DECONTAMINATION OF MICROBES USING METAL DOPED TITANIA NANOPARTILCLES

By

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2010-NUST-MSPhD-Env Sci-09

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

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It is certified that the contents and form of the thesis entitled

"DECONTAMINATION OF MICROBES USING METAL DOPED TITANIA NANOPARTICLES"

Submitted by

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have been found satisfactory for the requirements of the degree.

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This thesis is dedicated to my beloved parents, brother and sister

For their endless affection, support and encouragement

TABLE OF CONTENTS

Abbreviations

Ag-TiO_2	Silver Doped Titanium Dioxide
BOHS	British Occupational Hygiene Society
CFU/ml	Colony Forming Unit per Millilitre
e^-	Electron
eV	Electron Volt
FDA	Food and Drug Administration
GDP	Gross Domestic Product
IOHA	International Occupational Hygiene Association
HAI	Hospital Acquired Infection
GPR	General Purpose Reagent
h+	Hole
ICPS	International Programme on Chemical Safety
LI	Liquid Impregnation Method
MRSA	Methicillin-resistant *Staphylococcus aureus*
NIOSH	National Institute for Occupational Safety and Health
O.D	Optical Density
$*O^{-2}$	Oxygen Radical
*OH	Hydroxyl Radical
OH-	Hydroxyl Ion
SARS	Severe Acute Respiratory Syndrome
TiO_2	Titanium Dioxide
UN	United Nations
UNFAO	Food and Agriculture organization United Nations
UNICEF	United Nations International Children's Emergency Fund
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
WSP	Water and Sanitation Program
λ	Wavelength

List of Figures

List of Tables

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SAMAN KHAN

Abstract

Hard, nonporous environmental surfaces in health care settings are now receiving due recognition for their role in the spread of several types of nosocomial pathogens.The corresponding increase in the means to decontaminate such surfaces to interrupt the spread of infections is leading to the marketing of a plethora of titanium nanoparticles with varying claims of microbiocidal activity, human and environmental safety, and materials compatibility. In this work, we established the photo-killing effects of 1% silver doped titanium dioxide TiO_2. The nanoparticles synthesized by liquid impregnation method were characterized using X-Ray Diffraction (XRD), Energy Dispersive Spectroscopy (EDS) and Scanning Electron Microscopy (SEM). The Ag-TiO_2 nanoparticle coatings have been applied on glass and venetian blind surfaces, tested for loss of viability of two bacteria (*Pseudomonas aeruginosa*, *Bacillus subtilis*). The formation of bacterial colonies above the TiO_2 nanoparticle-coated surfaces decreased significantly after two hour of illumination under normal light in the visible spectrum. Such surfaces may be applicable to medical and other facilities where the potential for infection need to be controlled.

Chapter **1**

1.0 Introduction

1.1 Background

There are roughly 2.7 billion people in world without proper sanitation, out of this 1.7 million exist in Pakistan. Nearly 45% of households have no access to hygienic living conditions due to the economic condition of these people. These unhygienic conditions promote the growth of bacteria which are often maligned as the causes of human diseases.

Many common infections can spread by airborne transmission including Anthrax (inhalational), Chickenpox, Influenza, Measles and Smallpox disease in congested and poor sanitized areas. Diseases acquired due to poor sanitation and unhygienic conditions are also a issue in hospitals of developing countries. Hospitals of both large and small cities of Pakistan are facing multifaceted problems due to rampant nosocomial infections and the emergence of multi-drug-resistant bacteria (Memon, 2007).

1.2 Poor sanitization and microbial infections

The problem which this study focus is on poor sanitization and unhygienic condition due to contamination by microbes. The airborne transmission of nosocomial pathogens is of clinical and public interest (Nielsen, 2009; Wong et al., 2010). The spread of airborne pathogens like measles, anthrax, legionella, influenza, smallpox, and rhinovirus is often regarded as major threats to public

health since they cause severe airborne infectious diseases with high mortality rates (Fiegel et al., 2006). As airborne-diseases are on the rise and deaths due to respiratory infections due to air borne microbial infection is being enhanced, and it is being noticed that the developing countries are the major victims. The human and economic cost of hospital acquired infections is becoming increasingly prohibitive the world over, with the worst brunt being felt by the developing countries where scarce resources, compromised environmental hygiene and rampant overuse of antibiotics in communities and facilities all contribute to an already undesirable situation. So it is necessary to find out the solution by which inhabitants of developing countries could have hygienic and sanitized conditions at an affordable cost.

1.3 Disinfection and sanitization

Disinfection and sanitation is achieved by different methods. Sanitizing may be accomplished manually or with equipment such as machines using heat (as steam or hot water) or chemicals. When sanitizing, using a higher temperature generally shortens the time required to kill bacteria. According to the 1995 Food Code (FDA), the temperature/time requirement for the sanitizing step in manual dishwashing is 171°F for at least 30 seconds. Chemical sanitizing generally involves either immersing the object in a sanitizing solution for a specific amount of time or spraying/wiping the object with the solution and allowing it to air-dry. Chemical sanitizers differ in their effectiveness on certain organisms and in the concentration, temperature and contact time required to kill bacteria. Common chemical sanitizers include chlorine, iodine and quaternary ammonium

compounds or “quats.” Scented bleaches are not recommended as sanitizers. Detergents, antibacterial agents, sanitizers and janitorial products are being sold in market to accomplish the needs of physical cleanliness but do not fulfill the demands of sanitation requirements (Applied Foodservice Sanitation, 1992).

UV disinfection has gained great importance for surfaces disinfection. Ultraviolet germicidal irradiation (UVGI) is a disinfection method that uses ultraviolet (UV) light at sufficiently short wavelength to kill microorganisms. UV has been a known mutagen at the cellular level for more than one-hundred years (NIOSH, 2008).

1.4 Proposed solution

With the outbreak of infectious diseases caused by pathogenic bacteria and the rise of antibiotic resistance of bacteria, much attention in pharmaceutical and medical fields has been focused on creating new antibacterial agents (Fauci et al., 2005; Sondi & Salopek, 2004). In order to prevent the transmission of such airborne infectious diseases and control the spread of several types of airborne pathogens, self sanitizing environmental surfaces are receiving due recognition in public places and dwellings (Kimmerle et al., 2011; Sattar et al., 2010).

TiO_2 photocatalyst has been shown to inhibit bacterial growth**.** TiO_2 is used in form of powder with a strong UV light. Research work has been done regarding coating of TiO_2 nanoparticles. Conventional methods of manual disinfection with wiping are not effective in the longer term, cannot be standardized, and are time intensive and labor intensive. In addition, there are problems associated with the use of aggressive chemicals (Kuhn et al., 2003). There certainly is an urgent need

of “green” products and procedures that can serve as environmentally surface disinfectants.

TiO_2 photocatalyst has been shown to inhibit bacterial growth**.** TiO_2 is used in form of powder with a strong UV light. Research work has been done regarding coating of TiO_2 nanoparticles aqueous solutions on substrates. Recently, nanosize (<100 nm) TiO_2 particles have attracted a lot of attention of many researchers. These nanometer-size TiO_2 particles exhibit many special properties due to the fact that the band gap of the nanoparticles increases with the decrease of their size, and the small TiO_2 particles offer a very large surface area (Sunanda et al., 1998, Wolfrum et al., 2002).

1.5 The present study

Photocatalytic disinfection is known to be very effective in order to killing microbes in water. A well-known photo catalyst, TiO_2, in its anatase form can be used for the purpose of disinfection. Several studies have proved its biocidal activity in different ways (Daoud et al., 2005, Makhluf et al., 2005, Qi et al., 2004, Morones et al., 2005).

In this study, we demonstrate the metal doped TiO_2-mediated photocatalytic and bactericidal activities. Silver doped titanium (Ag- TiO_2) nanoparticles in aqueous solution prepared by liquid impregnation method and dispersed to form coatings found to inhibit microbial growth on various substrates. The nanoparticles were characterized by X-Ray Diffraction (XRD), Energy Dispersive Spectroscopy (EDS) and Scanning Electron Microscopy (SEM). The antibacterial effect of those nanoparticle suspensions and coatings were investigated, using

Pseudomonas aeruginosa (Gram-negative) and *Bacillus Subtilis* (Gram-positive) bacterium.

Chapter 2

2.0 Literature Review

2.1 Sanitation: The necessity of life

2.1.1 Need of sanitation and disinfection

Hygiene is the science that deals with the promotion and preservation of health by reducing harmful levels of germs through cleanliness, sanitization and sterilization.

Sanitation controls the source of contaminants in the environment, preventing contamination of food products. In this sense, sanitation removes soil and microorganisms (bacteria, yeast or molds, etc) from the environment and prevents bacterial build up, i.e., biofilm, reducing the possibility of cross contamination. Good sanitation, including cleaning and sanitizing, is essential to food safety and quality and is a foundation for all food safety systems (The Agricultural Policy Framework, Canada, 2012).

Even though sanitation has always been related to food safety, good sanitation has also a positive impact on other aspects of the product such as appearance, flavor, longer shelf life and overall acceptability of the product. Some critical elements of environmental sanitation are described below.

2.1.2 Clean vs. sanitize

Cleaning and sanitizing are two separate steps in an effective operation. Cleaning consists of removing the product residue (soil) from surfaces. It involves washing and rinsing and is usually performed using detergents and soaps. On the other hand, the purpose of sanitizing is to eliminate or substantially reduce the number of pathogenic bacteria and food spoilage microorganisms to acceptable levels (Cleaning and Sanitation Guidebook, Ontario Ministry of Agriculture, Food and Rural Affairs, 2006).

2.2 Problems due to lack of sanitization

The airborne transmission of nosocomial pathogens is of clinical and public interest (Nielsen, 2009; Wong et al., 2010). The spread of airborne pathogens like measles, anthrax, *Legionella*, influenza, smallpox, cholera and rhinovirus is often regarded as major threats of public health since they cause severe airborne infectious diseases with high mortality rates (Fiegel et al., 2006).

2.2.1 Possible bacteriological contamination

Due to poor hygiene the chances of disease occurrence is much increased. Bacteria, Viruses and parasites are the primary inhabitants of unhygienic places and they are the ones that cause deadly infectious diseases such as measles, anthrax, Legionella, influenza, smallpox, and rhinovirus, Cholera etc. These deadly germs use the human body as a host for reproduction and multiplying in no time thereby forcing the body succumb to their act. Air-borne diseases are about twice as significant as water and food-borne. Some of the bad hygiene diseases caused by viruses (via their carriers such as rats, mosquitoes, fleas, bats,

dogs) include Common Cold, Influenza, Hepatitis, Herpes, Dengue fever, AIDS, Rabies, Polio, Mumps, Measles, Yellow fever, Small pox, Warts etc.(Lewis, 2011).

2.2.2 Hospital acquired infections

Hospital-acquired infection (HAI) is an important public health issue with unacceptable levels of morbidity and mortality, over the last 5 years when unacceptable levels of morbidity and mortality became associated with poor hand hygiene and inadequate cleaning. Every year in the U.S., there are 1,7 million in-hospital infections, resulting in 99000 deaths, more U.S deaths in Vietnam War (58,209) and the Korean war (36,574) combined, and exceeds annual U.S. deaths caused by both breat cancer (40, 230) and prostate cancer (Ku, 2010).

Hospitals and health centers has special requirements for sanitation as they may have to deal with patients who are infected with cholera, typhoid and hepatitis A (Sanitation in hospitals and health centers, fact sheet 3.15). The hospital environment is specifically a place where there is a mixture of sick, infected and immune compromised individuals sharing the same building. Transmission of microorganisms from the environment to patients may occur through direct contact with contaminated equipment, or indirectly as a result of touching by hands (Eames et al., 2009).Body of clinical evidence derived from case reports and outbreak investigations suggested an association between poor environmental hygiene and the transmission of microorganisms causing HAIs in hospital (Dancer, 1999; Garner and Favero, 1986). Disease can be transmitted by air (over large distances), by direct/indirect contact on surfaces or combination of both routes. The contact transmission of disease forms the majority of HAI cases (Denton et

al, 2004; French et al, 2004; Wilcox et al, 2003; Griffiths et al, 2002; Boyce et al, 1997).

2.3 Sources of pathogens in air and surfaces

2.3.1 Generation of formites in the air and their microbiological component

The sources of pathogens exist in the air and surfaces. Environmental factors also affect survivability of microbes and their potential for expressing infection. Pathogens in the air and surfaces are spread on particles or droplets. The solid matter may come from skin, while the droplets may be generated from the upper or lower respiratory tract, mouth, nose and circumstances such as vomiting, dripping water taps and diarrohea. The physical mechanism of the generation of droplets and particles carrying pathogens is largely unknown, though indirect measurements are reported in this volume. Respiratory droplets can carry microorganisms such as bacteria and viruses and constitute a medium for the transmission of infectious diseases (Wells, 1955).

