DEVELOPMENT OF TITANIA NANOPARTICLE COATED TAPE FOR THE DECONTAMINATION OF SHOPPING CART HANDLES

By

Samia Taj

(NUST201463471MSCEE65214F)

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Submitted by

Samia Taj

Has been found satisfactory for the requirements of the degree of
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Supervisor: _____

Dr. Ishtiaq A. Qazi

Professor

IESE, SCEE, NUST

Member: _____

Professor Dr. Imran Hashmi

Associate Dean

IESE, SCEE, NUST

Member: _____

Dr. Zeshan

Assistant Professor

IESE, SCEE, NUST

DEDICATION

This thesis is dedicated to my Parents

For their endless affection, support and encouragement

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Allah Most High has said: ***Say: Praised be Allah!*** *[27:59] and:* ***If you would count the blessings of Allah you would not be able to reckon them,*** *[14:34] and:* ***Of the blessings of your Lord, speak out,*** *[93:11] and:* ***Remember Me, and I will remember you, give thanks to Me,*** *[2:152]. I fully realize the blessings upon me by the most gracious and divine force of all forces that enabled me, and gave me sense and insight to accomplish this research objectively and successfully. My special praise to* ***HAZRAT MUHAMMAD (PBUH****) who is forever the torch of knowledge for whole mankind.*

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Samia Taj

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LIST OF ABBREVIATIONS

SCs	Shopping Cart
CFU	Colony Forming Unit
TNPs	Titania Nanoparticle
Ag-TNP	Silver Doped Titania Nanoparticle
UV	Ultraviolet
ICUs	Intensive Care Units
nm	Nanometer
ROS	Reactive Oxygen Species
VOCs	Volatile Organic Compounds
FDA	Food and Drug Administration
MEK	Methyl Ethyl Ketone
XRD	X-Ray Diffraction
SEM	Scanning Electron Microscopy
EDS	Energy Dispersive Spectroscopy
mins	Minutes
IESE	Institute of Environmental Sciences & Engineering
mL	Milliliter
g	Gram
rRNA	Ribosomal Ribonucleic Acid
NCBI	National Centre for Biotechnology Information's
DBR	Drimarene Brilliant Red
MB	Methylene Blue
BLAST	Basic Local Alignment Search Tool
EMB	Eosin Methylene Blue

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ABSTRACT

Shopping Carts, a facility provided to the consumers to transport their purchased product, is one of most contaminated surface that harbors bacteria especially on their handles. With the passage of time, the extent of contamination of shopping cart handles is increasing which leads to transmission of infectious diseases among people. To overcome this problem, different strategies have been mentioned in literature like hand washing, antibacterial agents, disposable plastic barriers, and photocatalysis etc. Among all these, photocatalysis is becoming an emerging technique due to its low cost, non toxicity, and being energy independent and environment friendly technique. In the present study, titania nanoparticle suspensions were immobilized on double sided tape and wrapped around the shopping cart handles to provide self cleaning surfaces. Whereas the major species of microbial contamination found on shopping cart handles include *Staphylococcus sciuri, Bacillus pumilus, Bacillus aerius, Pseudomonas aeruginosa* and *Agrobacterium tumefaciens.* The coated tapes were tested against *E. coli* and the isolated bacterial strains (*Staphylococcus sciuri* and *Agrobacterium tumefaciens*) with 2 hrs exposure to UV and visible irradiation. The outcomes of the present study indicated that titania nanoparticle embedded tapes are effective in killing *E. coli* as well as resistant microbes (*Staphylococcus sciuri* and *Agrobacterium tumefaciens*) under normal light. Bacterial count was 9.75×10^6 CFU/ml at the start of experiment while after 2 hrs it was 7.4×10^6, 1.8×10^6, 9.0×10^5 CFU/ml for uncoated, 5%TNPs coated and 1% Ag-doped NPs, respectively. Finally it has been demonstrated that the technique may be effectively used for continual disinfection of the shopping cart handles.

Chapter 1

INTRODUCTION

Shopping Cart (SC) is a facility which is provided to the customers for transportation of their purchased products inside a store. Besides this, they may transfer their products to their cars through these carts. These are used by a large number of customers and the chance of contamination of shopping cart handles is considerable. Not surprisingly handles are one of the most contaminated sites where several bacteria are present that may have harmful impact on human health (Irshaid *et al*., 2014). This increases the chance of transmission of infections from person to person.

Arizona State University reported that shopping cart handles have more bacteria and dirt as compared to bus handles, public telephones, ATM corner and public restrooms (Side *et al*., 2014). Besides this, in another study, it has been found that shopping cart handles and bases have numerous infectious agents as compared to mice of internet cafe, door handles of public restrooms and bus handles. The results showed that shopping cart handles had 1100CFU/1.55 square inches followed by internet cafe mice 690 CFU/1.55 square inches, bus handles 380 CFU/1.55 square inches, and restroom door handles 340 CFUs/1.55 square inches. Moreover, resistant bacteria like *Campylobacter, E. coli,* and *S. aureus* have been found on handles of shopping carts (Duh and Tai, 2015).

Different strategies have been proposed to disinfect such surfaces as an approach to control the resistant bacteria. For this purpose, knowledge is also collected to kill the bacterial pathogens but the mortality rate with respect to bacterial infections is still high. Hence, there is a need to use innovative strategy for selection of antibacterial agents to destroy the resistant bacteria and control microbial infections (Ravishankar and Jamuna, 2015).

Without cleaning and disinfecting a shopping cart handle on a regular basis, more bacteria could spread potentially causing illness. So, it is necessary to develop protectors that have an anti-bacterial property. This research study provides a solution in this regard by exploiting the advantages of nanotechnology to develop antibacterial sticking tape for shopping cart handles which may disinfect its surface from pathogenic microorganisms by using photo-catalytic activity of pure TiO_2 nanoparticles (TNP) and 1% silver doped titania naoparticles (Ag-TNP).

1.1 CONTAMINATION OF SHOPPING CART HANDLES

The human skin has direct contact with air borne microbes and also made colonies with some other pathogenic bacteria. The microorganisms present on human skin are complex and variable. They can be gram positive such as *Staphylococcus aureus* and gram-negative organism like *Pseudomonas aeruginosa*, which can reside on human skin for long period of time and can be considered as a source of bacterial infections. *Salmonella, Campylobacter and Escherichia coli* are pathogenic bacteria which cause several bacterial infections on regular basis (Bier *et al*., 2004).

Shopping cart handles can be contaminated due to direct handling of moisturized food or may be contaminated due to carts used by previous users. Besides this, it has been investigated through epidemiological studies that the chances of enteric bacterial infections are highest by placing children riding shopping carts. Therefore, there is a need to improve the sanitation of shopping cart handles for the reduction of exposure of pathogenic bacteria and spreading of infectious diseases among shoppers (Patrick *et al*., 2010).

1.2 TREATMENT METHODS IN PRACTICE

Bacteria, viruses and fungi are destroyed or inactivated using disinfectant wipes but they are neither reliable nor cost effective so have to replace with some cost effective techniques. Similarly, bacterial infection can be reduced through UV irradiation or by exposing to ozone, but these options have negative impacts on human health (Huang *et al.*, 1999). Another study was conducted in which a shopping cart with an anti-bacterial handle was proposed that employed ultraviolet sterilization to prevent bacterial infections (Duh and Tai, 2015). While, on the other hand UV radiations are hazardous for human beings, the procedure is inconvenient and may not be cost effective. Finding a low cost, and energy independent viable solution is therefore important.

For this purpose, a safe and sound technology has been proposed by Azam *et al*. (2012) involving titania nanoparticle coated surface for photocatalytic degradation of resistant bacteria**.** In this regard, photocatalytic coatings have been aparingly used as

self sanitizing surfaces to decrease the extent of exposure to contaminated sites due to inexpensive source, non toxicity, good stability and easy availability (Liou and Chang, 2012; Gao *et al*., 2015).

Different metallic oxides are being used as a photocatalytic substance such as TiO_2, ZnO, SiO_2, etc. Among all these TiO_2 is one of the most used semiconductor photocatalyst to destroy microbes because it has good activity, is cost effective and has a long lasting impact on bacteria in the presence of normal light (Xing *et al*., 2012; Othman *et al*., 2014). It is white in color, is itself nontoxic and can be easily used for sanitization purpose (Khan *et al*., 2013).

The photocatalytic behavior of TiO_2 has been studied using a number of microorganisms like fungi, bacteria, algae, and viruses. Ravishankar and Jamuna (2015) reported inactivation of *E. coli*, *S. aureus* and *Listeria monocytogenes* by application of TiO_2 nanoparticles. Similarly, titania nanoparticles TNPs were found to be effective against *E. coli*, fungi and bacteria (Hu *et al*., 2006; Chawengkijwanich and Hayata, 2008). Besides this, these nanostructures were also investigated against food surfaces and proved a good choice for reduction rate of bacteria and improved food safety (Chorianopoulos *et al*., 2011). Photocatalysis is, thus one of the most useful methods because it works at room temperature and normal pressure. Besides this, it is an environment friendly technique as there is no production of secondary pollutants and photocatalysts are activated under solar and visible light as well (Ochiai and Fujishima, 2012).

The photocatalytic behavior of TiO_2 improves considerably when the size of particle is reduced to nano range by increasing its band gap on illumination of light. Therefore, the self-sanitized surfaces commonly use the material in commercial products like ceramic tiles, fabrics, air filters and glass surfaces (Karimi *et al*., 2010). Hence, immobilization of TNPs has been done on various substrates such as activated carbon, polymeric material, clays, silica, glass, quartz, and stainless steel (Shan *et al*., 2010). For decontamination of shopping cart handles different strategies like manual disinfection using wipes and wrapping of disposable plastic barriers around the handles were supposed to be used (Gerba and Maxwell, 2012). But these techniques are expensive or inconvenient. Therefore, the present study focused on developing titania nanoparticle coated tape for decontamination of shopping cart handles.

1.3 THE PRESENT STUDY

In the present study, bacterial samples were collected from shopping cart handles and identified through 16S rRNA Gene sequencing technique. Pure TNPA and 1% Ag doped TNP were prepared and immobilized on double sided tape. Furthermore, the antibacterial efficiency of coated and uncoated tapes was evaluated against selected bacterial strains.

1.4 DOUBLE SIDED TAPE

It is a tape coated with an adhesive on both side of the tape. It is the pressure sensitive tape which sticks two surfaces together. Typical materials used to make the adhesive include:

- Acrylate polymer
- Rubber, either natural rubber or synthetic thermoplastic rubber
- Silicone rubber (Silva, 2011).

1.5 OBJECTIVES OF THE STUDY

The research had the following objectives:

- Develop Titania nanoparticle coated tape
- Identify major bacterial strains present on shopping cart handles
- Assess the effectiveness of said tape against bacterial species found on the shopping carts

Chapter 2

LITERATURE REVIEW

2.1 BACTERIAL CONTAMINATION OF SHOPPING CARTS

Shopping cart is a facility which is provided by super markets and widely used to transport the products inside the shopping stores as well as customers used them to transport their purchased goods to their cars. It has been investigated through literature that shopping cart handles are one of the most contaminated surfaces as compared to other public places like bus stations, airports and shopping malls that became a source of bacteria and increased the transmission of bacterial infections among (Ghamdi *et al*., 2011; Reynolds *et al*., 2005).

Salmonella, Campylobacter and Escherichia coli are pathogenic bacteria which cause several bacterial infections on regular basis (Bier *et al*., 2004) and the children that ride shopping carts are at increased risk of these pathogenic bacteria (Jones *et al*., 2006; Fulterton *et al*., 2007; Mizumachi *et al*., 2010). Therefore, children exposed to these pathogenic bacteria on regular basis to be the victim of several disease. Similarly, Mizumachi *et al*. (2010) also reported the presence of some other resistant microbes on shopping cart handles such as *Staphylococcus aureus* and proposed that it was a hidden reservoir of handles. Shopping cart handles can be contaminated due to direct handling of moisturized food or may be contaminated due to carts used by previous users whosoever don't bother to sanitize their hand.

The range of bacteria present on shopping cart handles is very wide. Gerba and Maxwell (2012) reported that 110 to 11,000,000 heterotrophic and 7259 coliform bacteria were present on shopping cart handle. Out of total carts sampled 72% carts had coliforms on handles. Among those coliform, *E. coli* was detected in the form of colonies. Besides this, it was found on 18 carts out of 35 carts which proved the presence of coliforms on handles. On contrary it has been reported by Reynolds *et al*. (2005) that only 7% coliforms were present on table used for diaper changing, ATM corner, bus stations and restaurant tabletops and containers. Two solutions were proposed to decrease the exposure of pathogenic bacteria includes; use of disinfectant wipes or disposable barriers on shopping cart handles. In some regions like Arkansas, legislation was passed in grocery stores for provision of high level sanitation (Gerba and Maxwell, 2012).

Coliforms are group of bacteria which has been caused a number of diseases in health persons such as gastroenteritis, pneumonia and infections of urinary (Madigan *et al*., 2012). Dust, moisture, and food leftover are the sources of transmission of microbial populations (Hara and Zhang, 2012). Therefore, if we clean our surfaces on regular basis, the chances of transmission of bacteria will be reduced as compared to dirty places.

The presence of both gram positive as well as gram negative bacteria is an indication of poor hygienic conditions of SCs. So, if these surfaces are not cleaned on daily basis they may become an ultimate source of spreading of bacterial infections among health people as well. According to World Health Organization report, the

people who are affected by poor sanitation or hygienic conditions graded higher cause of hospitalization and ill health (World Health Organization, 1999).

P. aeruginosa is one of the bacterial isolate of shopping cart handles that is an opportunistic pathogen. This bacteria is directly linked to hospital based infections. They may be due to secretion of toxins or biofilms formation (Shanthi and Sekar, 2009; Ruxana *et al*., 2005; Driscoll *et al*., 2007). Hence, *P. aeruginosa* can reach at shopping cart through direct contact of an infected individual or may come from air or soil due to some construction activities.

