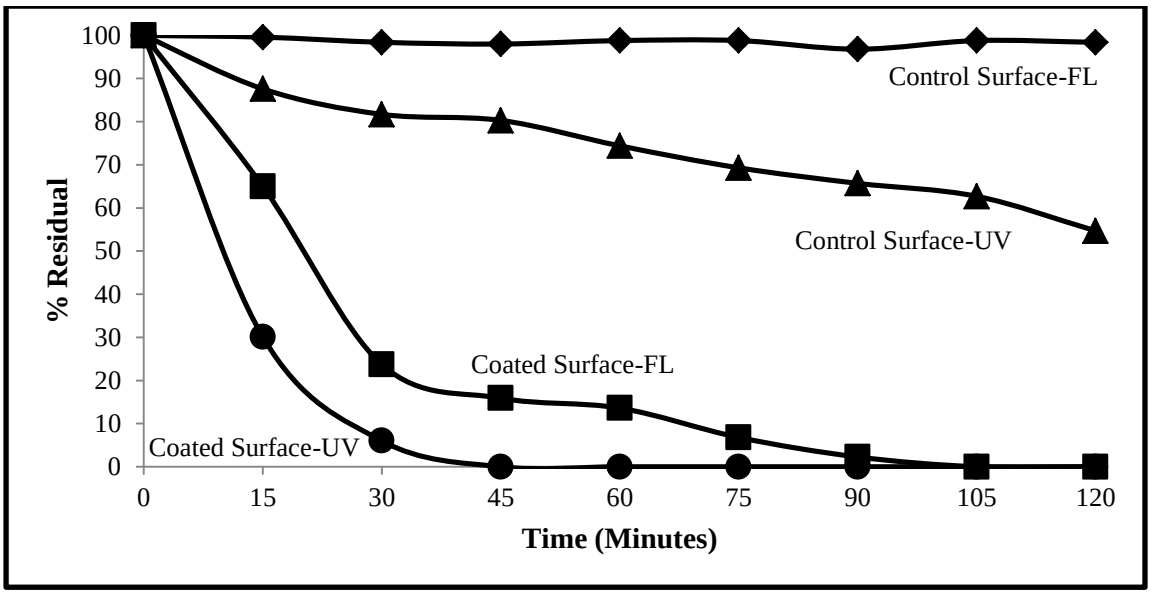


Figure 4.20: *Staph. aureus* Disinfection by 1% Iron Doped Titania Nanotubes Coated Surface with respect to time

4.2.3. Bactericidal effect of TNTs Coated Wood Surfaces for General Microbial Flora of Laboratory Air

For general disinfection efficiency testing, pure and doped TNTs coated and an uncoated wood surfaces (placed at a height of 3 feet from floor) were exposed to general microbial flora of Wastewater Laboratory air at IESE, NUST for 24 hours. Swab cultures from these exposed surfaces were taken after exposure and streaked on prepared agar plates using laminar flow hood. After incubation period of 24 hours, grown colonies were noted using colony counter.

Figure 4.21 shows that there were numerous colonies of bacteria on uncoated surface. Figure 4.22 shows that colonies were reduced by 80% in the case of wood surface coated with pure Titania Nanotubes, while there was no colony growth on the surface of wood coated with 1% Iron doped TNTs.