2.3.2 Respiratory droplets containing pathogens

The majority of the respiratory droplets are less than 100 μm in diameter (Duguid 1946; Loudon & Roberts 1967; Papineni & Rosenthal 1997), and these evaporate rapidly in the surrounding environment (Wells, 1934) and become droplet nuclei, which suspend in the air or are transported away by airflow. The size distribution of the droplets is a matter of great debate, largely because their size distribution

spans the limit of measurement techniques. There are many possible steps between the production of droplets by a human source or index case and the resulting infection and disease in another individual. Droplets that carry infectious agents can be formed in many ways. Natural means include breathing, talking, sneezing, singing and, in particular, coughing (Pantelic *et al.,* 2009, Xie *et al.*, 2009 and Tang *et al.,* 2009). Artificial means of producing potentially infectious aerosols are abound in hospitals, particularly when taking high-risk respiratory samples like nasopharyngeal aspirates or when using respiratory assist equipment, such as nebulizers (Hui *et al.*,2009), ventilators (Hui *et al.* 2006*a,b*) or oxygen masks (Hui *et al.*2006, *a,b,* 2007, Ip *et al.* 2007) for patients in respiratory distress.

2.3.3 Survivability of pathogens in the surrounding environment; air and surfaces

The survivability of pathogens in the air or surfaces depends on many factors, including residence time the level of moisture (which in part depends on temperature), atmospheric pollutants and UV light (if outdoors in the sun, for example). Both temperature and humidity affect the lipid envelope and protein coat, affecting the period of survival. Temperature and humidity will work together to either destroy the organisms or stabilize them. Chemical pollutants in the air such as carbon monoxide and sulphur dioxide, together with UV light, will add to this disruption and may decrease survival in such an environment (Cox, 1989). And, although movement in air may play a role in moving pathogens

between spaces, they have a potential to act as secondary sources when they sediment onto inanimate or animate surfaces.

Evidence has been provided to support the hypothesis that microorganisms detaching from biofilms on indwelling medical devices could overcome the host immune system and cause an infection (Ward et al., 1992)

The survival of any infectious agent (viruses, bacteria or fungi) depends partially on ambient environmental factors such as temperature and humidity (relative or absolute), as well as UV light and other atmospheric pollutants (Tang, 2009). The transport of such airborne droplets can be driven by various other environmental factors, such as local ventilation airflows (Nielsen, 2009 and Eames *et al.*, 2009), as well as the movement of people (and their clothing) and thermal gradients produced by various pieces of electrical equipment (Clark & de Calcina-Goff, 2009).

2.4 Diseases in Pakistan due to poor hygiene

Unsafe drinking water, inadequate sanitation and poor hygiene practices cause over 80 per cent of the diseases in the world, while the same cost Rs 112 billion per annum in Pakistan. Every year in the Pakistan there are 1.7 million in-hospital infections resulting in 99,000 deaths per anum. Crunching on the numbers it seems that the economic losses incurred from inadequate sanitation in Pakistan hit Rs 343.7 billion each year which is equivalent to 3.9% of country's GDP as per accordance to a new report published by the Water and Sanitation Program (WSP) (Haider, 2012, DAWN, 2012).

2.5 Hygiene Standards

2.5.1 Occupational hygiene

Occupational hygiene is generally defined as the art and science dedicated to the anticipation, recognition, evaluation, communication and control of environmental stressors in, or arising from, the workplace that may result in injury, illness, impairment, or affect the well being of workers and members of the community. These stressors are divided into the categoriesbiological, chemical, physical, ergonomic and psychosocial.

The British Occupational Hygiene Society (BOHS) define that "occupational hygiene is about the prevention of ill-health from work, through recognizing, evaluating and controlling the risks". The International Occupational Hygiene Association (IOHA) refers to occupational hygiene as the discipline of anticipating, recognizing, evaluating and controlling health hazards in the working environment with the objective of protecting worker health and well-being and safeguarding the community at large.

In the 19th century, Dr. Ignaz Semmel weis became the first to document that improved sanitization and hygiene is among the most important factors for reducing the spread of illnesses. Today this belief is commonly accepted in all areas of public health, including the healthcare and the foodservice industries. In fact, poor personal hygiene is the second leading cause of food borne illness outbreaks, only behind bacterial agents. With hand washing emphasized as a major component of personal hygiene in the U.S. Food Code, a good hand

washing program is needed for proper food safety (Starobin, 2006). A number of studies have shown that outbreaks can be terminated by improved hygiene compliance and better cleaning of the environment (Eames et al., 2009).

2.6 Hospital environmental hygiene and hand hygiene

There are standard principles for infection prevention and control focusing on hospital environmental, which is crucial to the prevention of HAI due to microbes. Recommendations for hospital environmental hygiene is based on relevant standards with the guidelines include cleaning the general hospital environment; cleaning items of shared equipment; and education and training of staff. There is some evidence that improved cleaning regimens are associated with the control of outbreaks of HAIs. In one study (Rampling et al, 2001) the control of an outbreak of an epidemic strain of MRSA was linked with increased cleaning hours and an emphasis on the removal of dust. However, often a range of interventions are introduced in order to control an outbreak and it is difficult to clearly distinguish the effect of a single component such as cleaning. Some evidence suggests that routine cleaning methods may not be sufficient to eliminate surface contamination with MRSA (Rampling et al, 2001). Disinfectants have been recommended for cleaning hospital environment, but a systematic review failed to confirm a link between disinfection and the prevention of HAIs, though contamination of detergent and inadequate disinfection strength could have been an important confounder (Dettenkofer et al., 2004).

The use of hypochlorite for cleaning has been associated with a reduction in incidence of *Clostridium difficile* infection in one study but this was in the

absence of a detectable change in environmental contamination when either detergent or hypochlorite was used (Wilcox et al., 2003).

In laboratory tests a combination of cleaning with detergent followed by hypochlorite was required to consistently eliminate norovirus from surfaces and prevent cross contamination. Dusting and cleaning using detergent was reported to have no effect on the number of MRSA isolated from the hospital environment, but the organism was virtually eliminated by exposure to hydrogen peroxide vapour (French et al, 2004).

Many microorganisms recovered from the hospital environment do not cause HAI. The relationship between these proposed standards and the risk of acquiring infection through contact with the environment have not been established. Since cleaning will only have a transient effect on the numbers of microorganisms, regular cleaning of hospital surfaces will not guarantee complete elimination. Hand decontamination before every patient contact is therefore required to ensure that pathogens acquired by touch are not transferred to patients (www.nursingtime.com, 2007).

2.7 Gram Positive and Gram Negative Bacteria

Bacteria are divided into two major groups, called gram-positive and gram-negative. These can be distinguished by the Gram-staining procedure but differences in cell wall structure are the most important in the reaction. The gram-negative cell wall is a complex, multilayered structure, while the gram-positive bacteria have much thicker single layer. The gram-positive cell wall consists of 90% peptidoglycan layer with 20 – 80 nm average thickness. The reminder 10% consists of teichoic acids, proteins and lipopolysaccharides.

The gram-negative cell wall is much more complex with 2 – 6 nm thick layer of peptidoglycan, comprising of 10% of cell wall. The cell wall also consists of an outer membrane, consisting of 50% lipopolysaccharides, 35% phospholipids and 15% proteins.

2.7.1 Microbes on surfaces

Microorganisms have primarily been characterized as freely suspended cells. Rediscovery of a microbiologic phenomenon, first described by van Leeuwenhoek, that microorganisms attach to and grow universally on exposed surfaces led to studies that revealed surface-associated microorganisms (biofilms) exhibited a distinct phenotype with respect to gene transcription and growth rate (Donlan, 2002).

2.7.2 Biofilm preparation

A biofilm is an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface by the excretion of complex polysaccharide-like compounds (Donlan, 2002). Biofilms are formed by pathogenic and spoilage microorganisms that can contaminate surface. Noncellular materials such as mineral crystals, corrosion particles, clay or silt particles, or blood components, depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix. Biofilms can be found on any surface: cutting boards, stainless steel surfaces, conveyors; but specifically in those areas where accumulation of product residues occurs: cracks in floors, concave surfaces, elbows, junctions in piping, etc. The variable nature of biofilms can be illustrated from scanning electron micrographs of biofilms from an industrial water system and a medical device, respectively (Figures 2.1 and 2.2).

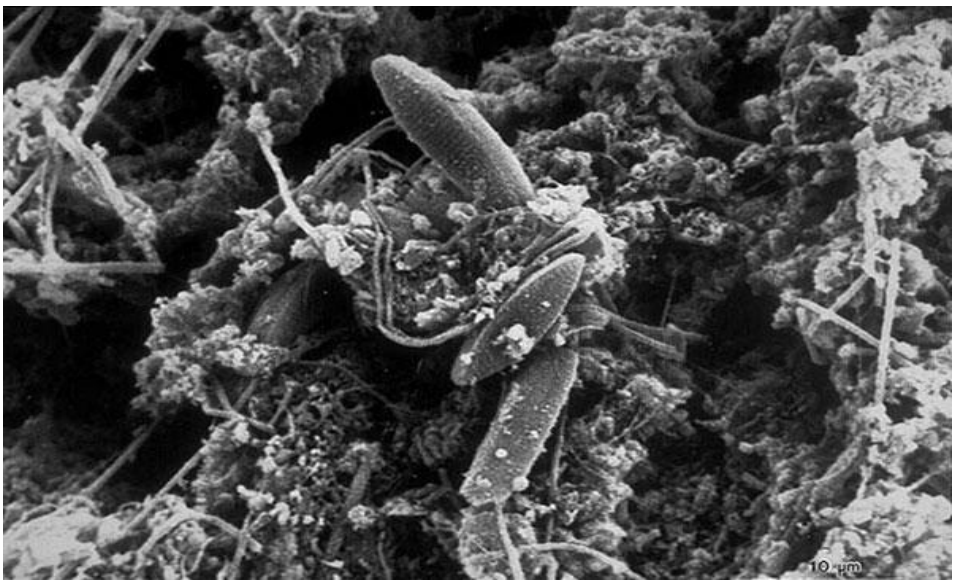


Figure 2.1 *Scanning electron micrograph of a native biofilm that developed on a mild steel surface in 8-week period in an industrial water system (Donlan and Gibbon, 2010)*

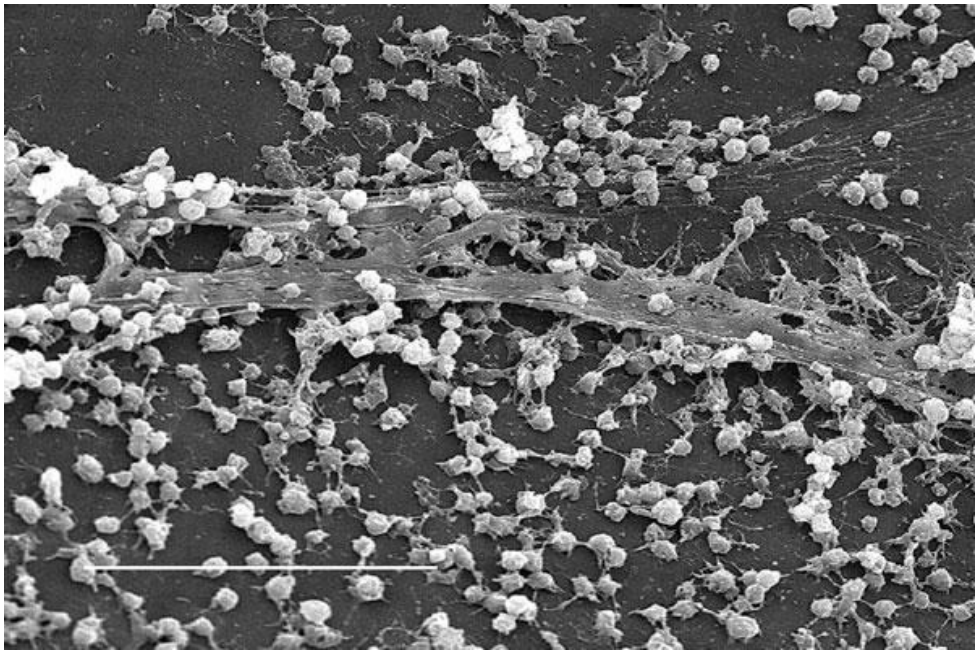


Figure 2.2. *. Scanning electron micrograph of a staphylococcal biofilm on the inner ; surface of an indwelling medical device. Bar, 20 µ. (Williams & Wilkins, 2010)*

Because biofilms form with time, prompt and effective cleaning is required to ensure elimination of bacteria in the early stages of biofilm formation. Unfortunately once a biofilm is formed; it is extremely difficult to remove (www.biofilm.org).