B. pumilus is a gram positive bacteria that may cause several bacterial infections in healthy human as well. It has been reported that cutaneous infections are caused due to this microbe (Tena *et al*., 2007). Their source may be infected customers or employees with other pathogenic bacteria or poor sanitization inside the mall.

Gerba and Maxwell (2012) studied the bacterial contamination of shopping cart handles and approaches to control and found a number of bacterial species on shopping cart handles. Similarly, Irshaid et al. (2014) studied bacterial contamination of shopping cart handles and bases and found that bases are more contaminated surfaces as compared to handles.

2.2 SHOPPING CART HANDLES AND THEIR DISINFECTION

Studies show that large amounts of bacteria like *Campylobacter, Salmonella*, and *E. coli* can often be found on the handles of shopping carts and baskets. So it is a

good idea to use clinical wipes to clean the handles before using the carts. Dr. Charles Gerba of the University of Arizona found the amounts of bacteria on these surfaces were higher than those found in public restrooms. However, that is likely due to restrooms being regularly cleaned, which is usually not the case with shopping carts and baskets. In addition to bacteria, there may also be concerns when people with colds or the flu touch shopping carts or baskets. The Centers for Disease Control and Prevention noted that the cold and flu viruses could generally survive on hard surfaces from two to eight hours. Practicing good health habits by cleaning the handles and even the seats on shopping carts with disinfecting wipes reduced the chances of infections. Besides this, regular hand washing with a detergent may also reduce the transmission of bacteria. Moreover, if you touch potentially contaminated surfaces, avoid touching your face until after thoroughly washing your hands. And if this is not possible, use disinfecting wipes or hand sanitizer.

Bacteria, viruses and fungi are destroyed or inactivated using disinfectant wipes but they are neither reliable nor cost effective so have to replace with some cost effective techniques. Similarly, bacterial infection can be reduced through UV irradiation or by exposing to ozone, but these options have negative impacts on human health (Huang *et al*., 1999). Moreover, another study was conducted in which a shopping cart with an anti-bacterial handle was proposed that employed ultraviolet sterilization to prevent bacterial infections. The system comprised the cart body, an anti-bacterial unit, and a charger unit. The anti-bacterial unit on the handle could help prevent shopping carts from spreading bacteria. The charger unit underneath the cart

served as an additional power source (Duh and Tai, 2015). But on the other hand UV radiations are hazardous for human beings when exposed to it. Therefore, nanotechnology can be considered as more effective technique as compared to other disinfection methods.

2.3 NANOTECHNOLOGY

The U.S Environmental Protection Agency defines nanotechnology as; "Research and technology development at the atomic, molecular, or macromolecular levels, in the length scale of approximately 1-100 nanometer (nm) range in any direction"

Nanotechnology, thus refers to working with atoms or molecules at nanoscale. Nanoscale ranges from 1 – 100 nm. Nanoscale materials have different physical, chemical and biological properties as compared with their larger structures (e.g., titania, carbon, iron, etc.). Nanomaterials are more reactive because they have greater surface area to mass ratio as compared to the bulk material. Therefore, they have high photocatalytic activity due to more surface area (Davies, 2009).

2.4 WHAT IS PHOTOCATALYSIS?

The word photocatalysis is a composite word which is composed of two parts, "photo" and "catalysis". Basically, it is a chemical reaction that occurs in the presence of light as light activates the catalyst and increase the reaction rate. Therefore, the

substance which increases the rate of chemical reaction is known as photo catalyst (Othani, 2011).

Photo catalysts are usually solid and not consumed or used during a reaction. They only tend to enhance the reaction kinetics. Photocatalysts are semiconductor in nature for example CeO_2, Fe_2O_3, TiO_2, WO_3, ZrO_2, and ZnO etc. (Benabbou *et al*., 2007; Kalidindi and Subasri, 2015).

2.4.1. The Ideal Photocatalyst

Photocatalyst is usually solid and semiconductor in nature, not consumed during a reaction. Some of the semiconductors which have been investigated for use during heterogeneous photacatlytic reactions are CdS, CdSe, CdTe, ZnS, ZnSe and some metal oxides like TiO_2, Fe_2O_3, ZrO_2, ZnO, SnO_2 and WO_3 (Benabbou *et al*., 2007). An ideal photocatalyst should be inert chemically as well as biologically, and also stable under light. It should exhibit a narrow band gap thus being photoactive under visible light. Most importantly, it should be non-toxic to both man and environment (Bhatkhande *et al*., 2002).

2.4.2. Titania as a Photocatalyst

Titanium is the ninth most plentiful element on the earth's crust and Titania is its most stable oxide with high photocatalytic activity and no toxicity. Due to its abundance on earth's crust, it is not very costly. These properties are responsible for

the extensive use of Titania as photocatalyst in the world of nanotechnology (Herrmann, 2005; Benabbou *et al*., 2007).

2.4.3. Polymorphs of Titania

Titanium dioxide commonly known as Titania is basically present in three polymorphic forms; Anatase, Rutile and Brookite. Anatse has the highest photocatalytic activity among all the polymorphs of titania (Carp *et al*., 2004; Malato Rodrıguez *et al*., 2004). Some studies have reported better results for the photocatalytic reactions by using a mixture of anatase and rutile polymorphs (Giolli *et al*., 2007; Sun *et al*., 2011).

2.4.4. Technical Limitations of TiO_2 and Their Solution

TiO_2 is an effective photocatalyst but its band gap energy of 3.2 eV, equivalent to 388 nm wavelength restricts its use in only UV region (Linsebigler *et al*., 1995). This issue has been addressed with the help of doping of Titania (Gaya and Abdullah, 2008). Various metals (Iron, Silver and Copper etc) and non-metals (Carbon, Nitrogen and Boron etc) have been used as dopants to reduce the band gap energy of Titania (Arana *et al*., 2004; Hamal and Klabunde, 2007). Doping must be controlled carefully as high dopant concentrations may result in electron and hole recombination (Carp *et al*., 2004; Coleman *et al*., 2005).

The major problem with TiO_2 as a functional self-sterilising coating indoors is that the band onset of the semiconductor is ~3.2 eV. It therefore requires UV light for

activation, which is present in sunlight abundantly but not inside the buildings. Therefore, there is a need to modify titania by doping of different noble metals (Ag, Au, Fe, etc) (Dunnill and Parkin, 2012). Moreover, Extensive effort has been made to develop the antibacterial TiO_2 coatings of different metals included Ag^{+}, Zn^{2+}, Co^{2+}, $Al^{3+}$and Hg^{2+} in TiO_2 coatings. Among all these silver doped titania showed best antibacterial activity when titania coating was incorporated with silver nanoparticles (Zhao *et al*., 2011). Similarly, silver doped titania nanoparticles surfaces showed 95% reduction in *E. coli* and *S. aureus* growth as compared to uncoated surfaces (Gao *et al*., 2015). Venieri *et al*. (2014) also investigated the inactivation of *E. coli* and K. *pneumonia* by immobilization of cobalt and manganese doped titania nanoparticles. Pham and Lee (2015) investigated the disinfection efficiencies of TiO_2 and Cu-doped TiO_2 photocatalysts, supported on glass fibers (GFs) under UV and visible irradiation, against *Staphylococcus aureus (S. aureus)* contained in an indoor aerosol.

2.5 PHOTOCATALYTIC ACTIVITY OF TiO_2

Fujishima and Honda in 1972 invented Photocatalytic activity of TiO_2 (Fujishima *et al*., 1972). They discovered that from valence band electron are accelerated towars conduction band in the presence of light. This movement of electrons creates an electron-hole pair (e- - h+).

$TiO_2 + hv \rightarrow e^- + h^+$ (Tan *et al*., 2011)

This electron-hole (e- - h+) pair can recombine or interact with other molecules (Behnajady *et al*., 2008). Holes created in the valance band take part in oxidation

reactions while electrons in the conduction band take part in reduction reactions of interacting molecules (Stamate *et al*., 2007).

Oxidation reaction:

$h+ + H_2O \rightarrow \cdot OH + H+$

Reduction reaction:

$e- + O_2 \rightarrow O_2 \cdot -$ (Tan *et al*., 2011)

Holes created in the valence band may interact with water molecules and hydroxyl ions (OH^-) and other Reactive Oxygen Species (ROS) can be generated. These hydroxyl (OH^-) or Reactive Oxygen Species (ROS) interact with any organic molecule present at or near the surface of TiO_2 and oxidize it leaving behind CO_2 and H_2O as an end product (Osburn, 2008).

$\cdot OH + Pollutant + O_2 \longrightarrow CO_2 + H_2O$ (Tan *et al*., 2011)

2.6 PHOTOCATALYTIC ACTIVITY OF Ag-TiO_2

Chances exist for the recombination of the electron-hole (e- - h+) pair which may reduce the photocatalytic activity of TiO_2. Metal (Ag, Pt, Fe, etc.) doping of semiconductor (TiO_2) reduces the recombination of these electron-hole (e- - h+) pairs. Studies have shown that doping of silver metal with TiO_2 has reduced recombination of electron-hole (e- - h+) pair significantly and Ag doping of TiO_2 nanoparticles increases photocatalytic ability (Behnajady *et al.*, 2008). Photocatalytic activity only

triggers when incident light has energy greater than band gap of the semiconductor (Fujishima *et al.*, 2000; Qureshi, 2012). TiO_2 has band gap of 3.2 electron volts (E = 3.2eV) which requires incident light having wavelength of lower than 388 nm for photocatalytic activity (Stamate *et al.*, 2007; Blake *et al.*, 1999). In ordinary circumstances, photocatalytic activity of TiO_2 begins when UV radiation falls on it while sunlight has only 5 % UV radiation (Osburn, 2008). So, band gap of TiO_2 has to be reduced to start photocatalytic activity even in ordinary sunlight. For this purpose, doping of TiO_2 is done with some suitable metal which ultimately narrow downs the band gap.

2.7 APPLICATIONS OF TIO_2 NANOPARTICLES

TiO_2 nanoparticles have emerged as an excellent photocatalyst semiconductor which is being used in many fields of life. Especially, it has wide applications in the field of environment (Fujishima *et al.*, 2000).

Some practical applications of TiO_2 photocatalysis are mentioned below (Stamate *et al.*, 2007):

- Anti bacterial property;
- Self sanitizing property;
- Air cleaning property;
- Water treatment

TiO_2 is an ideal photocatalyst which has wide applications; water purification, degradation of volatile organic compounds (VOCs), because of strong oxidation ability, low cost and non toxicity (Zhan *et al*., 2014). The versatile use of titanium dioxide for multiple purposes made it the most popular photocatalyst to resolve environmental problems (Wang *et al*., 2014).

Studies have shown that the use of titania nanoparticles for air quality control can be highly effective. Photo-catalytic oxidation of volatile organic compounds (VOCs) was achieved by TNPs by coating on large surfaces of advertisement boards (Tejasvi *et al*., 2015). Aghighi & Haghighat, (2015) evaluated the efficiency of titania nanoparticles in photocatalytic oxidation air cleaning devices using VOCs. The synthesized TNPs exhibited high removal efficiency of VOCs.

Besides this, due to photocatalytic effect titania nanoparticles coating on steel surfaces prevent corrosion. A study was investigated that the deposition of TiO_2 NPs thin films on stainless steel has improved the anticorrosive properties of stainless steels (Ćurković *et al*., 2013; Barati *et al*., 2014). Liu *et al*., (2012) also highlighted the prospects of titania nanoparticles for CO_2 conversion.

Moreover, the conventional water disinfection method involved chemical treatment such as hydrogen peroxide treatment, and chlorination; but, it has disadvantage due to its toxicity and other environmental problems. Hence the development of non-toxic, environment friendly and cost effective method for efficient

water disinfection has been proposed by using titania nanoparticles alone and modified (Sethi *et al*., 2014).

2.8 PHOTOCATALYTIC STERILIZATION

Photocatalytic property of TiO_2 makes it an ideal agent to be used in manufacturing of self-sterilizing surfaces. Oxidative species produced during photocatalysis of TiO_2 interact with cell membrane of microorganisms. Cell membrane oxidizes and gets damaged which ultimately results in death of microorganisms. A number of studies have been conducted in this regard. First bactericidal study of this nature was studied with *Escherichia coli* and was observed that all *Escherichia coli* bacterial species were disinfected after a period of one hour (Fujishima *et al*., 2000). Another study reveals that 96 and 100 % *Escherichia coli* species died after 30 min and 60 min exposure to TiO_2 respectively (Haung *et al*., 1999). *Streptococcus mutans* is a pathogenic microorganism which causes infection during dental surgery. It‟s 15 min exposure to TiO_2 results in death (Kim *et al*., 2007). TiO_2 also shows high photocatalytic efficiency against *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* bacterial species (Ibanez *et al*., 2003).

Ag-TiO_2 has better photocatalytic activity as observed during a study comparing the efficiency of pure and Ag doped titania coated surfaces by killing *Pseudomonas aeruginosa* and *Bacillus subtilis* (Khan *et al*., 2013). Fe nanotube coated surfaces also proved effective regarding disinfection of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Latif, 2013).

Semiconductor photocatalysis has emerged as a promising technique for microbial inactivation in various aqueous matrices, including diverse types of bacteria, fungi, viruses, and spores (Vereba *et al*., 2013). TiO_2 is nontoxic and the American Food and Drug Administration (FDA) has approved TiO_2 for use in human food, drugs, cosmetics, and food contact materials. Besides this, it is a photocatalyst which has been widely utilized as a self-cleaning and self-disinfecting material for surface coatings in many applications. The photocatalytic reaction of TiO_2 has been used to inactivate wide spectrum of microorganisms. A study was conducted to demonstrate the antimicrobial effect of TiO_2 photocatalyst on *Escherichia coli* in water and its photocatalytic activity against fungi and bacteria (Chawengkijwanich and Hayata, 2008). The antimicrobial agent inhibits spoilage and reduces pathogenic microorganisms. Similarly, the bactericidal and fungicidal effects of TiO_2 on *Escherichia coli* (*E. coli), S. aureus, Listeria monocytogenes* have been widely reported (Hu *et al*., 2006).