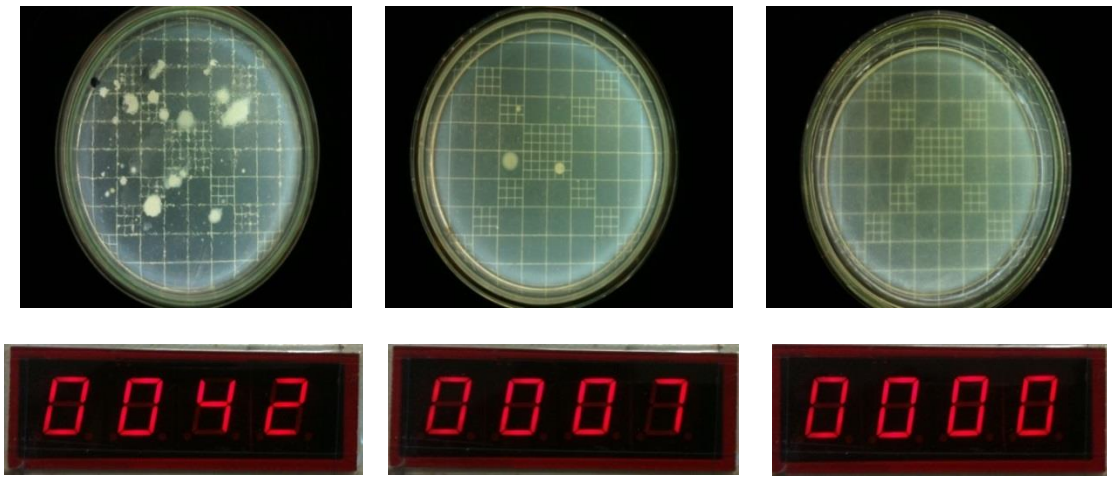


Figure 4.21: Bacterial Colonies on a) Control Wood Surface b) Pure TNTs coated Wood Surface c) 1% Iron Doped TNTs Coated Wood Surface

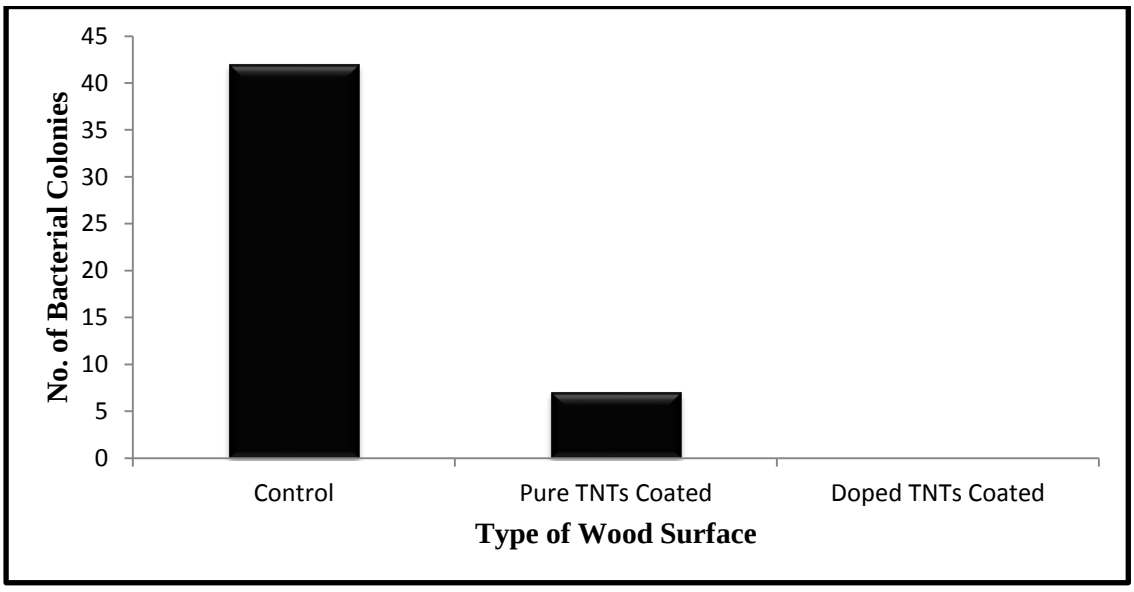


Figure 4.22: Reduction in Bacterial Colonies with different types of Nanotubes Coatings

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

- The hydrothermal method with modifications, like induction of ultra-sonication is effective for synthesizing pure and 1% Iron doped Titania Nanotubes.
- The method of coating using a Natural resin (Gum Acacia) is not only cost effective but also environment friendly.
- Nanotube coated surfaces showed remarkable reduction in airborne bacterial population as summarized below.
    - The most important finding of this study is that 1% Iron doped Titania Nanotube coated wood surfaces are more effective in killing *P. aeruginosa* and *Staph. aureus* than pure Titania Nanotube coated wood surfaces under fluorescent light.
    - Under UV light, pure Titania Nanotubes exhibit complete disinfection with disinfection time ranging from almost 75 to 105 minutes.
    - Under UV light, doped TNT coated wood surfaces show faster killing of bacteria

5.2 Recommendations

1. Comparison of Different Nano-material coatings like Nano Flowers, Nano Wires, Nano Rods, Nano Ribbons in disinfecting bacteria may be carried out
2. Other self-sterilizing surfaces may be prepared like Titania Embedded self-sterilizing glass surfaces
3. TNTs embedded computer key boards and mobile screens may be produced to sanitize air.
4. Idea can be extended to degrade other Indoor Air Pollutants using similar coatings
5. The novel idea of coating suggested in this study may be implemented in an entire building to produced “sanitized environment”
6. Titania Nanoube embedded wood liquor may be applied in a hospital to control nosocomial infecions
7. Similarly, this idea of coating may be implemented in bakeries to reduce food spoilage.

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