2.7.3 Commonly found microorganisms

Indicators of cleanliness based on levels of microbial or adenosine triphosphate (ATP) contamination have been proposed but are based on arbitrary standards of acceptable contamination and do not distinguish between normal environmental flora and pathogens responsible for HAI (Malik et al, 2003; Griffiths et al, 2002). This concept of heterogeneity is descriptive not only for mixed culture

biofilms (such as might be found in environmental biofilms) but also for pure culture biofilms common on medical devices and those associated with infectious diseases. There is certain criteria or characteristics that could be considered descriptive of biofilms in general, including a thin base film, ranging from a patchy monolayer of cells to a film several layers thick containing water channels (Stoodley et al., 1997).

Following are some microbes that are commonly found in unhygienic places and hospitals.

- ***Methicillin-Resistant Staphylococcus aureus* (MRSA)**

Methicillin-resistant *Staphylococcus aureus* (MRSA) have been recovered from a range of surfaces commonly touched, such as door handles (Barker et al, 2004; Oie et al, 2002), computer keyboards (Schultz et al, 2003), soap dispensers (Griffith et al, 2000; Brooks et al, 2002), and sink taps (French et al, 2004; Griffiths et al, 2002; Griffith et al, 2000) and sites where dust is allowed to accumulate (Denton et al, 2004; Rampling et al, 2001). MRSA can survive on surfaces or skin scales for up to 80 days. MRSA can be transmitted in aerosol from the respiratory tract but commonly attaches to skin scales of various sizes. The distance of travel depends on the size of the scale, the larger falling to the floor within 1–2 m, the smaller travelling the entire length of the ward.

- ***Clostridium difficile***

Clostridium difficile spores are thought to spread in the air and can be found near a patient carrying the organism (Roberts *et al.* 2008).

- ***Pseudomonas aeruginosa***

P. aeruginosa is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several different epidemiological studies indicate antibiotic resistance is increasing in clinical isolates while *B.subtilis* commonly found in the soil and nature environment and can easily contaminate the wound during the episode of trauma (*Tsuang* et al., 2008).

- **Bacillus subtilis**

B. subtilis is known to cause disease in severely immuno-compromised patients, and can conversely be used as a probiotic in healthy individuals (Oggioni, 1998). *B. subtilis* has also been implicated in several cases of food poisoning (McCombs, 2011).

2.8 Disinfection and sanitization measures to improve hygiene

Wide array of chemicals used as disinfectants to disinfect hard, nonporous environmental surfaces in industrial and institutional settings (Fu et al., 2007).

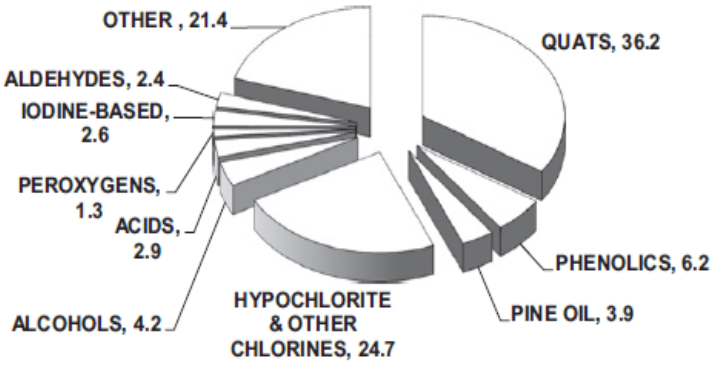


Fig 2.3 disinfectants in institutional & industrial settings in the world

2.8.1 Conventional disinfection methods

Disinfection and the use of chemical disinfectants is one key strategy of infection control. Disinfection refers to the reduction in the number of living microorganisms to a level that is considered to be safe for the particular environment. Typically, this entails the destruction of those microbes that are capable of causing disease.

Disinfection is different from sterilization, which is the complete destruction of all microbial life on the surface or in the liquid. The steam-heat technique of autoclaving is an example of sterilization. There are three levels of disinfection, with respect to power of the disinfection. High-level disinfection will kill all organisms, except for large concentrations of bacterial spores, using a chemical agent that has been approved as a so-called sterilant by the United States Food and Drug Administration. Intermediate level disinfection is that which kills myco-bacteria, most viruses, and all types of bacteria. The last type of disinfection is called low-level disinfection. In this type, some viruses and bacteria are killed using a chemical compound designated by the EPA as a hospital disinfectant.

The disinfectant that is selected and the use of the particular disinfectant depend on a number of factors. The nature of the surface is important. A smoother surface is easier to disinfect, as there are not as many crevasses for organisms to hide. Generally, a smoother surface requires less time to disinfect than a rough surface. The surface material is also important. For example, a wooden surface can soak up liquids that can act as nutrients for the microorganisms, while a plastic surface

that is more hydrophobic (water-hating) will tend to repel liquids and so present a more hostile environment for microbes.

Another factor in the selection of a disinfectant is the number of living microorganisms present.

Alcohol is a disinfectant that tends to be used on the skin to achieve a short-term disinfection. It can be used on surfaces as a spray. However, because alcohol evaporates quickly, it may not be present on a surface long enough to adequately disinfect the surface. A type of disinfectant known as tamed iodines, or iodophors, are also useful as skin disinfectants. In hospital settings, iodophors are used as a replacement for hand soap.

A better choice of disinfectant for surfaces is sodium hypochlorite. If left for five minutes, sodium hypochlorite performs as an intermediate level disinfectant on surfaces. However, chlorine bleach can be corrosive to metal surfaces and irritating to mucous membranes of the eye and nose.

Another surface disinfectant is compounds that contain a phenol group. A popular commercial brand known as Lysol is a phenolic disinfectant. Phenolics are intermediate level disinfectants, derived from coal tar, that are effective on contaminated surfaces. However, certain types of viruses and some bacteria are resistant to the killing action of phenolic compounds.

Another disinfectant is chlorhexidine. It is effective against fungus and yeast, but is not as effective against Gramnegative bacteria. Nor will it inactivate viruses

whose surfaces are water loving. In situations where the contaminant is expected to be fungi or yeast, chlorhexidine is a suitable choice of disinfectant.

Aldehyde compounds, such as formaldehyde and glutaraldehyde, are very effective disinfectants. Glutaraldehyde has other uses as well, such as preserving specimens prior to their examination by the technique of electron microscopy. Glutaraldehyde kills many microorganisms, and all known disease-causing microorganisms, after only a few minutes exposure. Another effective general disinfectant is those that contain quaternary ammonium.

2.8.2 Problems with conventional methods

Many disinfectants are non-specific in their action. They will act against any biological material that is present. These are referred to as broad-spectrum disinfectants. Examples of broad-spectrum disinfectants are glutaraldehyde, sodium hypochlorite (the active ingredient in common household bleach), and hydrogen peroxide. Disinfectants such as phenolics and quaternary ammonium compounds are very specific. Other disinfectants lie in between the highly specific and broadly based categories. For example, alcohol is effective against actively growing bacteria and viruses with a lipid-based outer surface, but is not effective against bacterial spores or viruses that prefer watery environments.

The potency of a disinfectant can also be affected by the concentration that is used. For example, pure alcohol is less effective than alcohol diluted with water, because the more dilute form can penetrate farther into biological specimens than the pure form can.

Another factor that can decrease the effectiveness of disinfectants can be the presence of organic (carbon-containing) material. This can be a great problem in the chlorine disinfection of surface water. The vegetation in the water can bind the chlorine, leaving less of the disinfectant available to act on the microorganisms in the water. Proteins can also bind disinfectants. So, the presence of blood or blood products, other body fluids, and fecal waste material can compromise disinfectant performance.

Microorganisms can develop resistance to disinfectants, or can even have built-in, or intrinsic, resistance. For example, application of some disinfectants to contaminated surfaces for too short a time can promote the development of resistance in those bacteria that survive the treatment (Cengage, 2003).

Because of the widespread use of antibiotics and the emergence of more resistant and virulent strains of microorganisms, there is an immediate need to develop alternative sterilization technologies. Particularly in microbiological laboratories and in areas of intensive medical use, regular and thorough disinfection of surface is required in order to reduce the number of bacteria and to prevent bacterial transmission. The widening use of conventional methods of manual disinfection is also raising concerns on their human and environmental safety at many levels along with the realization that routine surface disinfection procedures in health care settings are frequently inadequate and possibly counterproductive (Kuhn et al., 2003).

2.8.3 Other measures to improve hygiene

Use is largely based on history and tradition and much less on proven effectiveness of those chemicals in the field. Also, it is worth reiterating here that, all things considered equal, the microbicidal activity of any disinfectant is inversely proportional to the degree of soiling of the target surface and that laboratory-based testing of such disinfectants often gives only an indication of their performance in the field. Moreover, recent years have seen mounting concerns on the overall safety of several disinfectant chemicals and their potential to contribute to the already serious problem of antibiotic resistance. Many state, national and regional as well as advocacy groups are also now quite active in attempts to reduce the environmental loadings of many potentially harmful chemicals. All this is forcing a major rethink of what, when, and how we use disinfectants; and, if the anticipated changes do occur, the number of actives and their relative amounts will be substantially reduced in the near future (Sattar, 2012).

There is an urgent need of “green” products and procedures that can serve as environmental surface disinfectants for their microbicidal activity, label claims, registration requirements, overall safety, and routine practices of environmental surface decontamination (Carling et al., 2010).

2.9 Photo Catalysis

A photo degradation process is actually an oxidation reaction in the presence of light and oxygen, and, the photo catalyst is only the agent, capable of combining

light and oxygen (reactants) efficiently in order to enhance the degradation process. So, in spite of terminology, light is a reactant in photo catalytic process. Thus, photo catalysis can be defined as (Braslavsky, 2007).

A change in the rate of chemical reaction, or its initiation, under the action of ultraviolet, visible or infrared radiation in the presence of a substance, the photo catalyst, that absorbs light and is involved in the chemical transformation of the reaction partners. The overall process takes place in five independent steps (Herrmann, 2005).

i. Reactant diffusion from bulk phase to catalyst surface

ii. Adsorption of at least one of the reactants on the catalyst surface

iii. Reaction in adsorption phase

iv. Desorption of the product

v. Removal of the product from the interface region

Scientists have divided the photo catalysis process into two broad categories based on initial excitation process (Linsebigler et al., 1995)

2.9.1 An Ideal Photo Catalyst

There are certain properties which are considered while selecting a photo catalyst and presence of all of these in a single photo catalyst make it ideal (Bhatkhande *et al.*, 2002).

- Photoactive

- Excitable with visible and/or near UV light
- Biologically and chemically inert
- Photo stable
- Inexpensive
- Non-toxic

Furthermore, a photo catalyst is generally a semiconductor, and it will be chemically active as a sensitizer if the redox potential of hydroxyl radical will lie within the band gap of photo catalyst.

2.9.2 Catalysts for Photo Catalytic Reactions

In recent years, nanoscaled antibacterial materials as novel antimicrobial species have been seen as promising candidates for application owing to their high surface-to-volume ratio and their novel physical and chemical properties on the nanoscale level. Many kinds of nanometre-sized antibacterial materials such as TiO_2, ZnO, MgO, chitosan, calamine, copper and silver have been reported on in this area (Gong et al., 2007).

2.10 Titanium dioxide as a photo catalyst

2.10.1 Introduction

In the early 1970s Fujishima and Honda discovered photoinduced water cleavage on titanium dioxide (TiO_2) electrode (Fu et al., 2005). After that the use of photocatalysts to destroy organic compounds in contaminated air or water has

been extensively studied. In 1985, Matsunaga and coworkers reported that microbial cells in water could be sacrificed by contact with a TiO_2-Pt catalyst: bacteria cultures in contact with ultraviolet (UV)-irradiated TiO_2-Pt thin film with near-UV light for 60–120 min had a significant reduction in the number of cultivable cells (Sunda et al., 1998). Since this report, the photocatalytic property has been widely studied in a variety of microorganisms such as viruses, bacteria, fungi, and algae (Daoud et al., 2005, Ghule et al.,2006, Makhluf et al.,2005, Qi et al.,2004, Cubillo et al.,2006, Morones et al., 2005).

2.10.2 Photo catalytic activity of TiO_2

Titanium dioxide (TiO_2), a metal oxide semiconductor has been found to be one of the most effective photocatalysts due to its high efficiency and stability (Hassan et al., 2011). TiO_2 is white, inexpensive, and nontoxic (Nonami et al., 1988). It is one of the most widely used photo catalyst for disinfection (Wolfrum et al., 2002, Sokmen et al., 2001). Since the discovery of the photocatalystic splitting of water on a TiO_2 electrode under ultraviolet (UV) light (Fujishima and Honda, 1972) a great deal of research efforts have been made on semiconductor-based photocatalysts on both energy conversion and environmental applications (Tatsuma., 2003; Khan et al., 2002; Salih, 2002). Bacteria cultures in contact with TiO_2-Pt thin film irradiated with near-UV light had a significant reduction in the number of cultivable cells (Sunanda et al., 1998). The photocatalytic property has been widely studied in a variety of microorganisms such as viruses, bacteria, fungi, and algae. The main advantages of photocatalytic method are operation

under ambient temperature and pressure, high stability and the low cost of catalyst, completed mineralization without creating secondary pollution and possibility of using solar light (Wolfrum et al., 2002).