Microorganisms were killed by TiO_2 upon illumination due to its photocatalytic properties. Hydroxyl radicals and reactive oxygen species generated on the illuminated TiO_2 surface play a key role in inactivating microorganism by oxidizing the polyunsaturated phospholipid components of the cell membrane of the microbes. The use of nanometer sized TiO_2 particles has the potential to further enhance the antimicrobial activity of TiO_2 (Xing *et al*., 2012; Othman *et al*., 2014).

Moreover, the use of TiO_2 photocatalyst as alternative means of self-disinfecting contaminated surfaces by further development provided potent

disinfecting solutions for prevention of bio film formation. Similarly, they may be used as effective bio film disinfectant in food processing industries. Suspensions containing TiO_2 are effective at killing *E. coli*. This has led to the development of photocatalytic methods for the killing of bacteria and viruses using TiO_2 in aqueous media. TiO_2 nanoparticls are effective in *killing E. coli, S. aureus, Listeria monocytogenes* (Ravishankar and Jamuna, 2015).

Chorianopoulos *et al*. (2011) reported that that nanostructured TiO_2 on UV irradiation can be used as an effective way to reduce the disinfection time, eliminating pathogenic microorganisms in food contact surfaces and enhance food safety.

Photocatalytic disinfection of bacteria is currently gaining importance because of its effectiveness against highly virulent and antibiotic resistant strains of bacteria, especially in hospital environments, that demands newer disinfection methods. The capacity of titanium dioxide (TiO_2), to degrade organic contaminants in the air and water has been studied for more than 20 years (McCullagh *et al*., 2007). TiO_2 occurs naturally as rutile, brookite and anatase is the most efficient photocatalyst (Blake *et al*., 1999). However, most of the work utilized aqueous suspensions of TiO_2 to investigate its antibacterial properties (Evans and Sheel 2007; Foster *et al*. 2011; Gamage and Zhang 2010).

The biocidal action of the TiO_2 photocatalyst is frequently ascribed to OH• radicals and other reactive oxygen species (ROS) (Cho *et al*., 2004). In particular, some studies have demonstrated that the cell membrane is the primary site of reactive

photogenerated oxygen species attack, leading to lipid peroxidation (Kiwi and Nadtochenko 2005; Maness *et al*. 1999; Nadtochenko *et al*. 2006). The combination of cell membrane damage and further oxidative attack of intracellular components ultimately results in cell death (Rincon *et al*. 2004).

Other studies have suggested that the mode of action is the photooxidation of coenzyme A, leading to the inhibition of cell respiration and thus to cell death (Vohra *et al*., 2005). TiO_2 has been envisaged as one of the most effective disinfection technologies as no carcinogenic, mutagenic or malodorous compounds are formed during the process (Ibanez *et al.*, 2003). The antimicrobial effect of TiO_2 photocatalytic reaction was reported for the first time by Matsunaga *et al.* (1985). These authors investigated the effectiveness of the photocatalytic oxidation in water with several microorganisms, including *Lactobacillus acidophilus* (Gram-positive bacteria), *Saccharomyces cerevisiae* (yeast), *Escherichia coli* (Gram-negative bacteria) and *Chlorella vulgaris* (green algae). Since then, studies on photocatalytic killing have been intensively conducted on a wide spectrum of microorganisms, including viruses, fungi and many species of bacteria.

The use of the TiO_2 photocatalyst as an antimicrobial component of construction material has also been proposed. Kuhn *et al.* (2003) proposed for the first time to disinfect surfaces using a light-guiding material coated with a semiconductor (such as TiO_2). Vohra *et al*. (2005), have developed an advanced photocatalyst, which is a silver ion doped TiO_2 catalyst, and have obtained complete inactivation of various microbes.

Importance of disinfection of surfaces, particularly in the healthcare units and microbiology laboratories is, however, essential compared to the conventional methods of (Gamage and Zhang, 2010). Photocatalytic oxidation on such surfaces coated with titanium nanoparticles, provides an alternative solution to the problem as pathogenic bacteria like *E. coli*, *Staphylococcus aureus*, and *Enterococcus faecium* can be easily killed by titania coated surfaces (Kuhn *et al*., 2003). TiO_2 coated on glass and tiles, was found effective for killing of bacteria and self-cleaning (Pham and Lee, 2015). Similarly, 1% Ag-TiO_2 nanoparticles, water, and ethanol-based coating on substrates like glass and plastic venetian blinds kill 93 to 100% bacteria (Khan *et al*., 2013).

Since TiO_2 has been approved by US Food and Drug Administration for use in human food, food packing materials, medicines, and cosmetics (Chawengkijwanich and Hayata, 2008) and because it is nontoxic in nature (Liou and Chang, 2012), it may also be used as a photocatalyst in food industries and pharmaceuticals to reduce airborne bacteria, where the use of cleaning chemicals or biocides is prohibited or are ineffective (Sethi *et al*., 2014).

In the present study pure and 1%, silver-doped Titania nanoparticles were prepared by liquid impregnation method and TNPs suspensions were coated on the cellophane tape. The resulting coated tape was then wrapped around the handles of shopping carts and antibacterial efficiency of prepared tapes was assessed against some selected bacterial strains such as *E. coli*, *Staphylococcus scuiri* and *Agrobacterium tumefaciens* under UV and visible light

Chapter 3

MATERIALS AND METHODS

3.1 MATERIALS

Titania nanoparticles (TNPs) and silver doped titania nanoparticles (Ag-TNPs) were prepared by Liquid Impregnation Method. For this purpose, silver nitrate (Merck) and titanium (IV) dioxide (Sigma-Aldrich Laborchemikalien) were used as a source of silver and titanium, respectively. In Liquid Impregnation Method, titania powder was suspended in distilled water. Double sided tape was purchased from the local market for immobilization of titania nanoparticle suspension. Pure titania and silver doped titania nanoparticle were suspended in Methyl Ethyl Ketone (MEK). Solidified agar plates were prepared by using nutrient agar. Bacterial strains were isolated from shopping cart handles by continuous streaking. *E. coli* (ACC 25922) a gram negative, facultative, anaerobic, rod shaped bacterium of the genus *Escherichia* was used as a model culture to assess the effectiveness of coated and uncoated tape. Merck grade glassware was used throughout the experimental procedure.

3.2 SYNTHESIS OF PURE TITANIA AND Ag DOPED TITANIA NAOPARTICLE

3.2.1 Pure Titania Nanoparticle (TNP)

Titania nanoparticles were prepared by liquid impregnation method. For this purpose, 50g TiO_2 powder (GPR, BDH Chemicals Ltd. Poole England) was suspended

in distilled water and placed on magnetic stirrer for 24 hours. The suspension was allowed to settle overnight. After removing the supernatant, the resulting sample was placed in oven for 24 hours at 105 ºC to obtain a dried material. The dried material was crushed and annealed by placing it in muffle furnace for 6 hours at 400 ºC to obtain nanoparticles in anatase phase.

3.2.2 Ag Doped Titania Nanoparticle (Ag-TNP)

Slurry of 1 % Ag - TiO_2 nanoparticle was prepared in water by mixing 48.95 g of TiO_2 GPR and 1.05 g of $AgNO_3$ in a beaker and continuous stirring for 24 hours so that proper mixing of TiO_2 GPR and $AgNO_3$ could occur. The solution was allowed to settle for another 24 hours. After removing the supernatant, the solid material was placed in oven for 24 hours at 105ºC for water evaporation. The dried material was crushed properly in mortar and pastel and placed in china dish. The crushed material was annealed in muffle furnace at 400 ºC for 6 hours.

3.3 CHARACTERIZATION OF NANOPARTICLE

X-Ray Diffraction (XRD) analysis of the pure and doped TiO_2 nanoparticles was done to find out the crystalline structure and particle size of the pure and doped TiO_2 nanoparticles. Scanning Electron Microscopy (SEM) analysis revealed the morphology and provided images in this regard. Energy Dispersive Spectroscopy (EDS) analysis provided elemental composition of nanoparticles. It also provided percentages of composition for each element.

3.3.1 Morphology of TNPs (SEM)

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

In present study, morphology of samples was observed by using JEOL JSM 6460 SEM at different resolutions i.e. X5000 and X20000. SEM examined the prepared samples of pure and silver doped TNPs at an acceleration voltage of 20 kV.

3.3.2. Elemental Analysis of TNPs (EDS)

In order to determine the chemical composition (in percentages) of prepared pure and doped TNPs, Energy Dispersive Spectroscopy (EDS) coupled with SEM was used. Electron beam generated for the purpose of SEM, is also used for EDS. When electron beam strikes the sample, different elements present in the sample produced characteristic X-rays having different energies. Composition of different elements is found out by collecting and analyzing these characteristics X-rays. In the present study, Elemental Analysis of prepared TNPs was done by using EDS Oxford INCA X-sight 200.

3.3.3. Structure Analysis of TNPs (XRD)

X-Ray Diffraction (XRD) is a renowned and simple technique to determine 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 the same angle will reinforce each other giving a high peaks indicating the crystallinity of the sample. In the present study, the crystalline phase and size of prepared pure TNPs and Ag doped NPs was determined by using JEOL JDXII X-ray diffractometer

3.4 ISOLATION OF BACTERIAL SPECIES PRESENT ON SHOPPING CART HANDLE

Through literature it has been investigated that shopping cart handles harbor a number of bacterial species who are pathogenic and resistant as well. Gerba and Maxwell (2012) reported the presence of different species like *Escherichia coli*, *Klebsiella pneumonia,* and *Enterobacter sakazkii*. Similarly Irshaid *et al*. (2014) also reported bacterial species found on shopping cart handles like *Pseudomonas aeruginosa*, *Shigella sonnei*, *Bacillus cereus*, and *Bacillus pumilus*.

3.4.1 Preparation of Nutrient Agar Plates

14 g of Nutrient Agar (Merck) mixed in 1 liter distilled water with gradual mixing by glass rod. When molten nutrient Agar was completely dissolved, flask containing agar solution was transferred to autoclave for sterilization at 121 ºC for 15 mins. After sterilization, flask was placed in hot water bath at 47 ºC to avoid Agar solution from solidification. Finally, molten nutrient agar solution poured into petri plates (autoclaved at 121ºC for 15 mins) under Laminar Flow Hood cabinet and

allowed to cool down. Prepared petriplates, after solidification were transferred into incubator (for 24 hrs at 37 ºC) to check their sterility.

3.4.2 Sampling Area

A local cash & carry grocery store was selected for sample collection from shopping carts (SCs) (Figure 3.1). Random samples were collected from handles of six different SCs. Pre-sterilized cotton swabs were used to collect samples from shopping cart handles. One dry swab sample was collected from handle of each SC. The collected samples were immediately transported to the Environmental Microbiology Teaching Lab at Institute of Environmental Sciences and Engineering (IESE) for further processing.

Figure 3.1: Collection of bacterial sample from already used shopping cart handles

3.4.3 Bacterial Growth and Counting

3.4.3.1 Spread Plate Method

In this technique, approximately 0.1ml of diluted sample was spread on nutrient agar plate with the help of sterilized glass spreader as presented in Figure 3.2. Then plates were incubated at 37 ºC for 24 hours; growth of microbes was observed quantitatively using the colony counter. Disinfection capacity of various nanoparticles was hence measured by growth present on each plate and comparing these with the control surfaces.

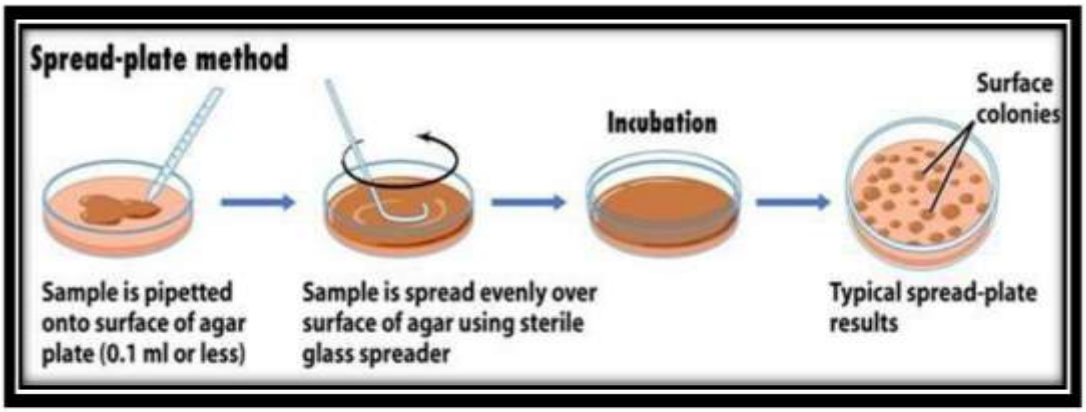


Figure 3.2: Spread plate technique for bacterial culture

3.4.4 Isolation of Pure Colonies

The morphology of isolated strains was studied. On the basis of their color, size, margins, and elevation, different colonies were identified, picked and streaked on separate agar plates. The pure cultures were isolated by continuous streaking of 2

weeks. The isolated strains were subjected to further characterization and preserved for gene sequencing analysis.

3.5 IDENTIFICATION OF THE ISOLATED BACTERIA

Identification of bacteria includes phenotypic characterization and genotypic characterization.

3.5.1 Phenotypic Characterization

It includes biochemical characterization, morphological characterization and gram staining.

3.5.1.1 Biochemical Characterization

Biochemical characterization was done using different biochemical tests. The details are presented below

Oxidase Test

Oxidase test determines the presence of an intracellular oxidase enzyme by bacteria. Some bacteria may produce more than one type of oxidase enzyme. (1%) of N, N, N', N'- tetramethyl-p-phenylenediamine reagent is used to detect the presence of oxidase enzymes produced by a variety of bacteria. Oxidase test was performed by allowing oxidase reagent to reach room temperature before its use. Few drops of oxidase reagent were placed on filter paper then a small piece of filter paper. The bacterial colony to be tested was touched with the help of loop and it was smeared onto

filter paper. Appearance of purple color on the filter paper within 30 sec showed positive result.

Catalase Test

For catalase test, 24 hrs fresh cultures were prepared. Few colonies of each culture were placed on glass slide and few drops of 3% hydrogen peroxide were poured on fresh cultures and observed for effervescence. Appearance of bubbles on the slide indicates the positive catalse test.

MacConkey Agar Test

MacConkey Agar is a media which is specifically used for gram negative bacteria as it stops the growth of gram positive bacteria. For this purpose, MacConkey Agar was prepared using specific protocol. Bacterial cultures were streaked and the plates were incubated for 24 hrs at 37 °C. Bacterial cultures which turned pink after the period of 24 h were Lactose fermenters. It is the bacteria which eats lactose and turns pink and not the media.

EMB Agar Test

Eosin Methylene Blue (EMB) Agar is used for the isolation and differentiation of Gram-negative enteric bacilli. For this purpose, EMB agar was prepared according to a specific protocol. Bacterial cultures were streaked and the plates were incubated for 24 hrs at 37 °C. Bacterial cultures which turned blue after the period of 24 h were Lactose fermenting bacteria.

Motility Test

Microscopy is the most accurate way to determine motility of fresh culture of bacteria. For this purpose, fresh culture of bacteria was placed on a glass slide and a smear was made with the help of a drop of autoclaved distilled water. It was covered with a cover slip. It was then observed under the microscope by using a drop of oil immersion. Bacteria could be seen moving under the microscope.

Simmon Citrate Agar Test

Some microorganisms use citrate as a carbon source by producing an enzyme citratase. Hence, Simmon citrate agar test is used for this purpose. Fresh culture of bacterial strains was streaked on already prepared Simmon citrate agar plates and incubated for 24-48 hours. The appearance of blue color indicates the positive test.

Urea Broth Test

Urease test is usually performed to confirm the gram negative bacterial strains. For this purpose, fresh culture of bacteria was inoculated in urea broth and incubated for 24-4 hours. The appearance of intense pink color indicates the positive urease test.

3.5.1.2 Morphological Identification

Colony morphology of the isolated strains was observed to identify and characterize them. All physiological and morphological identification were performed as per Bergey's Manual 23 of Determinative Bacteriology (Holt *et al*., 1994).

Following morphological characteristics were usually observed as reported by (Pelczar, 1957).

Table 3.1: Morphological Characteristics of Bacteria

Morphological Characteristics	Description
Size	Small, large, punctiform
Margin	Entire, curled, lobate, undulate, filiform
Texture	Creamy, dry, mucoid
Color	Yellow, orange, off white, pale yellow
Form	Rhizoid, circular, filamentous, irregular

3.5.1.3 Gram Staining

Bacterial colony was picked from and equally spread on the clean glass slide containing a small drop of water. Slide was air dried and heat fixed. 24 Then, crystal violet was applied on the smear for one min and washed with distilled water. Then, iodine solution was applied for a min and washed with distilled water. Then decolorizing agent was spread on the slide for 30 sec, then the glass slide was air dried and finally safranin was applied for one min as may be seen in Figure 3.3. Gram positive cells retained purple of crystal violet and seem purple while gram negative cells seem pink when observed under oil immersion at 100x resolution using microscope.

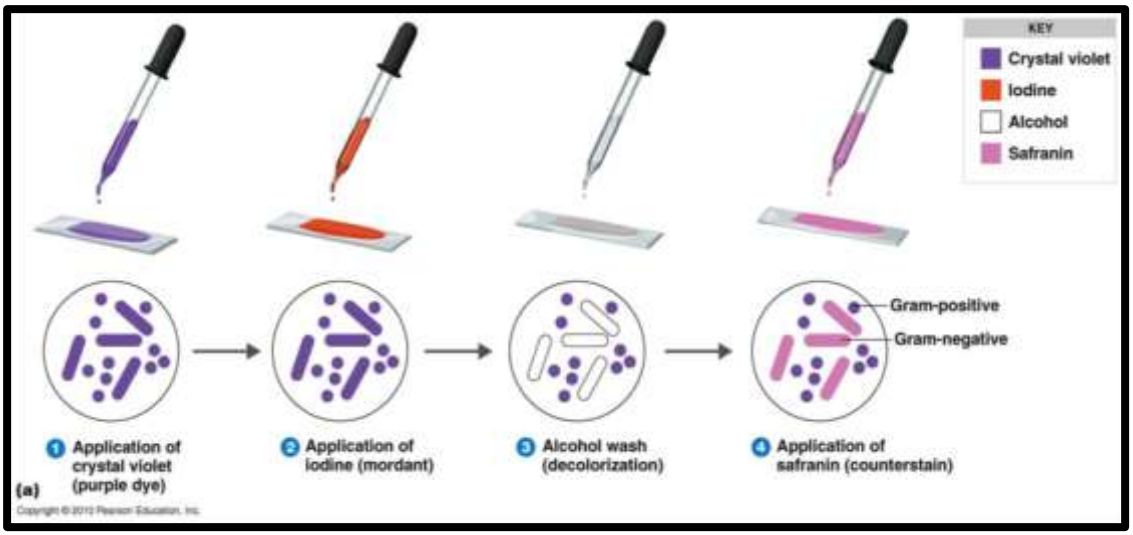


Figure 3.3: Gram staining technique to verify the purity of culture and differentiation of gram positive and gram negative bacterial strains

3.5.2 Genotypic Characterization of Pure Isolates by 16S rRNA Sequencing

For genotypic characterization, 16S rRNA sequencing technique was used. For this purpose, 24 hrs fresh cultures of isolated strains were prepared. Bacterial isolates were wiped gently with distilled water with help of glass rod and the inoculum was added to eppendorf tubes. Tubes were centrifuged for 10 mins to separate supernatant from bacterial culture. Supernatant was removed. For sample preservation 500µL of 50% glycerol and 200µL of nutrient broth were added to eppendrof tubes and preserved at -20ºC. The 16S rRNA sequencing was carried out as reported by Jacob and Irshaid (2012). Therefore, for 16S rRNA sequencing, the preserved isolates were sent to Genome Analysis Department Macrogen Inc. Korea (Figure 3.4). The resulted sequences were compared with Gen Bank database nucleotide sequence. This facility was availed by National Centre for Biotechnology Information's (NCBI) web BLAST

service. Furthermore, phylogenetic tree was formulated using a software of multiple sequence alignment program i.e. MEGA 4.0 (Saxena *et al*., 2014).

3.5.3 Phylogenetic Tree

A phylogenetic tree is an evolutionary tree having a branching diagram or "tree" which shows the evolutionary relationships among various species or other entities of biological origin, their phylogeny based upon similarities and differences in their physical or genetic characteristics. The taxa joined together in the tree are implied to have descended from a common ancestor. Phytogenic tree may be rooted having same ancestor or unrooted having unknown ancestors. In a phylogenetic tree, each node with descendants represents the inferred most recent common ancestor of the descendants and un rooted trees illustrate only the relatedness of the leaf nodes and do not require the ancestral root to be known or inferred (Morozova and Marra, 2008). Sequences obtained were analyzed using BLAST search at National Center for Biotechnology Information (NCBI) databases. The sequences were aligned using CLUSTALW after complete deletion of the mismatch sequences. A phylogenetic tree constructed by using the TREEVIEW program illustrates the phylogenetic relatedness of identified strains to the selected strains obtained from GenBank (NCBI). Furthermore, the 16S rDNA sequences of all bacterial isolates were aligned with reference sequences showing sequence homology from the NCBI database using the multiple sequence alignment program of MEGA 4.0 (Saxena *et al*., 2014).

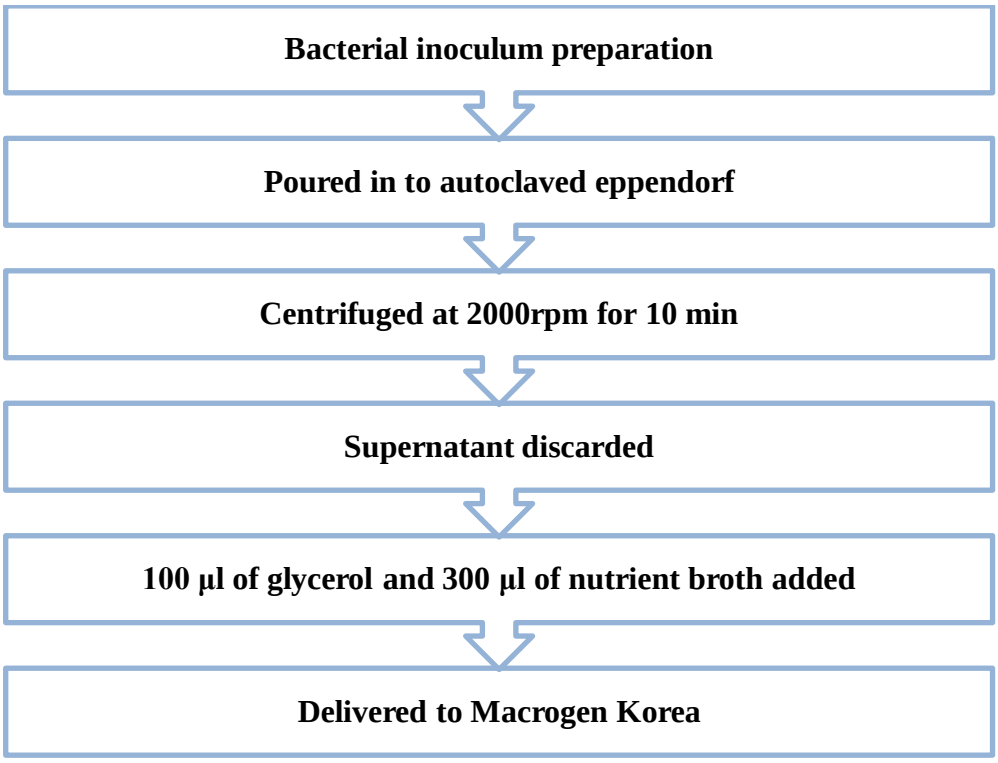


Figure 3.4: Sample Preparation for 16S rRNA Gene Sequencing

3.6 ANTIBACTERIAL EFFICIENCY OF COATED AND UNCOATED TAPES

Coated and uncoated tapes were exposed to different bacterial strains to assess their efficiency with respect to microorganism degradation.

3.6.1 Preparation of Bacterial Culture

1 mL of pure bacterial culture (*E. coli*) was inoculated in 100 ml of nutrient broth. The flask was placed on shaker incubator for 24 hours at 37 ºC to get dense culture. Atomic absorption spectrophotometer was used to determine the optical density of fresh bacterial culture at 600nm. For antibacterial efficiency testing,

optimized density range of 0.8-1.0 was selected. Besides this, colony forming unit (CFU/ml) for bacterial culture was also calculated using spread plate technique and a range of 25-250 was considered as countable colonies. Furthermore, 9.75×10^6 CFU/mL was used to assess the antibacterial efficiency of prepared tapes.

3.6.2 Immobilization of Nanoparticle

3.6.2.1 Preparation of Titania Nanoparticle Coated Tapes

Commercial TiO_2 powder was obtained from local market (average particle size: 43.1 nm, purity: ≥ 99.5% trace metals basis, crystalline phase: 94% anatase + 06% rutile). An amount of TiO_2 nanoparticles (1, 2, 3, 4, and 5g) was mixed with 100mL organic solvent, particularly methyl ethyl ketone (MEK), to produce TiO_2 nanoparticle concentrations of 1, 2, 3, 4 and 5%. Similarly, Ag doped nanoparticle were also mixed with methyl ethyl ketone (MEK) to produce Ag doped TiO_2 suspension. Furthermore, ultrasonic irradiation of suspension was done using temperature controlled sonicator for 30 minutes. The suspension was then manually coated onto one side of low density packing tape (dimensions: 16.5 cm × 17.8 cm, thickness: 0.01mm) using a K bar coater (RK Print Instruments, UK) at room temperature and dried in air for 10 minutes (Othman *et al.*, 2014).

3.6.3 Photocatalytic Study of Prepared Tapes

3.6.3.1 DBR Dye Degradation

DBR dye was used as a model compound to assess the effectiveness of coated and uncoated tapes. Pure titania and silver doped titania nanoparticle suspensions were immobilized on tapes and air dried at room temperature. Furthermore, 30 mg/L dye solution was prepared. Pre-washed petriplates were cleaned to carry out the proposed experiment. In each petriplate, about 30 ml of dye solution was poured into which prepared tapes were dispersed. Later the petriplates were placed under UV or visible chamber for specific time interval using orbital shaker. The experiment was conducted for 4 hours (UV irradiation) and 72 hours (visible irradiation). Samples were collected at different intervals of time throughout the experiment. Absorbance of collected samples was measured using UV/Visible Spectrophotometer at 542 nm. The photocatalytic degradation was calculated using the following formula

Percent Degradation = $A_0 - A_t / A_0 \times 100$

Where A_0 is the initial absorbance of sample before any light exposure while A_t is the absorbance of samples at specific intervals of time while exposing to light (UV and visible).

3.6.3.2 Disinfection Experiment

The antibacterial efficiency of coated and uncoated tapes was tested against *E. coli* and two other isolated bacterial strains (*Staphylococcus scuiri* and *Agrobacterium tumefaciens*). The prepared tapes (1cm × 1cm) were placed in laminar flow hood and bacterial suspension was spread using glass spreader. After that, the tapes were placed under UV and visible light for 2hrs. At different intervals of time (0, 60, 120) mins exposure of light, bacterial sample was collected from the prepared tapes. Samples were collected by rubbing cotton swabs on the entire surface of coated and uncoated

tapes at proposed intervals of time. The collected samples were diluted and spread on nutrient agar plates with the help of sterilized spreader. After spreading of sample, petri plates were incubated in incubator for 24 hours at 37ºC and CFU/ml were counted on a colony counter.