2.10.3 Modifications in TiO_2

The efficiency of TiO_2 photocatalytic properties and antibacterial activities should depend on our abilities (i) to make stable nanostructured TiO_2 particles or composites, (ii) to generate electron-hole pairs by extending the excitation wavelength to the visible light region, and (iii) to achieve a reduced recombination rate on the newly created electron-hole carriers. Several methods may be used to improve the photocatalytic efficiency, including increase of the surface area of TiO_2 through tailoring particle size and pore-size distributions, generation of defect structures to induce space-charge separation via metal dopants, and surface modification of the TiO_2 with a metal or another semiconductor. Many investigations have reported that the addition of noble metals such as gold and silver may enhance the overall photoefficiency of TiO_2, and a variety of noble metal capped TiO_2 nanocomposite solutions have been synthesized in recent years to improve the efficiency of the photocatalytic activity of TiO_2. Because the majority of photoexcited charge carriers [electrons (e-) and holes (h+)] may undergo a rapid recombination, single component semiconductor nanoparticles have shown to exhibit relatively poor photocatalytic efficiency. Semiconductor-metal nanocomposites, on the other hand, exhibit increased efficiency of photocatalytic activity because of a reduction in the e^- - h+

recombination rate due to better charge separation between the electrons, which accumulate on the metal, and holes, which remain on the photocatalyst surface (Haick & Paz, 2003).

2.10.4 The effect of doping content of silver

The detrimental effect of silver on TiO_2 photoactivity has several reasons.

- Excessive coverage of TiO_2 catalyst limits the amount of light reaching to the TiO_2 surface, reducing the number of photogenerated e^--h+ pairs and lowering consequently the TiO_2 photoactivity (Carp et al., 2004).
- Metal deposits may occupy the active sites on the TiO_2 surface for the desired photocatalytic reactions causing the TiO_2 lose its activity (Coleman et al., 2005).
- Negatively charged silver sites begin to attract holes and subsequently recombine them with electrons. In this case, the metal deposits become recombination centres (Carp *et al.*, 2004).
- The probability of the hole capture is increased by the large number of silver particles at high silver loadings, which decrease the probability of holes reacting with adsorbed species at the TiO_2 surface (Sobana *et al.*, 2006).

2.10.5 Photo catalytic mechanism in TiO_2

When TiO_2 catalyst is irradiated with light of energy greater than or equal to its band gap energy, electron-hole pairs are generated that can induce redox reactions

at the surface of the TiO_2 (Fu et al., 2005). The general scheme for the photocatalytic damage of microorganism cells by TiO_2 photocatalytic properties involves several steps: (1) the photo excited TiO_2 catalyst produces electron-hole pairs that migrate to the TiO_2 surface; (2) photo generated holes in TiO_2 can react with adsorbed H_2O or OH^- at the catalyst/water interface to produce the highly reactive hydroxyl radicals and the electrons can react with oxygen vacancies to form superoxide ions; (3) finally, the various highly active oxygen species generated can oxidize organic compounds/cells adsorbed on the TiO_2 surface, resulting in the death of the microorganisms.

TiO_2 under irradiation of light with wavelength lower than 390 nm produces e^-- h^+ pairs. Recombination of e^-- h^+ pairs reduces the rate of photocatalytic degradation. Doping of silver on titania nanoparticles increase their visible absorption capacity. The positive effect of silver on the photoactivity of TiO_2 is explained by its ability to trap electrons. This process reduces the recombination of light generated e^-- h+ at TiO_2 surface (Coleman et al., 2005). Therefore a more effective electron transfer occurs to the electron acceptors and donors adsorbed on the surface of the particle than in the case of undoped TiO_2. Oxygen adsorbed on photocatalyst surface traps the electrons and produces superoxide anion (Daneshvar et al., 2004). On the other hand holes at the TiO_2 surface can oxidize adsorbed water or hydroxide ions to produces hydroxyl radicals (Behnajady et al., 2007).

Self-cleaning surfaces can become a reality because of photocatalytic coatings containing titanium dioxide nanoparticles. These nanoparticles initiate

photocatalysis, a process by which dirt is broken down by exposure to the sun's ultraviolet rays (Elvin, 2007).

2.10.6 TiO_2 as ideal for sanitation purpose

Photocatalyst TiO_2 super disinfections power has been verified not only for killing bacteria, virus and fungi, but also for eliminating foul smell. It has been tested with a series of experiences by different authorities and academic bodies, food research centres, universities, etc, and having very good performance. TiO_2 can kill *Pseudomonas aeruginosa*, influenza virus, MRSA, *Tubercle bacillus* etc. All those bacteria and virus are highly transmitted in our environment, and will cause severe illness to weak and sick persons.

2.10.7 Past studies of TiO_2 for antimicrobial functioning

Photocatalytic activity of TiO_2 was first observed in 1972 under ultraviolet (UV) light (Fujishima and Honda, 1972) which open an era of use of TiO_2 for environmental applications.

In one research photoactivation of TiO_2 was evaluated, and the loss of viability of five clinical pathogenic bacteria suspensions (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococus aureus*, *Enterococcus hirae*, and *Bacteroides fragilis*) was examined by the viable count procedure. The bactericidal effect of TiO_2 nanoparticle coated metal plates was also tested. Result showed that the ultraviolet (UV) did not affect the viability of bacteria. The survival curve of microorganisms in the presence of TiO_2 nanoparticles showed that nearly

complete killing was achieved after 50 min of UV illumination (Tsuang et al., 2008).

Microbial disinfection has also been evaluated using TiO_2 in different types of water; surface water and distilled water. In this work the photocatalytic inactivation rates of *Escherichia coli* using immobilized nanoparticle TiO_2 films were found to be significantly lower in surface water samples in comparison to distilled water. The presence of nitrate and sulphate anions spiked into distilled water resulted in a decrease in the rate of photocatalytic disinfection. The presence of humic acid was found to have a more pronounced affect. Results showed that initial pH of the water did not markedly affect the photocatalytic disinfection rate (Dheaya et al., 2009).

In another research sol-gel chemistry approach was used to fabricate nanoparticles of TiO_2 in its anatase form. Different percentages of gold and vanadium-doped TiO_2 nanoparticles were successfully prepared. The synthesized nanoparticles have a size of about 12-18 nm and an anatase phase as characterized by XRD, TEM, AFM, and UV-vis spectroscopy. The TiO_2 nanoparticles coatings have been applied on glass slide substrates. The antibacterial activity of TiO_2 nanocomposites was investigated qualitatively and quantitatively. Two types of bacteria, *Escherichia coli* (DH 5R) and *Bacillus megaterium* (QM B1551), were used during the experiments. The quantitative examination of bacterial activity for bacteria was estimated by the survival ratio as calculated from the number of viable cells, which form colonies on the nutrient agar plates. Results showed good antibacterial activity on two types of bacteria, *E. coli* and *B. megaterium*, even

under room light. The good antibacterial effect may be due to those particles' small size, large surface area, large band gap energy, and more active sites for carrying out catalytic reactions (Fu et al., 2005).

The deterioration of indoor air quality is a widespread environmental problem because of airborne bacteria. Another study was conducted to evaluate the feasibility of applying nanoscale silver-doped TiO_2 particles as a photocatalyst to enhance the disinfecting capability of bacterial restraining equipment in a medical nursing institution. The photocatalyst TiO_2 was added to various air quality control equipments. Results from this study indicate that the instrument in which TiO_2 catalyst was added had the best bacterial restraining rate. Efficiency increase upto 81% as compared to bacterial restraining equipment without TiO_2 nanoparticles that has an efficiency of 66%. Similar results were obtained at different heights (90 and 180 cm) of the nursing institutions, revealing that freshly disinfected air is provided regardless of location as long as the air quality control equipment is in operation (Zhao et al., 2010).

TiO_2 has also been tested for photocatalytic inactivation of viruses. In one study, photocatalytic silver doped TiO_2 nanoparticles were investigated for their capability of inactivating bacteriophage virus MS2 in aqueous media. Nano-sized Ag deposits were formed on TiO_2 nanopowders using a photochemical reduction method. The MS2 inactivation kinetics of Ag-TiO_2 was compared to the base TiO_2 material. The inactivation rate of MS2 was enhanced by more than 5 fold depending on the non-doped TiO_2 nanopowders, and the inactivation efficiency increased with increasing silver content (Liga et al., 2010).

In another research TiO_2 nanoparticles were prepared by the hydrolysis precipitation method with $Ti(OBu)_4$, silver nitrate and ammonia. Crystal structure, particle size and interfacial structure of the prepared nanoparticles were characterized by X-ray diffraction measurements (XRD), Transmission electron microscopy (TEM) and Fourier-transform infrared spectroscopy (FTIR). The FTIR revealed that Ag and N doping of TiO_2 appeared to have strong absorption by ^-OH group and showed the characteristic absorption band of NH^{4+} and Ag. The antibacterial properties of nanoparticles were investigated by agar diffusion method toward *Escherichia coli* and *Bacillus subtilis*. The results indicated that both Ag^- and N-doped TiO_2 could increase the antibacterial properties of TiO_2 nanoparticles under fluorescent light irradiation. A 1% Ag-N-TiO_2 had the highest antibacterial activity with a clear antibacterial circle of 33.0 mm toward *Escherichia coli* and 22.8 mm toward *Bacillus subtilis* after cultivation for 24 hours (Yuan et al., 2010).

2.10.8 Environmental nanotechnology work done at IESE, NUST

Different researches have been conducted in IESE, NUST using titania for different environmental applications. It has been used for Arsenic (As) removal from water using titania coated glass beads (Danish, 2012) and sand (Nabi, 2009). Titania nanoparticles has also been used for degradation of polythene (Asghar, 2010),phenol (Ilyas, 2010), chloroform (Qureshi, 2012) and ciprofloxacin (Hayder, 2012). It has also been used for microbial disinfection in water (Younas, 2012).

2.10.9 TiO_2 Functions in Sanitation and Improving Hygiene

- **Sterilizing Effect**

TiO_2 photocatalyst decomposes the bacterial cells instead of only killing them. The titanium dioxide has been found to be most effective against microbes as compared to any other antibacterial agent, because titania photocatalytic reaction works even when there are bacterial cells covering the surface and the bacteria are actively propagating. Photocatalytic action decomposed the end toxin produced at the death of cell. TiO_2 does not destroy and it shows a long-term disinfection effect. Antibacterial property by titanium dioxide is 1.5 times stronger than ozone and three times stronger than chlorine (Rui, 2010).

- **Deodorizing Effect**

The hydroxyl radicals which are produced during photocatalysis accelerate the breakdown of any Volatile Organic Compounds by breaking the molecular bonds. This will help in combining the organic gases to form a single molecule that is not harmless to humans thus improve the air cleaning efficiency. Some of the examples of odor molecules are: Tobacco odor, formaldehyde, nitrogen dioxide, urine and fecal odor, gasoline etc. Air purifier with TiO_2 can prevent bacteria, soil, smoke, pollen, virus and detrimental gas as well as seize the free microbes in the air by 99.9% filtering percentage (Nozawa et al., 2001).

- **Air Purifying Effect**

TiO_2 can be applied for the reduction or deterioration of polluted compounds in air i.e., NOx, cigarette smoke, volatile compounds arising from various construction materials. Because of that high photocatalytic reactivity, TiO_2 can be

applied to protect walls and lamp-houses in tunneling, as well as to steer clear of white tents from becoming sooty and dark. Atmospheric constituents such as greenhouse gases, chlorofluorocarbons (CFCs) and its substitutes, nitrogenous and sulfurous compounds undergo photochemical decomposition by TiO_2 photocatalyst, either directly or indirectly in the presence of sunlight so these pollutants can eventually be removed (Ao and Lee, 2005)

- **Anti fogging, Self-Cleaning**

Most of the external walls of buildings become dirty from automotive exhaust fumes, which constitute oily components. When the exterior building materials are coated with a TiO_2, a protective film of photocatalyst with antistatic, super oxidative, and hydrophilic properties provides the self-cleaning building. The hydrocarbons in automobile exhaust fumes are oxidized and the dirt on the walls washes away with rainfall, keeping the external building clean at all times (Fujishima and Zhang, 2006).