Chapter 4

RESULTS AND DISCUSSION

4.1 CHARACTERIZATION

4.1.1. X-Ray Diffraction (XRD) Analysis of Nanoparticle

Crystal phase and crystalline size of pure TNPs and Ag doped NPs was determined using X-ray diffraction. Figure 4.1 and 4.2 represents the XRD patterns for pure and doped NPs. It may be observed through figures that the average sizes of pure TNPs and Ag doped NPs were 43.1 and 54.3 nm, respectively and the particles are completely in anatase phase. No other allotropes of titania were exhibited by those particles. The particles are in anatase phase because they were calcined for 6 hours at 400 ºC. It has also been proved that Titania in anatse form exhibit significantly better photocatalytic results than other forms of Titania i.e. rutile and brookite (Yao *et al.*, 2006). Besides this, anatase phase is also a powerful oxidizing agent having other characteristics like nontoxicity and long term photo-catalytic ability (Hoffmann *et al.*, 1995). Moreover, it has been reported that nanopartices when annealed or calcined at high temperature (almost 400ºC) are converted to anatase phase having two to three time more photo-catalytic ability than the commercially prepared TNPs (Sekino, 2010). Therefore, the prepared particles have shown improved photocatalytic degradation of dye as well as microorganisms.

4.1.2. Scanning Electron Microscopy (SEM) Analysis

The size and shape of the prepared particles was analyzed using Scanning Electron Microscopy at resolutions i.e. X5000 and X20000. JEOL JSM-6460 was used to capture images for pure TNPs and Ag doped NPs. The images are presented in Figure 4.3 and 4.4. Images of pure TiO_2 and 1% silver doped TiO_2 NPs indicate that particles are in spherical and irregular shape, respectively. Moreover, doped particles are showing porous structure with high degree of complexity. The SEM analysis reveals that these structures have more surface area that may increase the efficiency of fabricated structures.

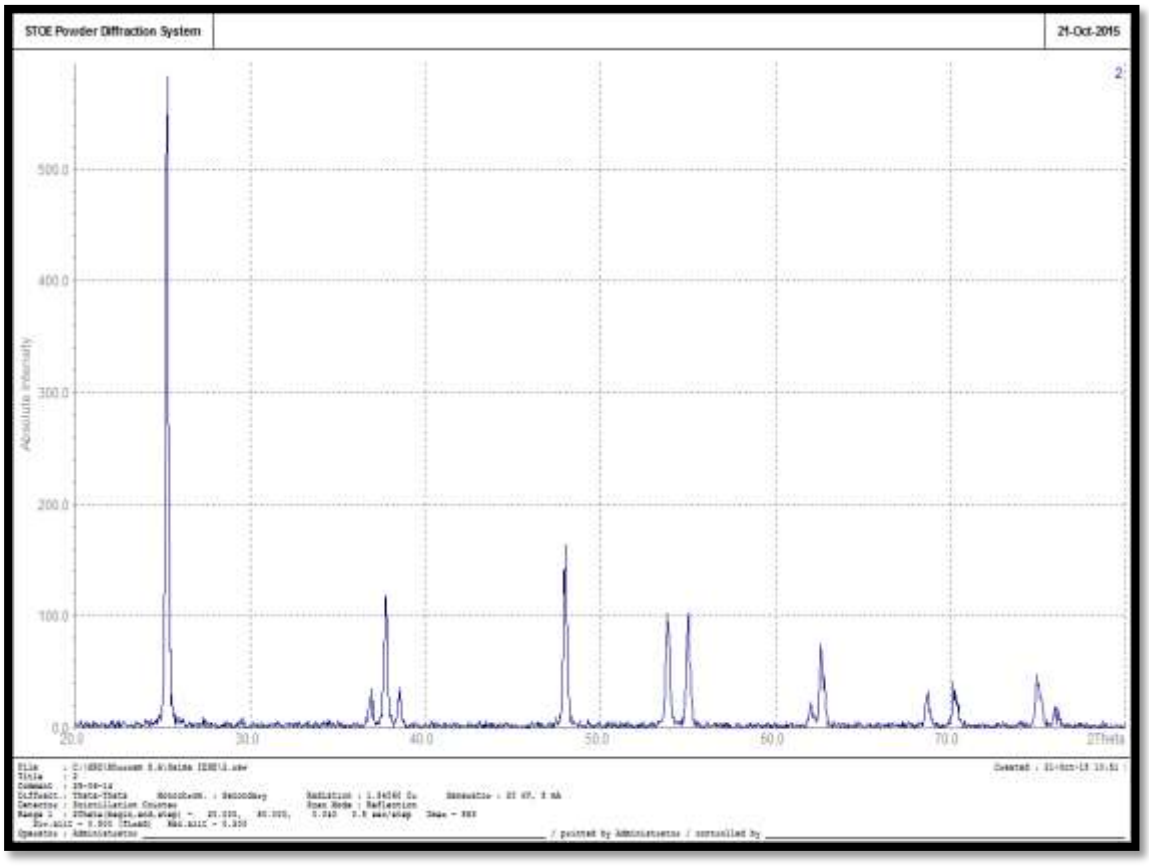


Figure 4.1: XRD pattern for pure Titania Nanoparticle

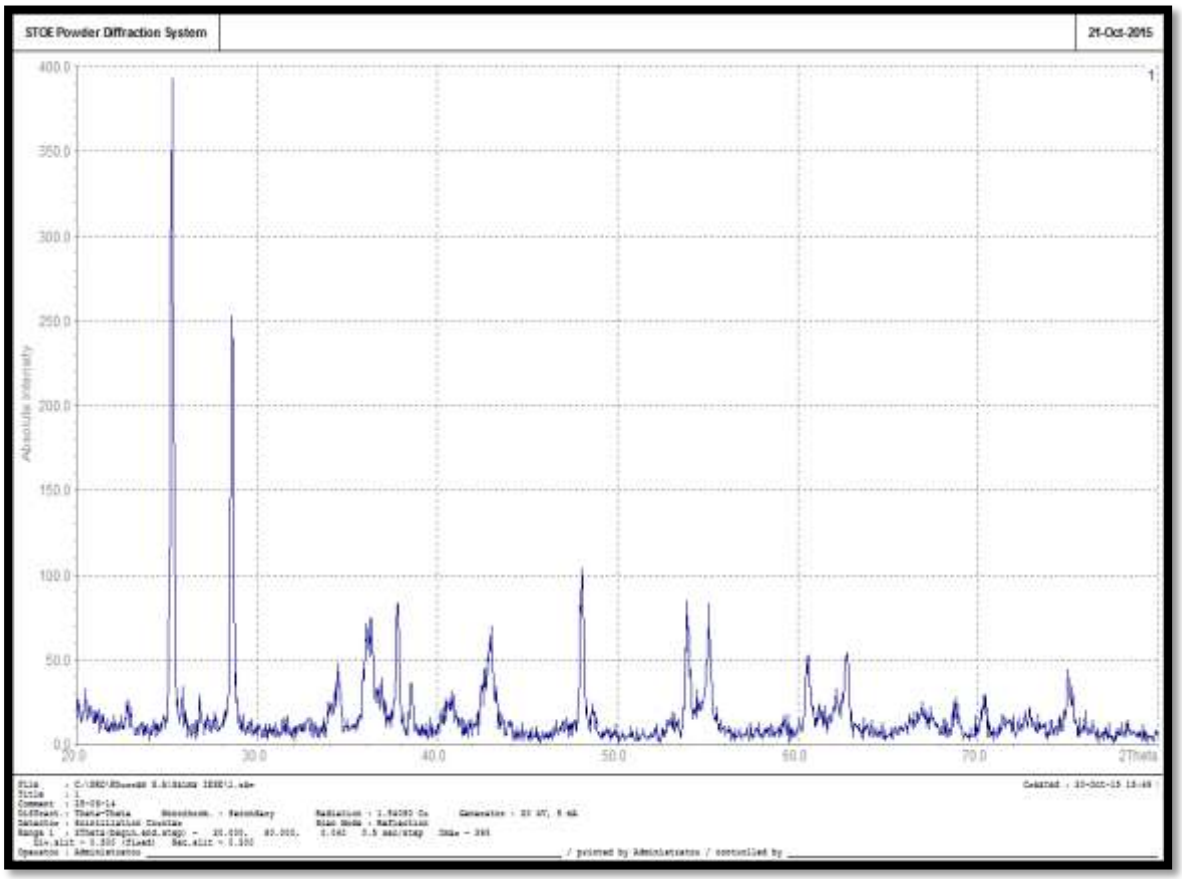


Figure 4.2: XRD pattern for Ag doped Titania Nanoparticle

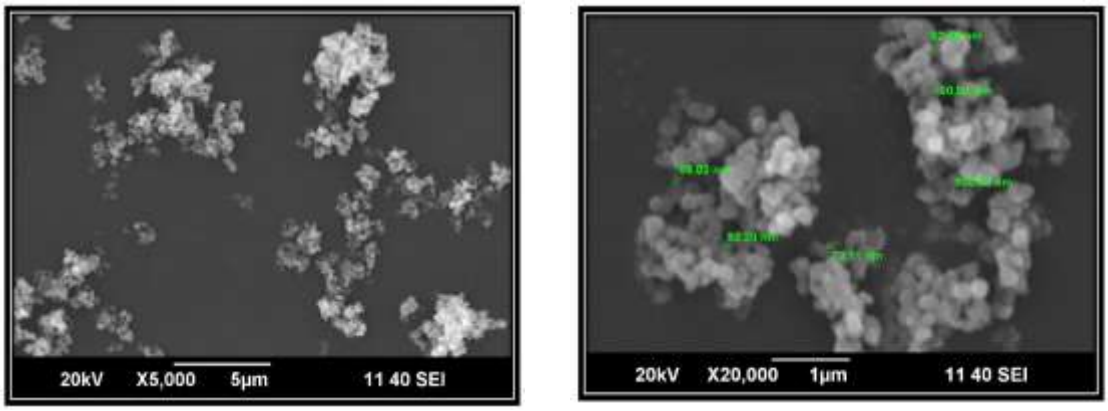


Figure 4.3: SEM images for Pure TNPs captured at X5000 and 20000

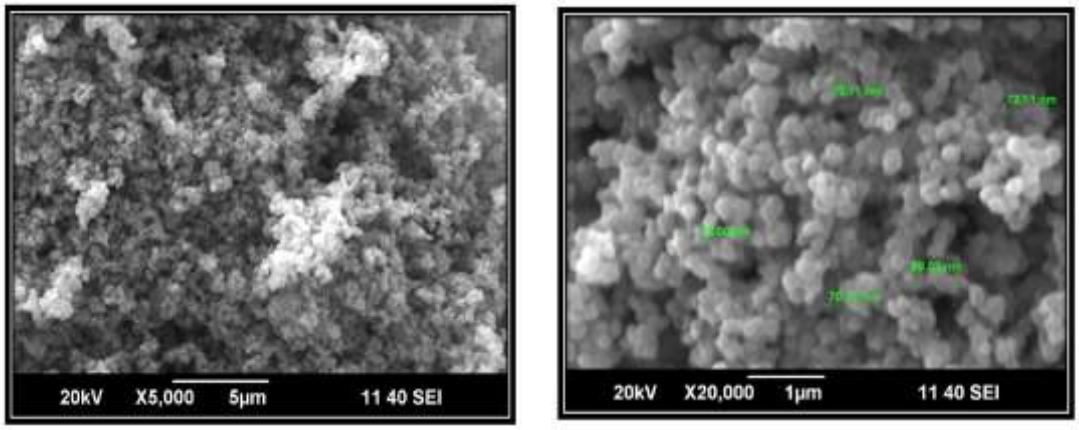
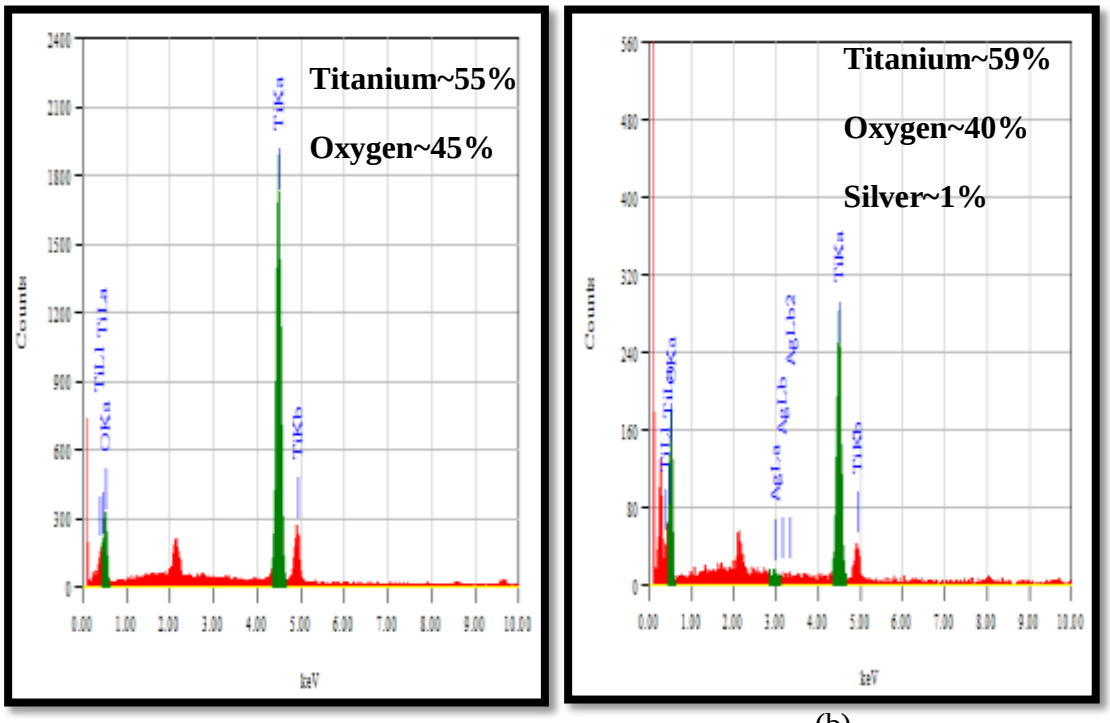


Figure 4.4: SEM images for 1% Ag doped TNPs captured at X5000 and X20000

4.1.3 EDS Analysis

Energy Dispersive Spectroscopy (EDS) analysis revealed the composition of sample i.e. both elemental and quantitative. In case of pure titania nanoparticles in Figure (4.5a), it may be observed that sample contains only titanium and oxygen with composition 55 and 45% respectively endorsing the successful fabrication of desired nano-material. Figure (4.5b) shows that the fabricated 1% silver doped titania nanoparticles constituted of silver, titanium and oxygen with molar ratios of almost 1, 59 and 40%, respectively.



(a) (b)

Figure 4.5: EDS analysis for Pure Titania Nanoparticle (a) and Ag Doped Titania Nanoparticle

4.2 BACTERIAL ISOLATION AND IDENTIFICATION

Five prominent colonies were picked and streaked on sterile agar plate separately. By continuous streaking, pure different isolates were obtained. These isolates were subjected to phenotypic and genotypic characterization.

4.2.1 Phenotypic Characterization

The results of gram staining are presented in Figure 4.6. Out of the 5 bacterial isolates, 2 bacterial strains were gram negative while the remaining strains (3) were gram positive. Among all these, most of them belonged to family Bacillaceae as they were rod shaped while 2 strains were circular in shape and belonged to family Cocci.

The isolated bacterial species include *Staphylococcus sciuri, Bacillus pumilus, Bacillus aerius, Pseudomonas aeruginosa* and *Agrobacterium tumefaciens*. The isolated bacterial strains were also subjected for biochemical characterization. The results for cell morphology and biochemical tests are presented in Table 4.1.

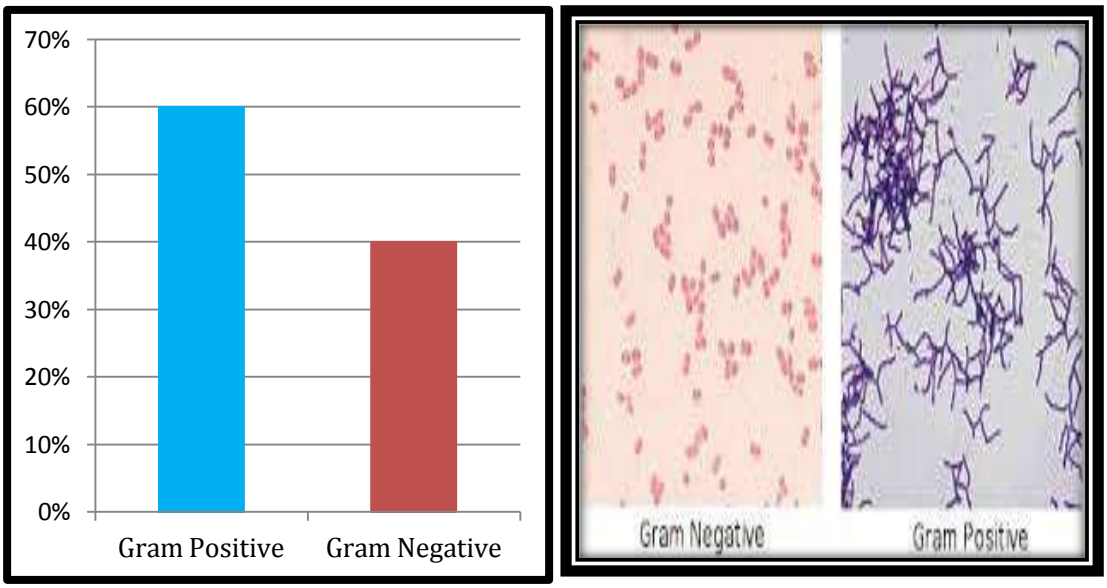


Figure 4.6: Percentage of gram positive and gram negative in isolated strains

4.2.2 Genotypic Characterization

Generally, 16S rRNA gene sequencing analysis was used for genotypic characterization. Therefore, partial sequences of 16S rRNA of 5 isolates (*Staphylococcus sciuri, Bacillus pumilus, Bacillus aerius, Pseudomonas aeruginosa* and *Agrobactreium tumefaciens*) were further analyzed to generate a phylogenetic tree.

4.2.3 Phylogenetic Analysis of Bacterial Isolates

Sequences obtained were analyzed using BLAST search at National Center for Biotechnology Information (NCBI) databases. The sequences were aligned using CLUSTALW after complete deletion of the mismatch sequences. A phylogenetic tree constructed by using the TREEVIEW program illustrates the phylogenetic relatedness of identified strains to the selected strains obtained from GenBank (NCBI). The accession number of isolated strains was assigned on submission of sequences of strains to NCBI. Isolated species along with their accession numbers are presented in Table 4.2. A phylogenetic tree, constructed through MEGA 4 program demonstrates the phylogenetic relatedness and linkage among identified strains, shown in Figure 4.7.

Table 4.1

Cell morphology and biochemical characterization of isolated bacterial strains

Biochemical Test	***Staphylococcus scuiri***	***Bacillus pumilus***	***Bacillus aerius***	***Pseudomonas aeruginosa***	***Agrobactreium tumefaciens***
Cell Morphology	Gram +ve	Gram +ve	Gram +ve	Gram −ve	Gram −ve
Motility	Non Motile	Motile	Motile	Motile	Motile
Oxidation/ Fermentation	Facultative Anaerobic	Aerobic	Aerobic	Aerobic	Aerobic
Urease	−ve	−ve	−ve	−ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve
Oxidase	+ve	−ve	+ve	+ve	+ve
McConkey Agar	−ve	−ve	−ve	+ve	+ve
EMB Agar	−ve	−ve	−ve	+ve	+ve

Table 4.2

Isolated bacterial species and their accession number

Sr. No	Bacterial species	Accession Numbers
1.	*Staphylococcus sciuri*	KU962123
2.	*Bacillus pumilus*	KU962124
3.	*Bacillus aerius*	KU962125
4.	*Pseudomonas aeruginosa*	KU962126
5.	*Agrobactreium tumefaciens*	KU962127

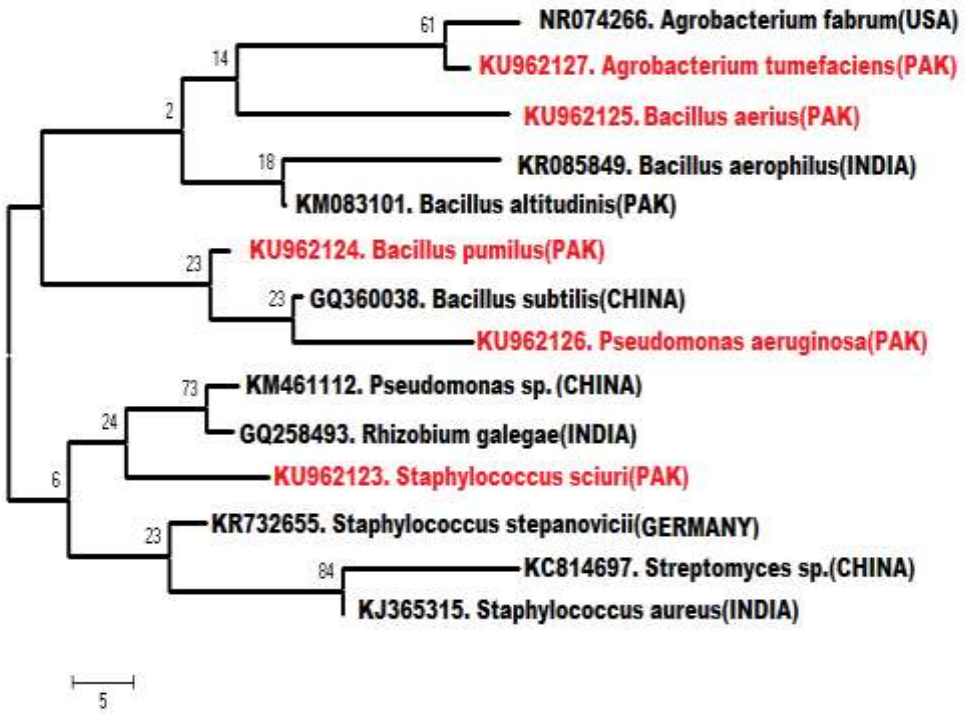


Figure 4.7: Phylogenetic Tree Demonstrating the Relatedness and Linkage of Bacterial Strains

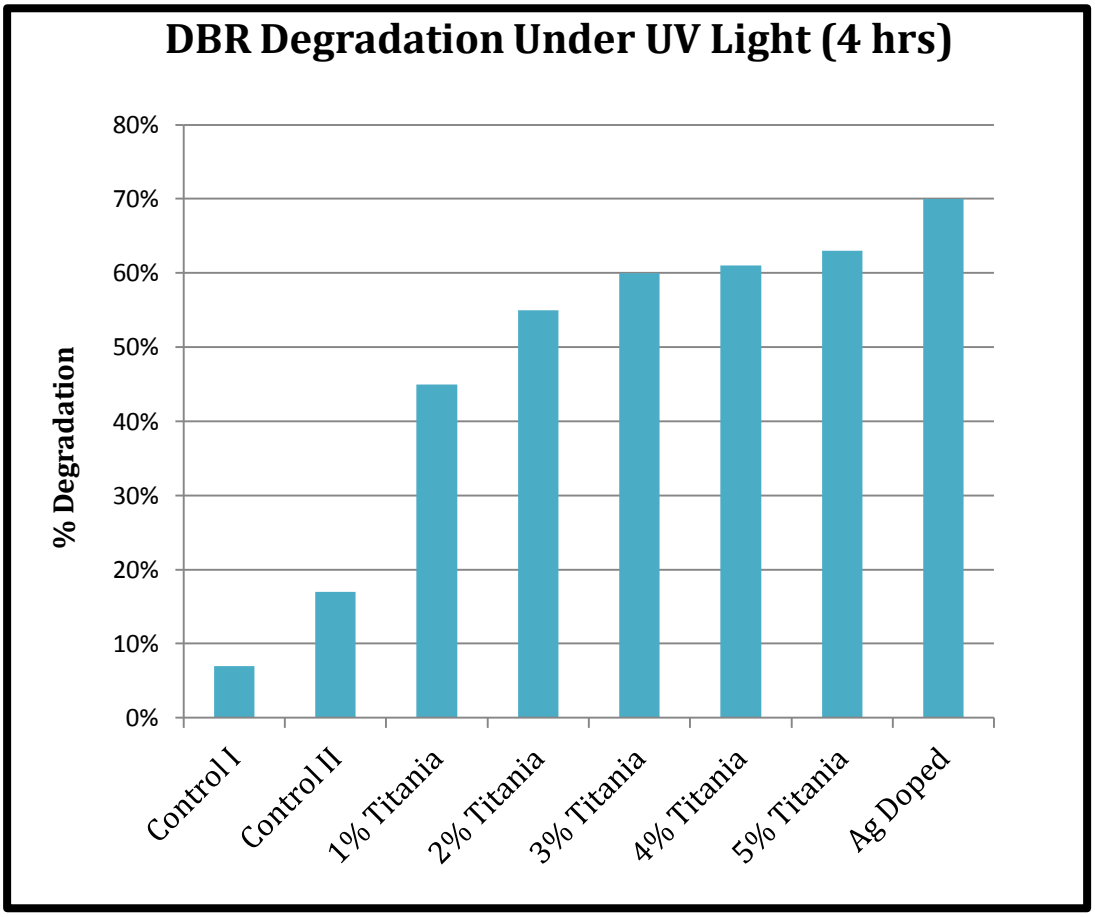
The 16S rRNA based phylogenetic analysis of all bacterial isolates demonstrated 99.00% sequence similarity with the blast hit. As mentioned in the literature that strains were identified through BLAST search (Morozova and Marra, 2008) available at National Center for Biotechnology Information (NCBI) databases revealing 99% similarity to different bacterial species. Schloss market the limit of 97% for identification of species (Schloss, 2004).

4.3 DRIMARENE BRILLIANT RED (DBR) DYE DEGRADATION

4.3.1 Under UV Light

Photoreduction of DBR may occur in the presence of pure TNPs and silver doped NPs. Hence, DBR dye was selected as a model to assess the effectiveness of coated and uncoated tapes. The prepared samples were exposed to UV light for 4 hours. The results for percent degradation of dye with coated and uncoated tapes are significant and presented in Figure (4.8a). The results depict that by increasing titania content, per cent degradation of dye also increased. The maximum removal was obtained with 5% titania coated tape as compared to other prepared tapes. Although titania is a suitable photocatalyst because of non toxicity, excellent efficiency, stability, and environmental friendly innovative technique to degrade resistant pollutants and in killing microorganisms (Pasqui and Barbucci, 2014), its photocatalytic activity increases when it is doped with metals like Ag, Au etc. In the present study, DBR dye was also treated with Ag doped TNPs. As expected, higher per cent degradation (70%)

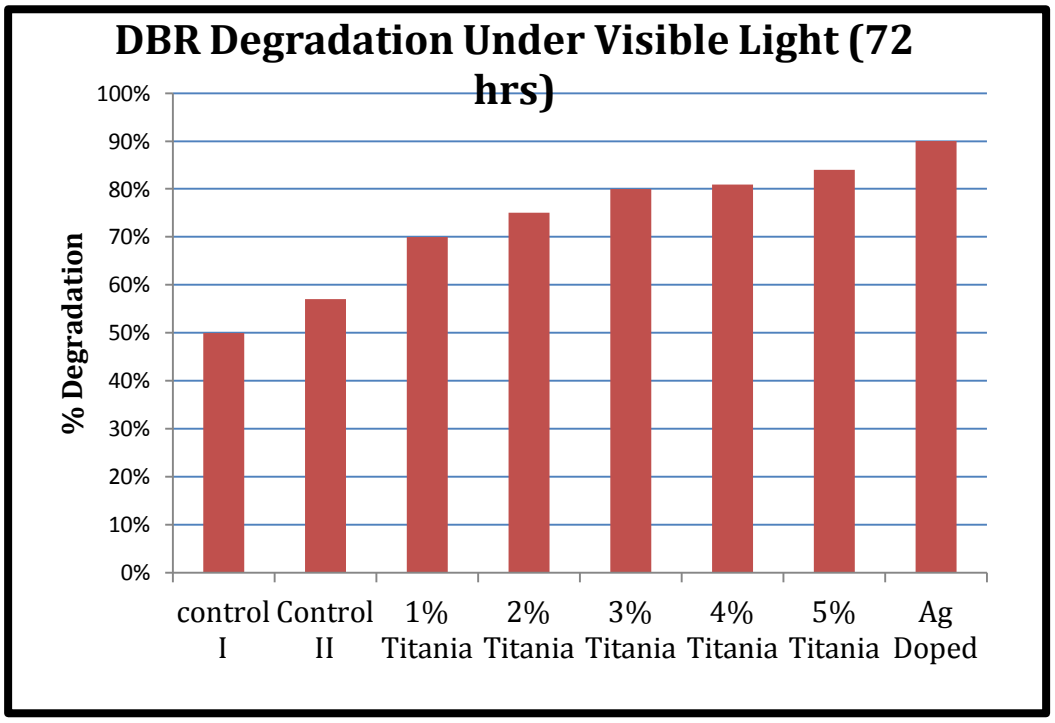
was observed with 1% Ag doped titania nanoparticle coated tape in comparison with pure titania naoparticle coatings. The probable reason behind is that doped nanoparticle traps electron and increased the separation of electron hole. Therefore, it increased the photocatalytic performance of nanoporous substrates. Similarly, enhanced degradation for Methylene Blue (MB) has already been reported when treated with Ag/I/ AgCl/ TiO_2 composites under UV and visible light (Rehan *et al.*, 2013).