- **Water Purification**

Metal doped TiO_2 photocatalyst coupled with sun lights can oxidize organic pollutants into nontoxic materials, such as CO_2 and water and can disinfect certain bacteria. This nanotechnology can be very efficient at removal of hazardous organic compounds (TOCs) and decontamination of a variety of microorganisms and some viruses in the secondary wastewater treatment method. Pilot projects have demonstrated that photocatalytic detoxification systems can effectively kill bacteria in secondary wastewater treatment (Dheaya et al., 2009*).*

Chapter 3

Material and Methods

3.0 Experimental Procedure

3.1 Materials System

One percent Ag-TiO_2 nanoparticles were prepared by a liquid impregnation method. Titanium (IV) dioxide (Sigma-Aldrich Labor chemikalien) and silver nitrate (Merck) were used in the liquid impregnation process as sources of titanium and silver, respectively. Distilled water was used as solvent in the process. The water employed in all preparations was purified by a distillator. Two bacteria strains, *Bacillus Subtilis* (ATCC 1174) *Pseudomonas Aeruginosa* (ATCC 27853), were bought from Microbiologics, Inc USA. Other materials for bacteria cultivation, such as nutrient agar, nutrient broth, sodium chloride, test tubes and pyrex petri dishes were of Merck grade.

3.2 Instrumentation

The instruments used for the study were:

- HACH spectrophotometer DR/2400
- Philips tube light (light source)
- JSM-6490A (JEOL Japan) analytical scanning electron microscopy (SEM)
- Theta-theta (Stoe Germany) X-ray diffraction (XRD)

- TX323L weighing balance (SHIMADZU cooperation Kyoto Japan)
- NEY M-525 muffle furnace
- Labtech oven (Daihan)
- Memmert incubator
- SUNTEX colony counters 560
- CG 843 pH meter (SCHOTT instruments)
- JAC 1505 Sonicator (JINWOO)
- Systronics digital pH meter
- Stuart SB162 magnetic stirrer

3.3 Synthesis of Ag-TiO_2 Nanoparticles

3.3.1 Preparation of photocatalyst; liquid impregnation method

In the liquid impregnation method (Saha et al., 2012) silver ion (Ag^+) doped on TiO_2 was prepared according to the following steps:

We prepare 80 g of 1% Ag doped TiO_2 nanoparticles. 79.2 g of TiO_2 was added to 500 ml of deionized water. Then for preparation of silver doped TiO_2 nanoparticles, 1.7 g of $AgNO_3$ for doping was added to TiO_2 suspension; the silver concentration was of 1% (molar ratio) versus TiO_2. The slurry was stirred well for 6 hour and allowed to rest for 24 h and then dried in an air oven at 100 ^oC for 12 h. The dried solids were crushed to fine powder in an agate mortar and calcined at 400 ^oC for 6 h in a muffle furnace. In this method the metal gets

deposited on the surface of the photocatalyst (Behnajady et al., 2008).

3.3.2 Characterization of Ag-TiO_2 nanoparticles

The crystal structure of the photocatalyst Ag-TiO_2 nanoparticles was analyzed by X-ray diffractometer (Theta-theta, Stoe Germany). X-ray diffraction (XRD) measurements which were carried out at room temperature with Cu Kα radiation (λ = 0.15478 nm) at 60 keV and 15 mA.

The topography, chemical composition, crystalline structure and metal deposition effect of the TiO_2 determined using scanning electron microscopy (SEM). SEM examines the morphology of these nanoparticles at an acceleration voltage of 20 kV. The SEM samples were previously sputter-coated with a gold film. For identification of the elements present in the nanoparticles and for determination of its chemical composition Energy-dispersive spectroscopy (EDS) was used. EDS system embedded with JEOL JSM 6490A was used in this study for the characterization of synthesized nanoparticles by assessing the elemental composition.

3.4 Bacterial cultures and antibacterial activities test in solution phase

3.4.1 Bacterial cultures

Two types of bacteria, *Pseudomonas aeruginosa* ATCC 27853, a Gram negative bacterium and *Bacillus subtilis* ATCC 1174, a Gram positive bacterium, were used as model bacteria in this study.

P. aeruginosa is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several different epidemiological studies indicate antibiotic resistance is increasing in clinical isolates while *B. subtilis* commonly found in the soil and natural environment and can easily contaminate the wound during the episode of trauma (Tsuang et al., 2008). *B. subtilis* is known to cause disease in severely immuno-compromised patients, and can conversely be used as a probiotic in healthy individuals (Oggioni et al., 1998). *B. subtilis* has also been implicated in several cases of food poisoning (McCombs, 2011).

Liquid culture of *P. aeruginosa* (*P. aeruginosa* strain ATCC 27853) was grown aerobically in Nutrient Broth (NB) at 37°C for 16 hours. Liquid culture of *B. Subtilis* (*B. subtilis* strain ATCC 1174) was prepared by same method. The density of the *P. aeruginosa* and *B. subtilis* cells in liquid cultures were estimated by optical density (OD) at 600 nm wavelength. The OD was chosen in a range of 0.8-1.0, which is the optimal optical density of the cells for conventional bacterial activity testing. The cell suspensions used for antibacterial activity was approximately 9×10^9 colony-forming units cfu/ml.

We observed the turbidity of the broth to determine when we should put the bacteria on nutrient agar (Merck) plates after serial dilutions of the culture in 0.85% saline solution. The bacteria concentration was determined by a viable count procedure on nutrient agar plates after serial dilutions of the culture in 0.85% saline solution.

3.4.2 Photo-catalytic reaction

3.4.2.1 Bacterial Activity Test of Ag-TiO_2 Nanoparticles on P.aeruginosa and B.subtilis

The photocatalyst used in this study was Ag-TiO_2 nanoparticles; a surface area of 25 m^2/g (Groen, 2012) and a primary particle size of 79 nm. In the photocatalytic experiments the flask containing 16 hours old culture of *P.aeruginosa* was adjusted on magnetic stirrer. The required concentration of Ag-TiO_2 nanoparticle (10 mg/ml) was weighed and applied to the *P. aeruginosa* culture. The Ag-TiO_2 *P.aeruginosa* culture slurry was placed on a magnetic stir plate with continuous stirring at 250 rpm and was illuminated with simple room light. Bacterial activity test of TiO_2 nanoparticles on *B. subtilis* was tested with same method.

3.4.2.2 Bacteria viability assay

The loss of viability was examined by the viable count procedure. The Ag-TiO_2 nanoparticles and bacterial culture slurry was exposed to simple fluorescent light with continuous stirring. A *P. aeruginosa* culture without Ag-TiO_2 was illuminated as a control. Samples aliquotes were taken at 0, 10, 20, 40, 60, 90, 120 min intervals for two hours. The viable count was performed on nutrient agar plates after serial dilutions of the sample in saline solution. All plates were incubated at 37°C for 24 hour.

Similar viable culture and count procedures were performed for the *B. Subtilis* (ATCC 1174) the plates were incubated at 37°C for 24 hour.

3.5 Preparation of TiO_2 nanoparticles-coated substrates and antibacterial activity tests

3.5.1 Immobilization of Ag-TiO_2 nanoparticles on substrates

3.5.1.1 Ag-TiO_2 nanoparticles coating on pyrex glass petri dish

All pyrex glass petri dishes were etched with dilute hydrofluoric acid (20% v/v) for 24 h and washed thoroughly with deionized water, making a rough surface for better contact of TiO_2 on the glass surface. Then the surface of the petri dish was first treated with acetone and distilled water to remove organic and inorganic materials attached to the surface and was dried under atmospheric conditions. Immobilization of Ag-TiO_2 nanoparticles was done on these pyrex glass petri dishes using following two methods.

3.5.1.1.1 Water based coating

In this method distilled water was used as solvent. The schematics of experiment are shown in Figure 1. Ag-TiO_2 slurry was prepared with 1.5 g Ag-TiO_2 in 200 ml of deionized water and the suspension was placed in an ultrasonic bath for 15 min for complete dissolution as it will break aggregates particles of Ag-TiO_2 and help in making proper slurry.

The petri dish were immersed in the resultant slurry of Ag-TiO_2 for one hour and then removed from the suspension and placed in an oven for 1.5 hour at 150 °C. These were subsequently placed in a furnace for 2 hour at 500°C. The coated petri dishes were thoroughly washed with double distilled water to remove any free Ag-TiO_2 particles (Khataee, 2009).

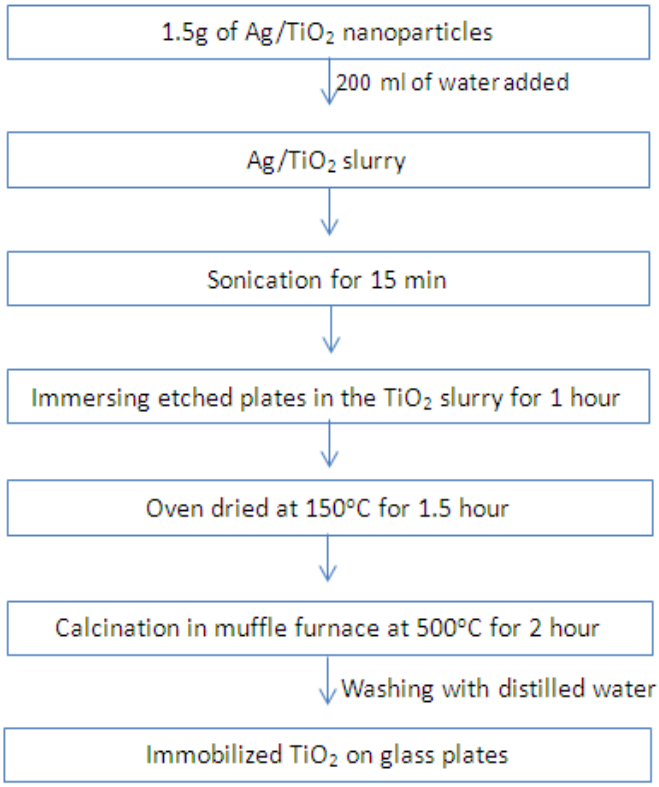


Figure 3.1 Immobilization of TiO_2 nanoparticles on glass plates; heat attachment method

3.5.1.1.2 Ethanol based coating

In this method ethanol was used as solvent. Five grams of Ag-TiO_2 nanoparticles were dissolved in 180 ml of 99% ethanol to form the base medium of the slurry. Vigorous stirring conditions ensured that the Ag-TiO_2 powder was properly dispersed to produce produced slurry. Then dilute nitric acid was added to adjust the pH to 3.5, which is necessary for better dispersion of Ag-TiO_2 powder. This was followed by sonication for 15 min in an ultrasonic bath.

The petri dish was immersed in the Ag-TiO_2 suspension for 60 min followed by drying in air for 24 h and then calcinated at 475 ^{o}C for 1 hour. Calcination allows

the Ag-TiO_2 nanoparticle to adhere more strongly to the support. Afterwards, the coated petri dish was washed in deionized water to remove the unattached TiO_2 particles from the petri dish (Vaez, 2012). The schematics of experiment are shown in figure 3.2.

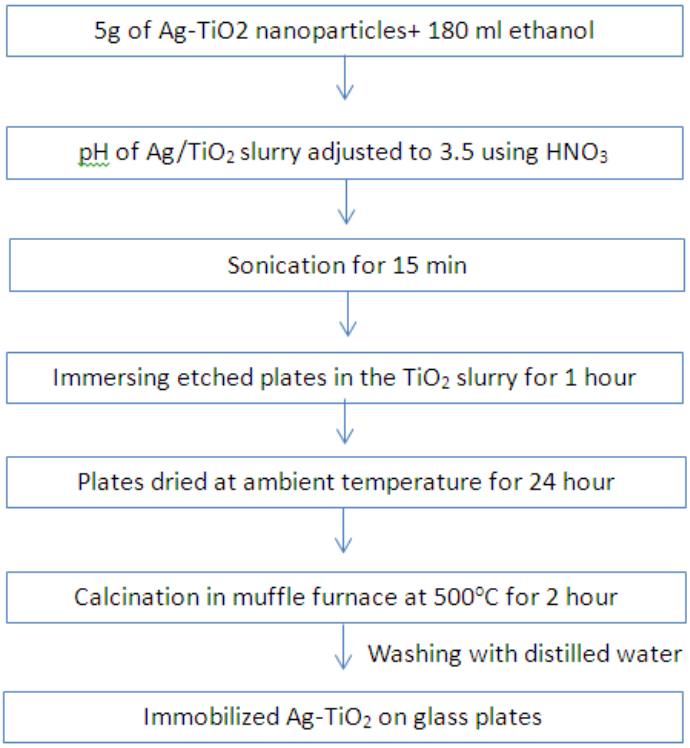


Figure 3.2. Immobilization of Ag-TiO_2 nanoparticles on glass plates by ethanol method; dip coating

3.5.1.2 Ag-TiO_2 nanoparticles coating on plastic venetian blinds

Plastic venetian blinds are type of plastic window treatment. It is made of PVC plastic (French, 1941). The surface of the plastic venetian blinds was first treated with acetone and distilled water to remove organic and inorganic materials attached to the surface and was dried under atmospheric conditions. Then these

plastic venetian blinds were cut in to 3 × 3 cm pieces. Immobilization of Ag-TiO_2nanoparticles was done on these plastic venetian blinds using following two methods.