(a)

4.3.2 Under Visible Light

The prepared samples were also exposed to visible light for 72 hours. The result for per cent degradation of dye was highly significant and presented in Figure 4.8 (b). The photocatalytic behavior of pure TNPs coated tape is less as compared to Ag Doped NPs. The results exhibited the same trend as in case of UV irradiation. The percent degradation increased by increasing the concentration of TNPs. Therefore, maximum removal (83%) was observed by 5% TNPs as compared to control. On the other hand, Ag doped particle has shown highest degradation (90%) of DBR dye.



(b)

Figure 4.8: Effect of pure and doped TNPs coated tape on percent degradation of DBR dye under UV (a) and Visible irradiation (b)

4.4 ANTIBACTERIAL EFFICIENCY OF COATED AND UNCOATED TAPES

4.4.1 *E. coli*

The bactericidal effect of pure TNPs and Ag doped NPs immobilized tape was also investigated by using *E. coli* (Gram negative bacteria). Fresh culture of *E. coli* was prepared in nutrient broth and sprayed on prepared tapes, separately. One ml of liquid culture was spread on 1×1 cm^2 piece of double sided tape and all tapes were placed in UV and visible chamber for UV and visible irradiation for 2 hours, respectively. The per cent reduction of *E. coli* on TiO_2 nanoparticle-coated tapes under UV and visible irradiation is shown in Figures 4.9 (a, b).

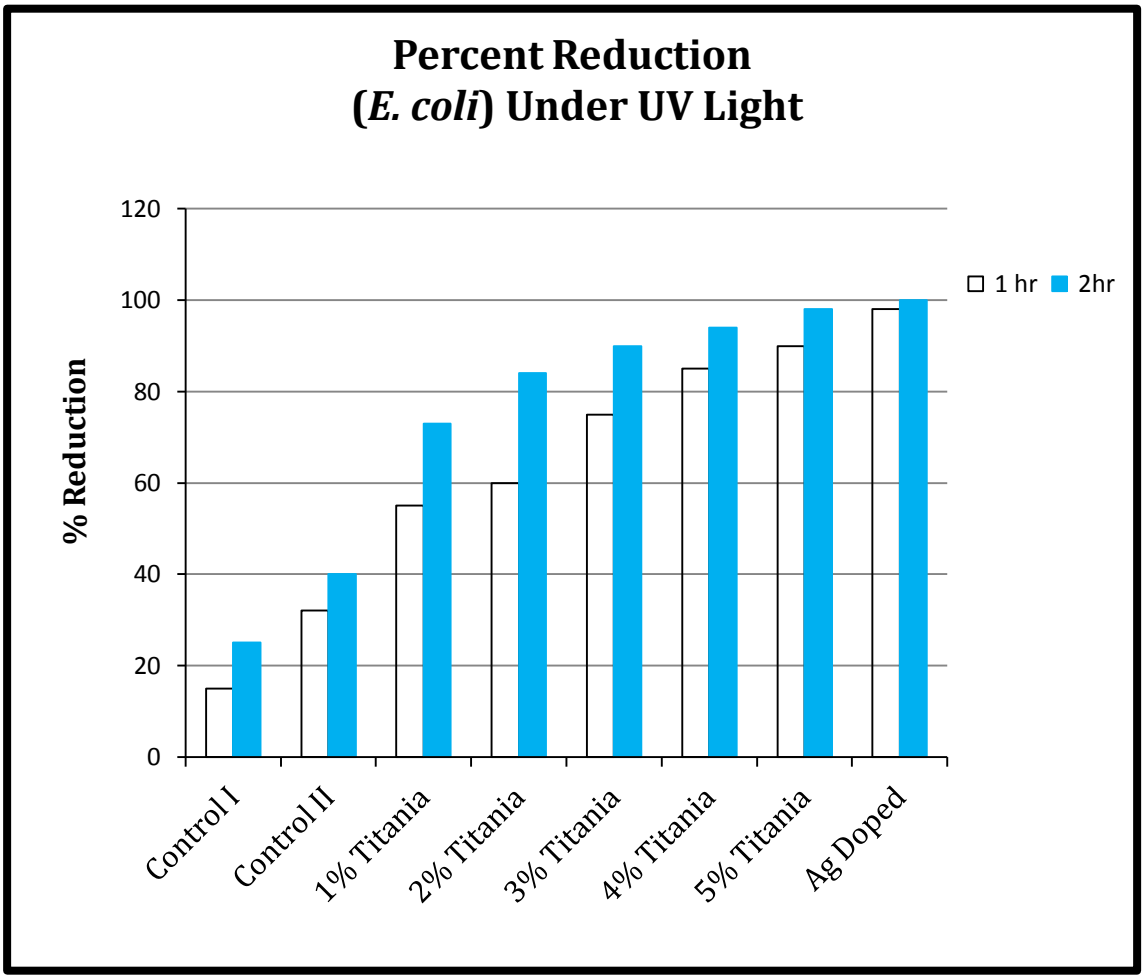
The bacterial colonies of *E. coli* were significantly reduced on TNPs immobilized tapes with the passage of time while remains constant on tapes which were not coated with pure and Ag doped NPs. Hence, it is concluded that number of colonies decreased significantly when exposed to light for 2 hrs. At the start of the experiment, about 9.75×10^6 CFU/mL were present but the number of colonies was reduced by almost 100% on Ag-TiO_2 nanoparticles-coated tape under UV irradiation and 85% under visible irradiation within 120 mins of light exposure. More than 90% decontamination was observed within 60 mins exposure of light on Ag-TiO_2 nanoparticles-coated layer substrates. Almost complete decontamination was achieved within 120 mins of treatment. Similarly, pure TNPs coated tapes were also investigated and showed highly significant reduction in bacterial colonies as presented in Figure

(4.9a). On contrary, bacterial count remained constant on uncoated tapes after exposure of light for 2 hrs. But, among all these, Ag doped TNPs coated tape showed significant reduction in bacterial colonies as compared to pure TNPs coated tapes and uncoated tapes.

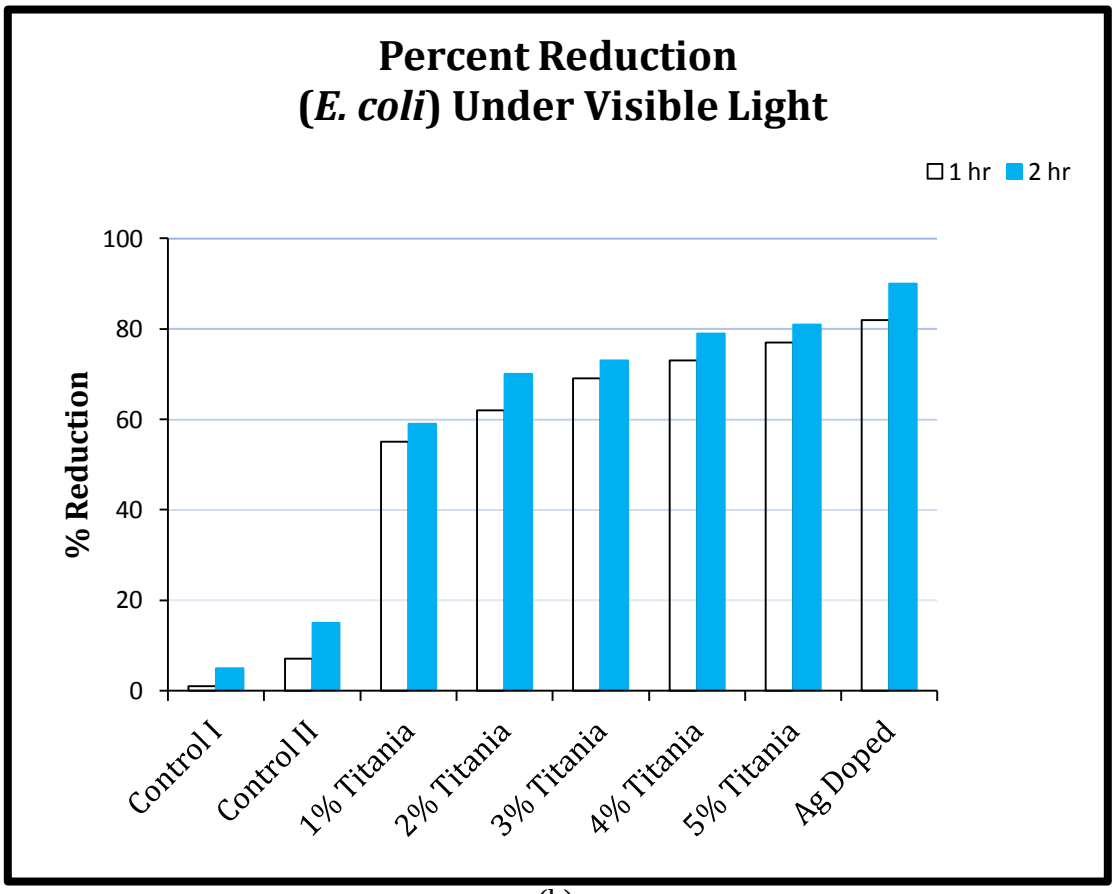
The graphs (4.9 a,b) are showing that number of bacterial colonies were very large i.e. 9.75×10^6 CFU/ml at the start of experiment as well as after 2 hrs of exposure to light in case of control tapes where no titania layer was present. On the other hand a significant decrease was observed in the number of bacterial colonies within 2 hours exposure in case of coated tapes. Hence, 73, 84, 90, 94, 98 and 100% reduction of *E. coli* colonies was observed in 1, 2, 3, 4, 5 and 1% Ag doped TNPS coated substrates under UV light irradiation, respectively. Similar trend was observed when these bacteria were spread on coated and uncoated tapes and exposed to visible light for 2 hours as shown in Figure 4.9 (b).

Therefore, it was concluded that if more than 2% pure TNPs or Ag doped NPs coated tapes are wrapped around any contaminated handles, they will provide a very good self cleaning surface. The reason behind the death of microorganism is that positive charge is present on metal oxides while microorganisms have negative charge, so due to electrostatic attraction between metal oxides and microbes that cause killing of microbes when exposed to such coated surfaces (Zhang and Chen, 2009). Actually, metal oxides damaged the cell wall of bacteria which increased the penetration of particles and finally caused the death of resistant microbes (Ravishankar and Jamuna, 2015). Moreover, TiO_2 nanoparticles interact with microbes and increased the antibacterial activity due to small size and high surface to volume ratio as compared to

mesoporous structures. Besides this, metal doped nanoparticle coated substrates showed significant results in the present study. Similar results were obtained when Ag doped TNPs were immobilized and tested against *E. coli* and *K. pneumonia* (Zhao *et al*., 2011). Moreover, Cu doped nanoparticle immobilized on glass fiber showed very efficient antibacterial activity when irradiated under UV as well as visible light (Venieri *et al*. 2014; Pham and Lee, 2015).



(a)



(b)

Figure 4.9: Percent reduction of *E. coli* colonies by treating with pure TNPs and Ag doped NPs coated tapes under UV (a) and Visible irradiation (b)

4.4.2 Isolate (*Staphylococcus sciuri*)

Liquid culture of isolated strains was prepared in nutrient broth and effectiveness of prepared tapes was assessed against selected strains. Among 5 bacterial strains, 1 gram positive (*Staphylococcus sciuri*) and 1 gram negative (*Agrobactreium tumefaciens*) bacterial strains were selected to assess the effectiveness of prepared tapes.

Isolates were separately sprayed on prepared tapes and exposed to UV and visible light for 2 hrs. Figure 4.10 (a,b) represents the degradation of ST-1 (*Staphylococcus sciuri*) under UV and visible light, respectively. Initially, bacterial colonies were 9.75×10^6 CFU/ml but reduced for pure TNPs and Ag doped TNPs coated tapes after exposure to light for 60 mins. After 60 mins 98 and 90% disinfection was observed for Ag doped NPs coated tape under UV and visible light exposure, respectively. While complete disinfection of *Staphylococcus sciuri* was achieved with Ag doped TNPs after 2 hrs as cell colonies significantly dropped upto zero. On the other hand, degradation efficiency with TNPs was slightly slower as compared to doped particles as shown in Figure (4.10). Among all these, maximum removal efficiency was obtained with 5% TNPs coated tape (99%) as compared to 1% TNPs (72), 2% TNPs (82%), 3% TNPs (89%) and 4% TNPs (95%) coated tapes under UV irradiation. Similar trend was observed when this experiment was repeated under fluorescent light. Moreover, fluorescent light manage to generate very few electron hole pairs from pure TNPs resulting in incomplete inactivation of *Staphylococcus sciuri* within one hour (Hallmich and Gehr, 2010).

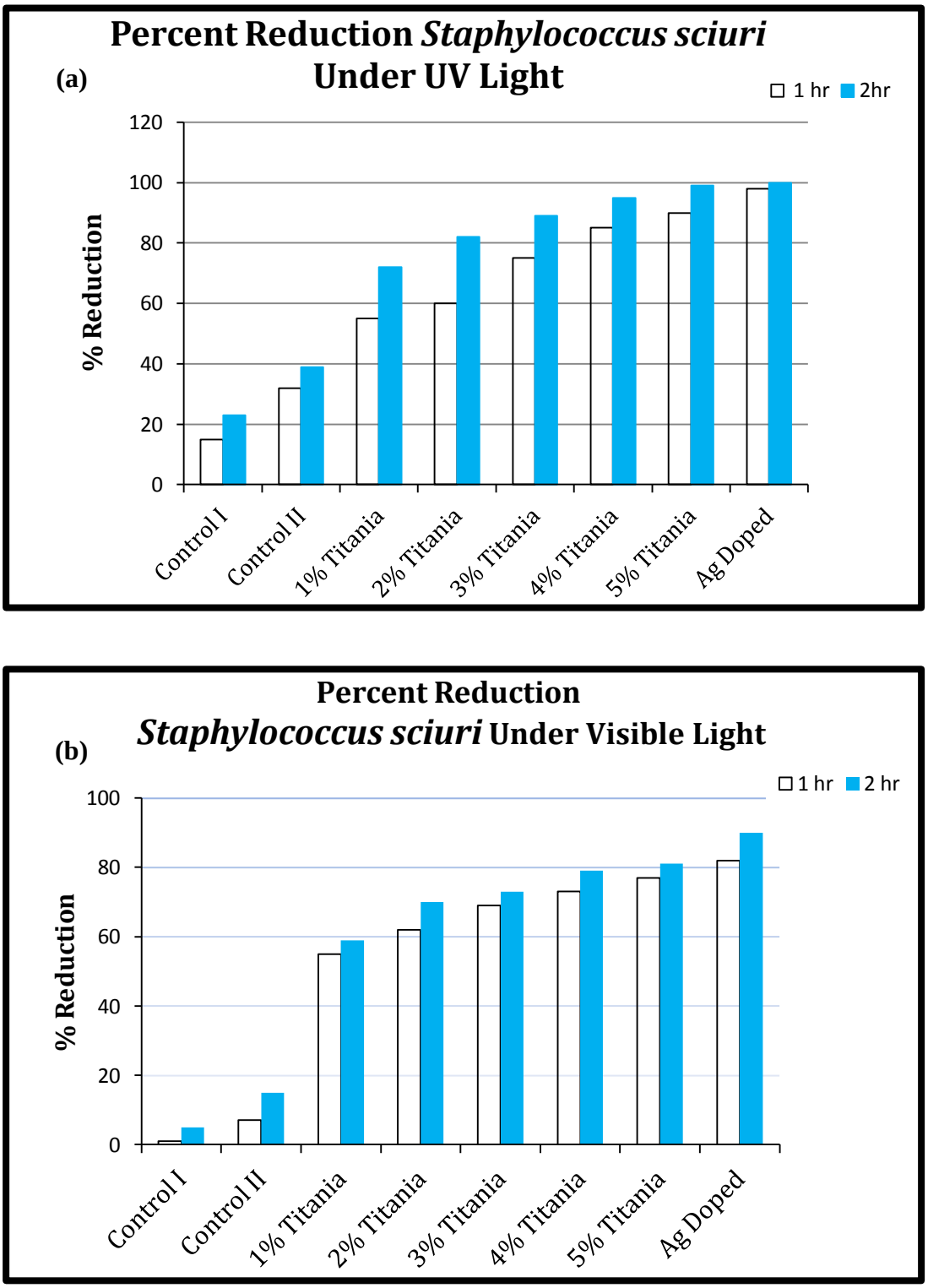
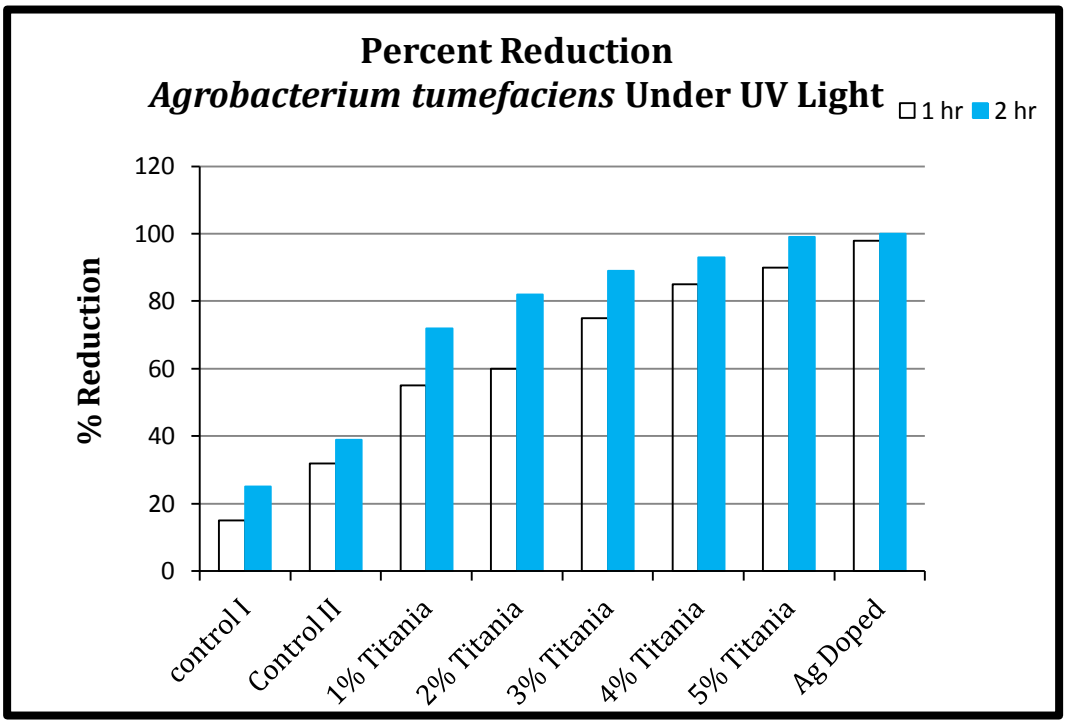


Figure 4.10: Percent reduction of isolate (*Staphylococcus sciuri*) colonies by treating with pure TNPs and Ag doped NPs coated tapes under UV (a) and visible irradiation

4.4.3 Isolate (*Agrobacterium tumefaciens*)

Broth of *Agrobactreium tumefaciens* was prepared and efficiency of coated and uncoated tapes was also investigated. Figure 4.11 (a,b) represents the percent reduction of bacterial colonies under UV and visible irradiation. The results depict that Ag doped NPs coated tapes removed almost 99 and 90% of bacterial colonies within 60 minutes of exposure to UV and visible light, respectively. On the other hand, the efficiency of pure TNPs was less as compared to doped particles. Under UV light all bacterial colonies were killed after 120 mins but almost 81% bacterial colonies reduction was observed with maximum titania content (5% TNPs) used under visible light irradiation. Control samples under visible light showed no reduction in bacterial colonies after 2 hours of exposure. The TiO_2 photocatalysts have been investigated extensively for the killing or growth inhibition of bacteria due to its powerful oxidation strength, good chemical stability and nontoxicity. As it has been reported that titania is a strong oxidizing agent so it produced reactive oxygen species that badly damage the cell wall of microorganisms and increased inactivation of new bacterial cells (Anandgaonker *et al*., 2015).

The use of TiO_2 photocatalyst has been increased with the passage of time. It has been reviewed through literature that these were used to combat nosocomial infections, water purification (Sethi *et al*., 2014), killing of viruses, fungi and bacteria (Vereba *et al*., 2013). Similarly, it has been reported that degradation of organic pollutants as well as growth inhibition of bacteria was also observed while using titania coated substrates alone (Yadava *et al*., 2014).



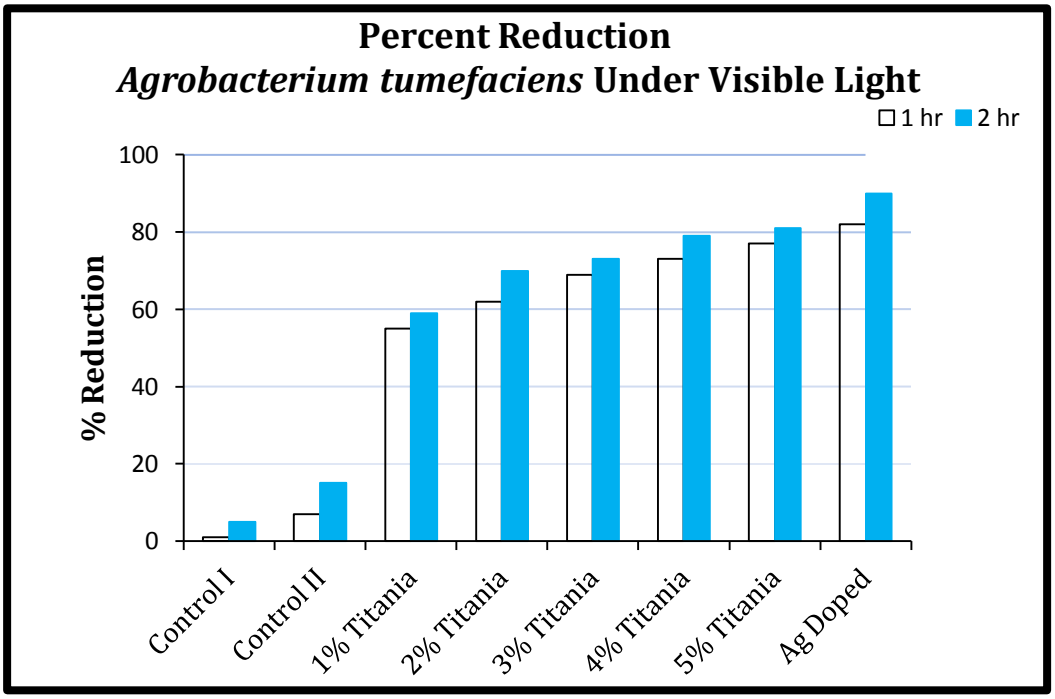


Figure 4.11: Percent reduction of isolate (*Agrobacterium tumefaciens*) colonies by treating with pure TNPs and Ag doped NPs coated tapes under UV (a) and visible irradiation

Therefore, these can be recommended to provide a self sanitized environment inside the whole building such as home, institution, hospitals, shopping malls, etc.

4.5 Actual Field Testing

The prepared tapes were tested against different bacterial strains at lab scale. Therefore, they were prepared for actual field testing in local Cash & Carry grocery store. At lab scale, the tapes were coated with pure TNPs and Ag doped NPs but due to some aesthetic difficulties TNPs and Ag doped NPs were embedded into the proposed tapes. Instead of coatings, hence, TiO_2 nanoparticle embedded tapes were prepared for actual field testing. Six carts were selected for actual field testing. Out of six, 2 shopping cart handles were wrapped with uncoated tape, other 2 were wrapped with pure titania nanoparticle coated tape while the remaining 2 were wrapped with Ag doped titania nanoparticle coated tape. The results for this study are highly significant and presented in Table 1. Three samples were collected from each shopping cart and poured on already prepared nutrient agar plates. Plates were incubated for 24 hours at 37º C. The colonies were counted with the help of colony counter for each plate and mean results are presented in Table 4.2. Maximum disinfection was observed in Ag doped titania nanoparticle coated tapes followed by pure titania nanoparticle coated and uncoated tapes. Therefore, it is concluded that Ag doped TNPs embedded tapes can be wrapped around the shopping cart handles to provide a self sanitizing surfaces. Besides this, it is very cost effective technique to kill the resistant microbes as well (Lai and Sreekantan, 2012; Miranda-García *et al*., 2014; Liu *et al*., 2014; Sethi *et al*., 2014).

Table 4.3

Bacterial colonies found on shopping cart handles during field study

Sr. No.	Coatings	Bacterial Colonies CFU/ml	Bacterial Colonies CFU/ml
1.	Control	1.25×10^3	1.625×10^3
2.	Pure TNPs Coatings (5%)	25	35
3.	Ag Doped TNPs Coatings (1%)	5	5

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

In the present study, pure and 1% Ag doped TiO_2 NPs were prepared using liquid impregnation method. Due to anatase crystalline phase of nanoparticles, these showed very efficient photocatalytic behavior against DBR dye, and bacterial strains as well, under UV and visible irradiation. The said nanoparticles were immobilized on double sided tape with various concentration of titania and tested against different bacterial strains. The results showed that 80 and 90% bacterial colonies were inhibited when sprayed on pure TNP coated tape and Ag doped NPs coated tape for 2 hours under normal light, respectively. On the other hand, the bacterial count was above the countable range on uncoated tape after 2 hours irradiation. Therefore, it was concluded that Ag-TNP embedded tape may be wrapped around the shopping cart handles to provide a self-sanitizing surface. The hypothesis was successfully field tested on the actual handles of the shopping carts in a local store.

5.2 RECOMMENDATIONS

Following recommendations are proposed on the basis of current research;

- Nanoparticle embedded transparent tape should be firmly wrapped around the shopping cart handles, used in local stores, to enhance their antibacterial

activity. More work should be carried out to verify the durability of nanoparticle embedded tape.

- This work may be evaluated for disinfection of pathogenic microorganisms other than *E. coli*, *Staphylococcus sciuri*, and *Agrobacterium tumefaciens.*
- Research study may be extended by using different metal (Fe, Pt, Cu, etc.) doped nanomaterials.

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