3.5.1.2.1 Water based coating

Ag-TiO_2 slurry was prepared in distilled water by same method as was prepared for coating on pyrex glass petri dishes. The plastic venetian blinds were immersed in the slurry of Ag-TiO_2 for one hour and then removed from the suspension and placed in an oven for 1.5 hour at 150 °C. These venetian blinds were subsequently placed in a furnace for 2 hour at 160°C. The coated plastic venetian blinds were thoroughly washed with double distilled water to remove any free Ag-TiO_2 particles (Khataee, 2009).

3.5.1.2.2 Ethanol based coating

Ag-TiO_2 slurry was prepared in 99% ethanol by same method as was prepared for coating on pyrex glass petri dishes. The plastic venetian blinds were immersed in the Ag-TiO_2 suspension for 60 min followed by drying in air for 24 h and then calcinated at 160^oC for 1 hour. Afterwards, the coated petri dish was washed in deionized water to remove the unattached TiO_2 particles from the plastic venetian blinds (Vaez, 2012).

3.5.1.3.1 Characterization of Ag-TiO_2 nanoparticles coated substrates

To determine the morphological characteristics for comparative study of change in appearance of non coated and Ag-TiO_2 nanoparticle coated substrates and nanoparticles embedment of the TiO_2 in glass and venetian blinds scanning

electron microscopy (SEM) analysis was used.

3.5.2 Bacterial decontamination effect of Ag- TiO_2 nanoparticle-coated substrates

3.5.2.1Bactericidal effect of Ag- TiO_2 nanoparticle-coated pyrex glass petri dish

3.5.2.1.1 *P. aeruginosa* bactericidal effect

The viability of *P. aeruginosa* was performed over coated pyrex glass substrates; water based coated pyrex glass, ethanol based coated pyrex glass and uncoated (control) substrates were kept in sterile fume hood and bacterial culture was sprayed on the plates as evenly as possible. The substrates were exposed to room tube light. The sample for bacterial count was taken from coated and uncoated substrates at 0, 10, 20, 40, 60, 90 and 120 min intervals for two hours. The sample was prepared by taking swab thoroughly from the whole plate. Microbial count from substrate surfaces was performed using standard method (Collins and lyne, 2004).

3.5.2.1.2 *B. Subtilis* bactericidal effect

The viability of *B. subtilis* ATCC 1174 was performed over water and ethanol based coated pyrex glass petri dishes and uncoated pyrex glass petri dishes in a similar fashion as was done for checking decontamination rate of *P. aeruginosa*.

3.5.2.2 Bactericidal effect of Ag-TiO_2 nanoparticle coated plastic venetian blinds

3.5.2.2.1 *P. aeruginosa* bactericidal effect

The viability of *P.aeruginosa* was performed over water based coated venetian blinds, ethanol based coated venetian blinds and uncoated (control) substrate was evaluated by similar method for checking *P. aeruginosa* bactericidal effect of coated venetian blinds.

3.5.2.2.2 *B. subtilis* bactericidal effect

The viability of *B. subtilis* ATCC 1174 was performed over coated plastic venetian blinds in a same way as was done for checking decontamination rate of *B. subtilis* over water based and ethanol based Ag-TiO_2 coated pyrex glass petri dishes.

Chapter 4

4.0 Results and Discussions

4.1. Characterization of Materials

4.1.1. Crystal Phase Composition of Ag-TiO_2 Nanoparticles; XRD Results

X-Ray diffraction was used to investigate the crystal phase composition and the crystallite size of Ag-TiO_2nanoparticles. XRD patterns showed that the nanoparticles contain pure anatase phase (JCPDS Card Number 73-1764). No rutile reflection was seen in XRD patterns. Usually, a heat treatment at approximately 400^oC is required for the phase transition of TiO_2 from amorphous to anatase phase in the solid state. The Ag doping made the peak of diffraction wider.The XRD patterns are shown in following figure 4.1.a) and b)

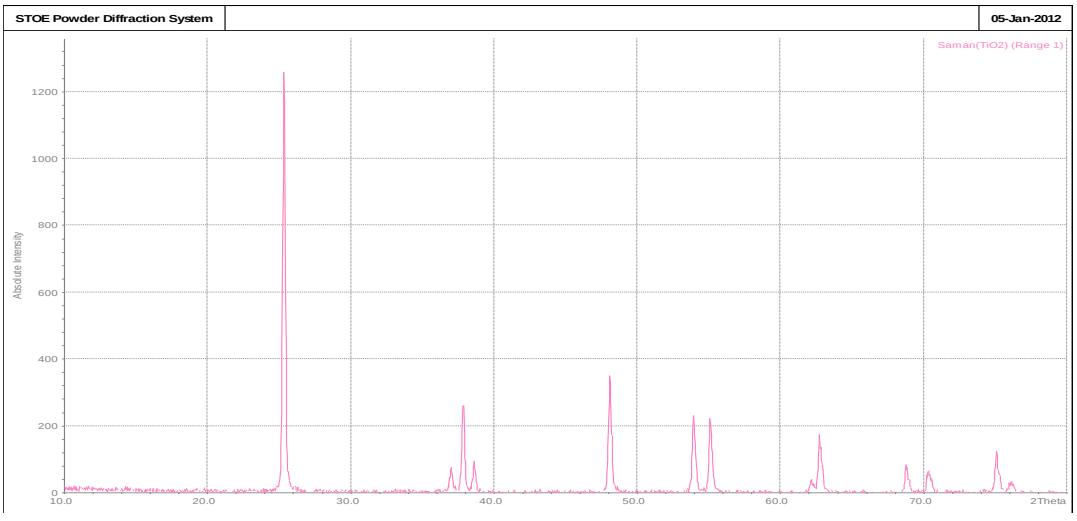


Figure 4.1 a) XRD patterns of metal-doped TiO_2 nanoparticles

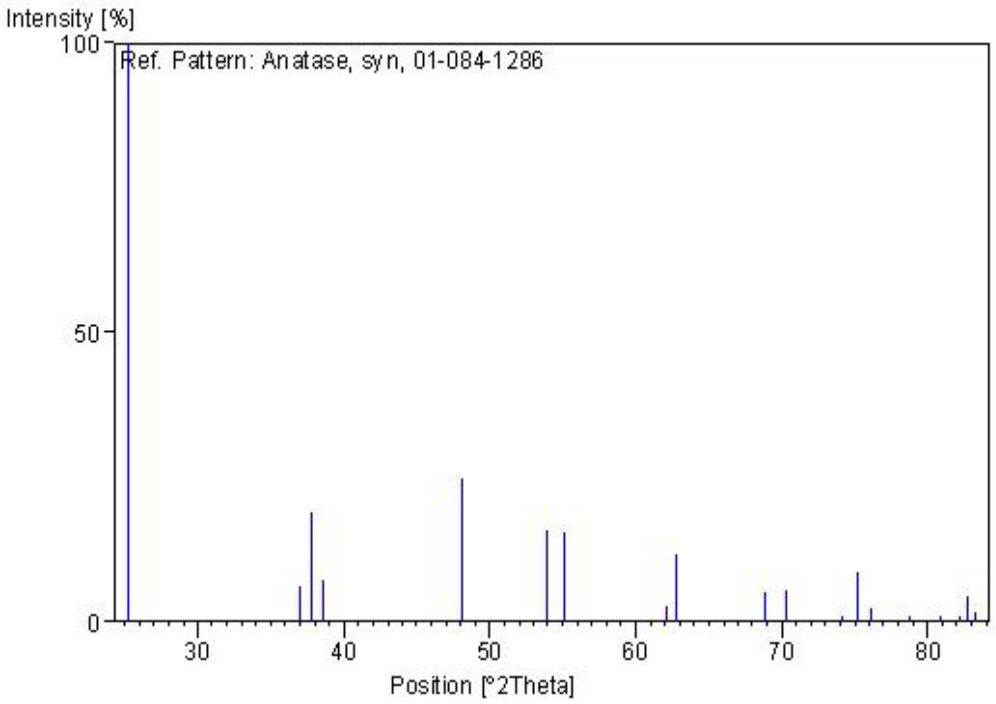


Figure 4.1 b) XRD patterns of metal-doped TiO_2 nanoparticles

4.1.2 SEM Observations

SEM was used for the direct observation of particle size and morphology of sample powders. Figures 4.2 show the images of 1% silver doped TiO_2 nanoparticles by JEOL JSM-6460 at 500 and 20,000 magnifications. SEM images of Ag-TiO_2 nanoparticles confirm the presence of porous, sponge like structure of high roughness and complexity.

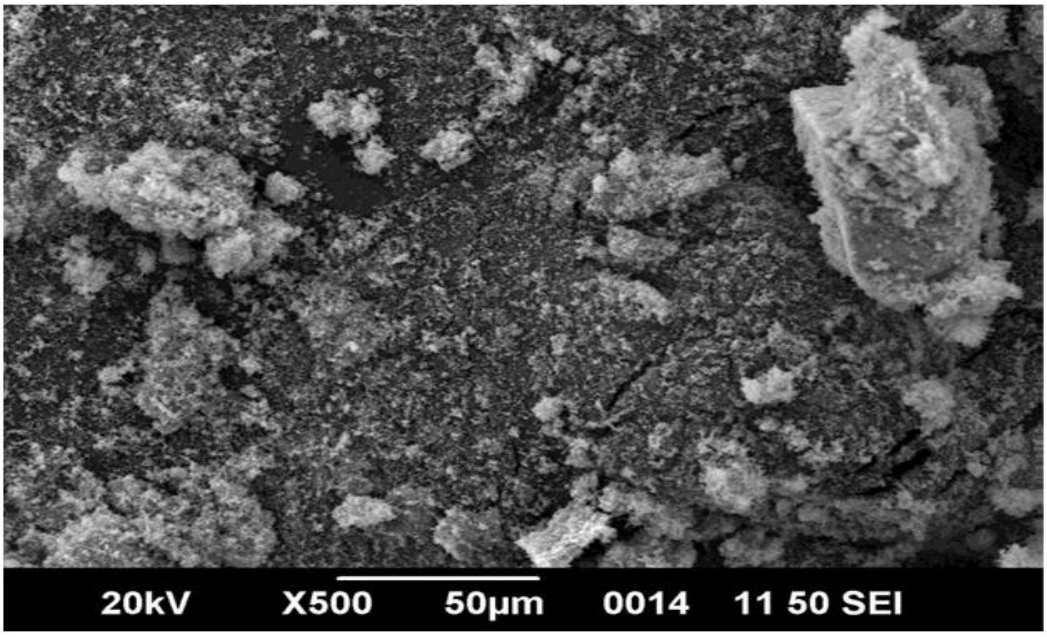


Figure 4.2 a)SEM image of 1% Ag doped TiO_2 taken at X500

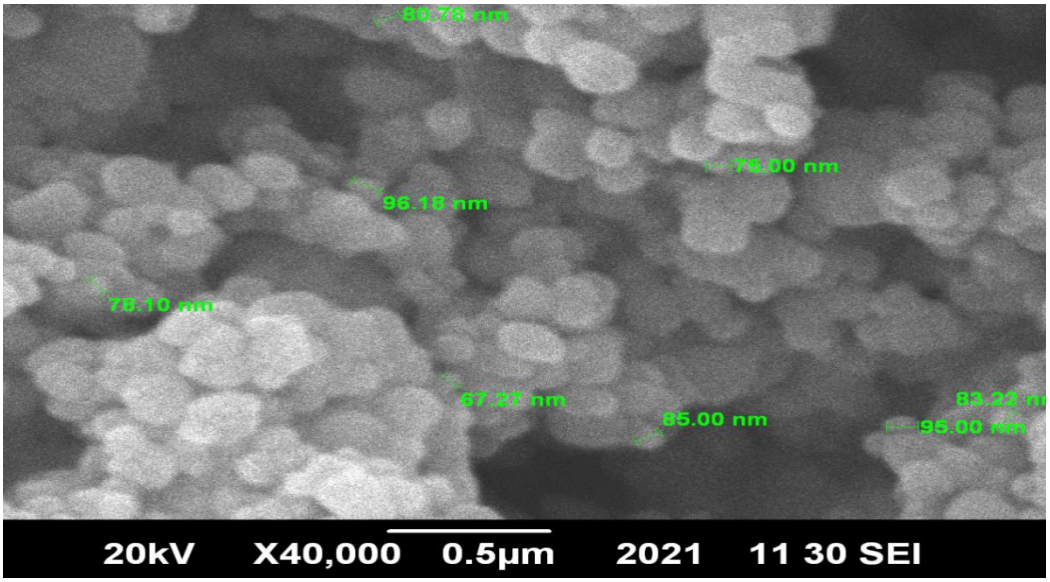


Figure 4.2 b)SEM image of 1% Ag doped TiO2 taken at X40000

Such structure indicates the high surface area which has been proven to be efficient for photo catalytic degradation purposes. Samples consisted of more fine particles but the surface morphology of all the silver-doped TiO_2 samples was

different from each other. The SEM pictures show that the distribution of silver on the surface of TiO_2 is not uniform and silver doped TiO_2 catalyst contains irregular shaped particles which are the aggregation of tiny crystals. Most of the particles were spheroid or oblate spheroid and loosed, macropore can be clearly seen in the SEM micrographs. These nanoparticles are aggregated into microsized particles. The aggregation of these nanoparticles is beneficial to their removal from aqueous environment after the treatment.

Figure 4.3 and 4.4 show the SEM images of TiO_2 coated pyrex glass petri dish and venetian blind in comparison with non coated substrates.

Non-coated glass

Ag-TiO_2 nanoparticle coated glass

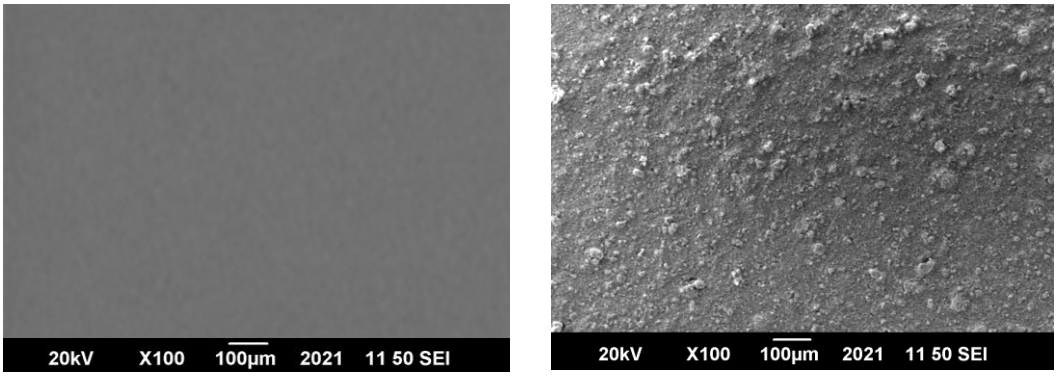


Figure 4.3 SEM images of non-coated and TiO_2 coated pyrex glass petri dish

SEM images of figure 4.3 shows Ag-TiO_2 nanoparticles coated glass surfaces have rough surface. Coating has form thick layer over surface of glass. Due to the high surface coverage of nanoparticles on the surface of the glass, it provides region for photodecomposition activity so act as a good self-sanitizing layer. Titania itself is not decomposed or being used in process of disinfection. So

weathering of layer is only limiting factor for that coating. Thick layer of nanoparticles over coated glass surface is advantageous as it is less affected to chance of weathering of coating as it consume more time to get removed from surface so it has more life time.

Non-coated venetian blind Coated venetian blind

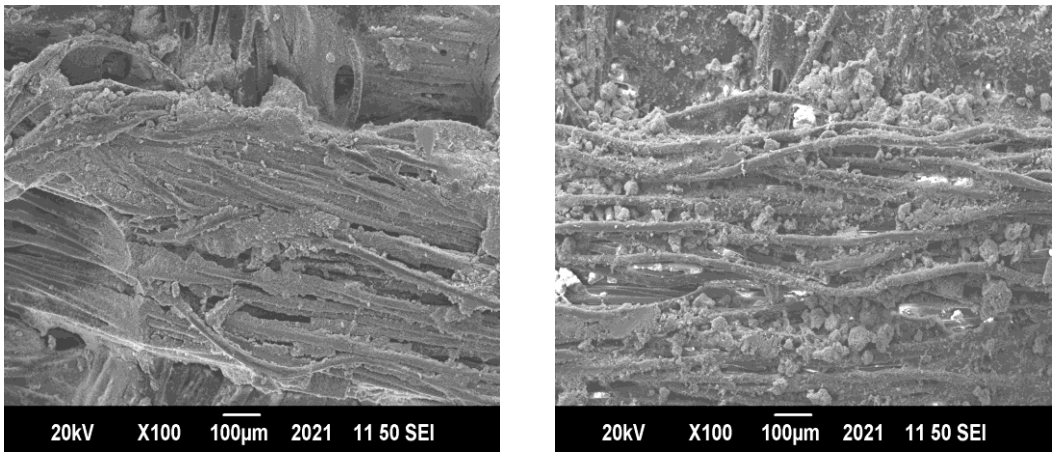


Figure 4.4 SEM images of non-coated and TiO_2 coated plastic venetian blinds

SEM image of figure 4.4 shows that nanoparticles get embedded into the plastic and fabric stands of plastic venetian blinds which provide a good surface of photocatalytic activity so disinfection is carried out in a better manner.

4.1.3 Energy Dispersive Spectroscopy Analysis

EDS analysis showed that the percentage composition of Ag-TiO_2 nanoparticles was not consistent. The results indicated that titanium, oxygen and silver were the constitutive elements of the nanoparticles prepared by the liquid impregnation method and no extraneous elements were present (Figures 4.3). EDS confirms that we have prepared one percent silver doped titania nanoparticles. It also varied

from point to point showing that the composition of the prepared nanoparticles was not homogenous, which confirmed from the SEM results. But whole molar ratio show that silver is one percent in TiO_2 nanoparticles.

Table 4.1 EDS analysis of doped nanoparticles

Element	Mass (%)	Mol (%)	Compound	Mass%
O	39.47			
Ti K	58.91	99	TiO_2	98.27
Ag L	1.61	1	Ag_2O	1.73
Total	100	100		100

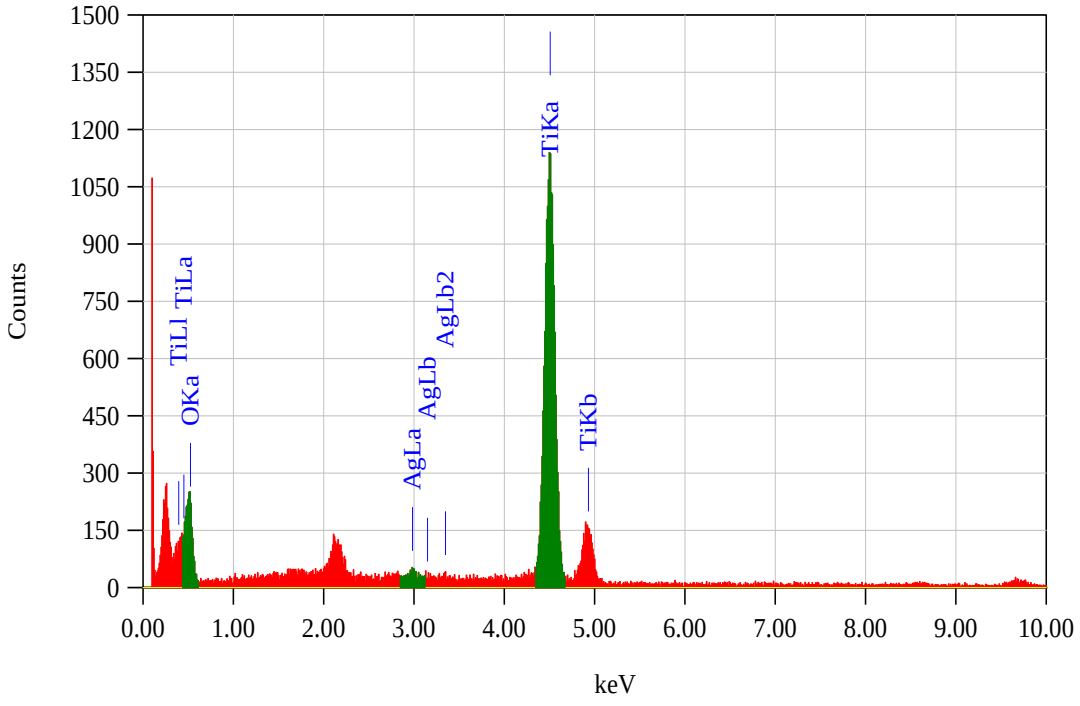


Fig 4.5 EDS pattern of Ag-TiO_2 nanoparticles prepared by liquid impregnation method

4.2 Loss of viability of *P.aeruginosa* and *B.subtilis* under TiO_2 photocatalytic reaction by Ag-TiO_2 nanoparticles

4.2.1 Photocatalytic disinfection by Ag-TiO_2 nanoparticles in solution phase

The antibacterial effect of nanoparticles on *P.aeruginosa* and *B.subtilis* was first tested for silver doped TiO_2 solutions.The viability of Ag-TiO_2 treated bacteria cells was determined by colony counting after 24 h (*P.aeruginosa* and *B.subtilis*) of incubation. The viability of bacteria was significantly inhibited by the treatment of TiO_2 photocatalytic reaction. In the photocatalyzedAg-TiO_2 nanoparticles, the survival of intact *P.aeruginosa* and *B.subtilis* colonies dropped in solution phase as a function of time as shown in figure 3.6 and 3.7.

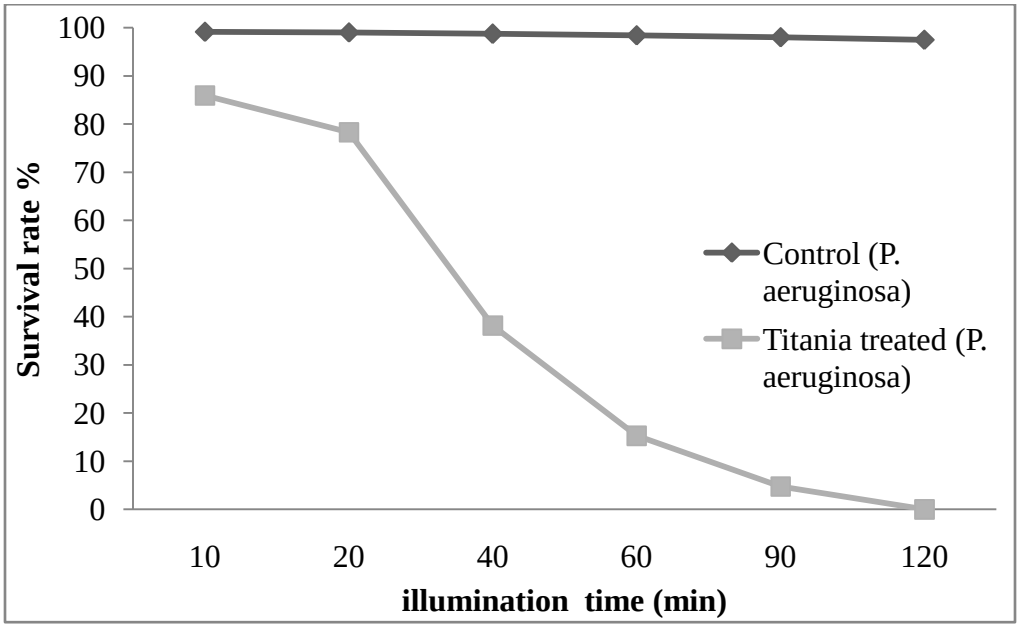


Figure 4.6 a) The survival of *P. aeruginosa* cells in solution phase versus illumination time

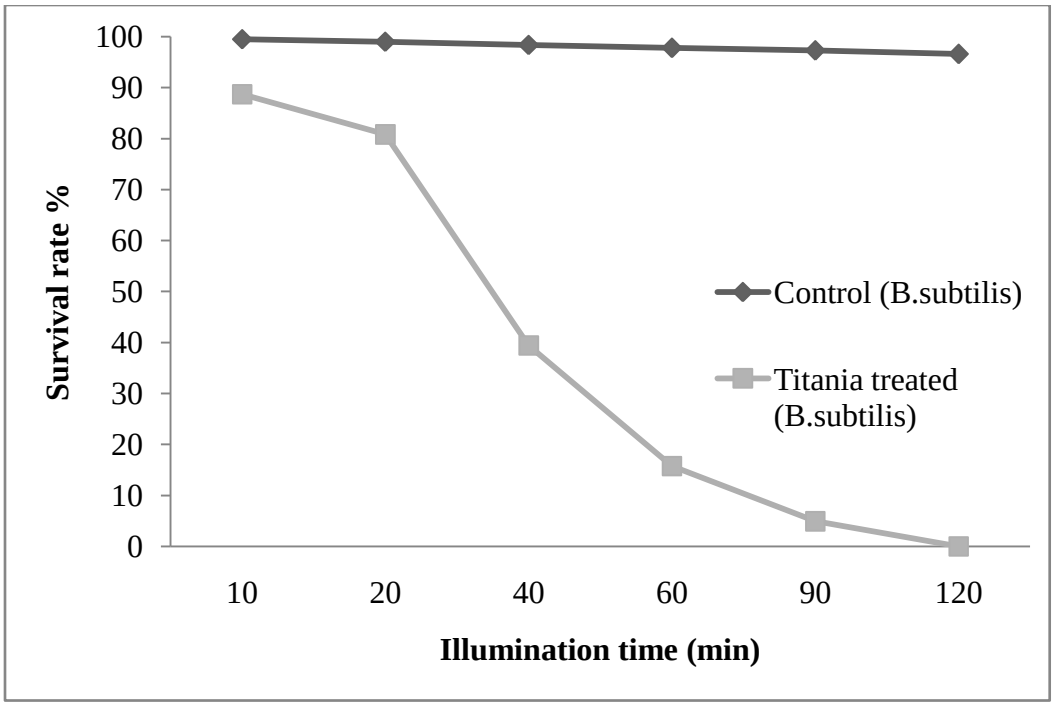
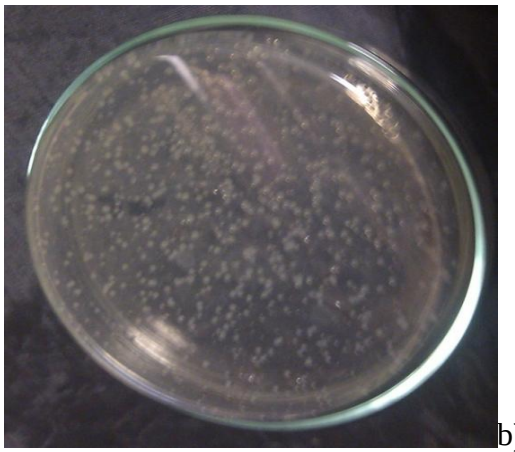
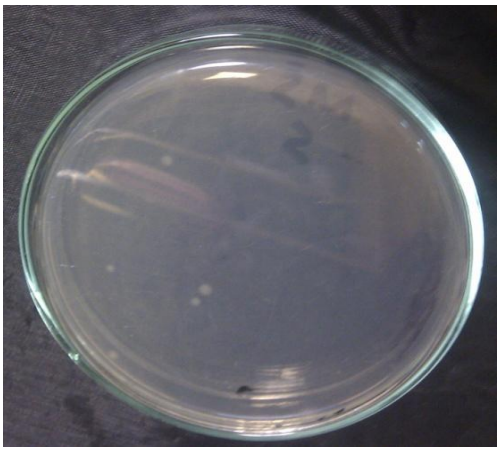


Figure 4.6 b) The survival of *B. subtilis*cells in solution phase Vs illumination time

When the initial bacteria concentration was 9 x 10^9 cfu/ml, the survival of intact *P.aeruginosa* and *B.subtilis* colony dropped significantly after 30 min photocatalytic reaction; bacteria killing were nearly completed within only 90 min under the present experimental conditions. A great decrease in the number of viable bacteria was observed on the illuminated TiO_2 nanoparticles, demonstrating their photokilling activity. The survival curve did not follow a simple single exponential decay process as a function of illumination time, but seemed to consist of two steps, a relative lower rate photokilling step, followed by a higher one (Figure 4.6).

Figure 4.6 shows the antibacterial effect on *P.aeruginosa* and *B.subtilis* respectively using cell culture and Ag-TiO_2 nanoparticles solution and control solution without Ag-TiO_2 nanoparticles. The representative agar plate is

manifestation of bacterial count (*P. aeruginosa* and *B. subtilis*) under various illumination time treatment of TiO_2 photocatalytic reaction. The results in figure 4.6 show that there was a remarkable decrease in bacterial count. The result shows that there was no inhibition on bacterial growth in control solution. The bacterial load was reduced by 90% for *P.aeruginosa* and 90.5 % for *B. subtilis* within our hour of treatment.

a)  b) 

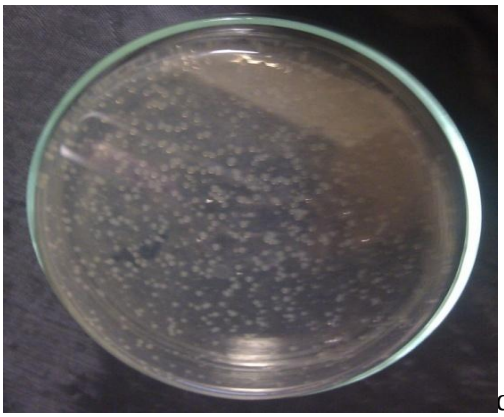
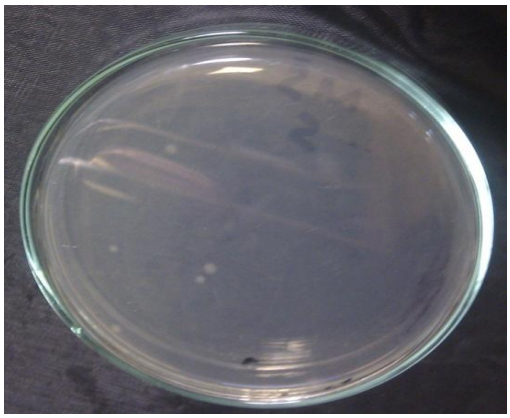
c)  d) 

Fig. 4.7Antibacterial effect in solution phase: (a) Control Solution of *P.aeruginosa* (B) Solution of *P.aeruginosa* with Ag-TiO_2nanoparticles (c) Control Solution of *B.subtilis* and (d) Solution of *B.subtilis* with Ag-TiO_2 nanoparticles

Anatase Ag-TiO_2 nanoparticles have shown bactericidal activities as they have inhibitory behavior to bacterial growth of *P.aeruginosa* (gram-negative bacteria) and *B.subtilis (*gram-positive bacteria) in presence of light. This is attributed to the increasing visible absorption capacity due to the doping of silver in titanium nanoparticles (Seery et al., 2007). The Gram-positive bacteria have a relatively cell wall composed of many layers of peptidoglycan polymer and only one membrane (plasma membrane). The gram-negative bacteria have only a thin layer of peptidoglycan and more complex cell wall with two cell membranes, an outer membrane, and a plasma membrane. The addition of the outer membrane of the Gram-negative bacteria cells influences the permeability of many molecules. Under certain conditions, the Gram-negative bacteria are more resistant to many chemical agents than Gram-positive cells (Tortora et al., 2001).

The photocatalytic process of anatase Ag-TiO_2 nanoparticles includes chemical steps that produce highly reactive species such as hydroxyl radical, hydrogen peroxide, and superoxides that can cause grave damage to microorganisms. Among these reactive oxygen species, the hydroxyl radicals are highlyreactive and therefore short-lived. The superoxide ions are relatively longer lived. Due to their negative charge they cannot penetrate the cell membrane. They must contact directly the outer surface of bacteria unless the TiO_2 particle has penetrated the cell. Hydrogen peroxide is less harmful compared to hydroxyl radicals and superoxide ions, but it can enter the cell (Blake et al., 1999).

Several proposed mechanisms for cell killing by the TiO_2 photocatalytic processes were reported (Lu et al., 2003).One research group reported direct evidence of

cell membrane damage by the irradiation of a thin transparent TiO_2 film to examine the photo-catalytic degradation of endotoxin from *P.aeruginosa*. The endotoxin is a component of the outer membrane of gram-negative bacteria and is released only when the cellular structure is destroyed. The results indicated that the TiO_2 photocatalyst destroys the outer membrane of the *P.aeruginosa* cell and causes the death of the bacteria as damage of the cell membrane directly leads to leakage of minerals, proteins and genetic materials, causing cell death (Sunada et al., 1998).

4.2.2 Bactericidal effect of TiO_2 nanoparticle-coated substrates

The survival rate of *P. aeruginosa* and *B.subtilis* on TiO_2 nanoparticle-coated substrates; pyrex glass petri dish, venetian blinds, under photocatalytic reaction is shown in following figures.

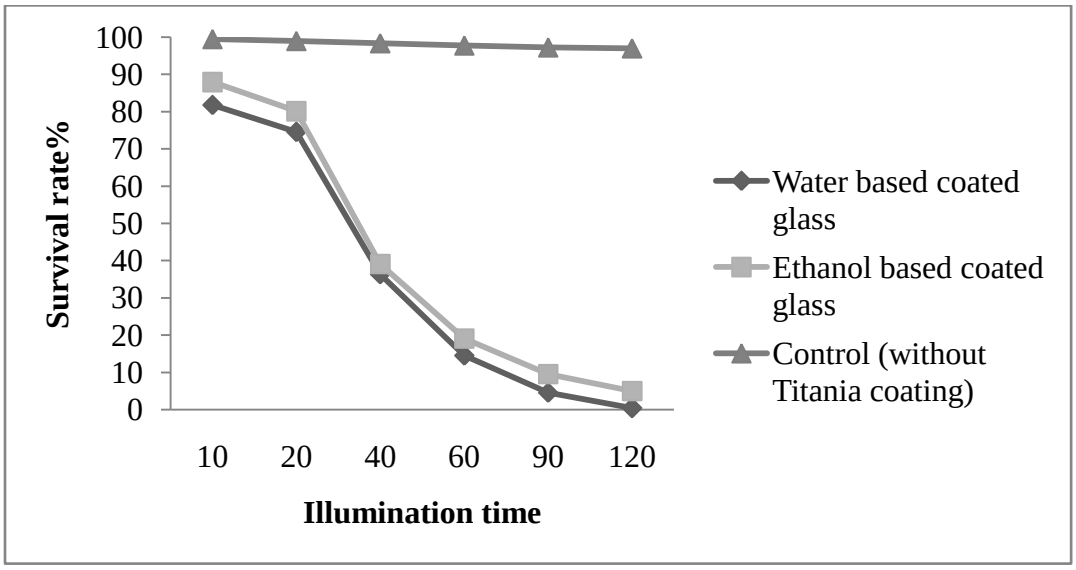


Figure 4.8 Survival rate of *P. aeruginosa* on coated pyrex glass as a function of time

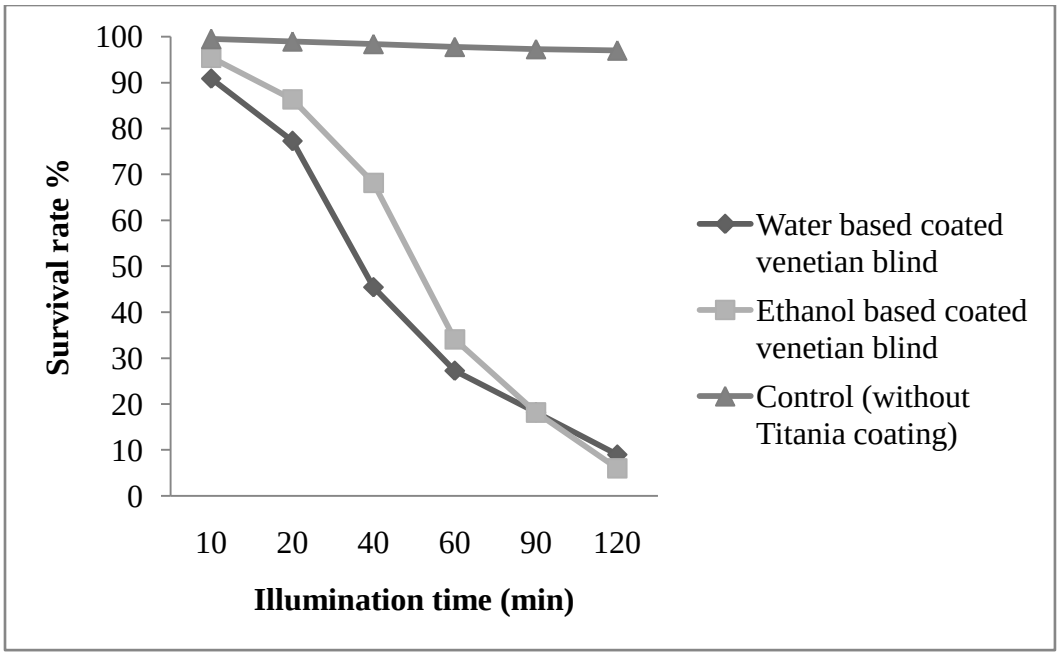


Figure 4.9 Survival rate of *P. aeruginosa* on coated venetian blinds as a function of time

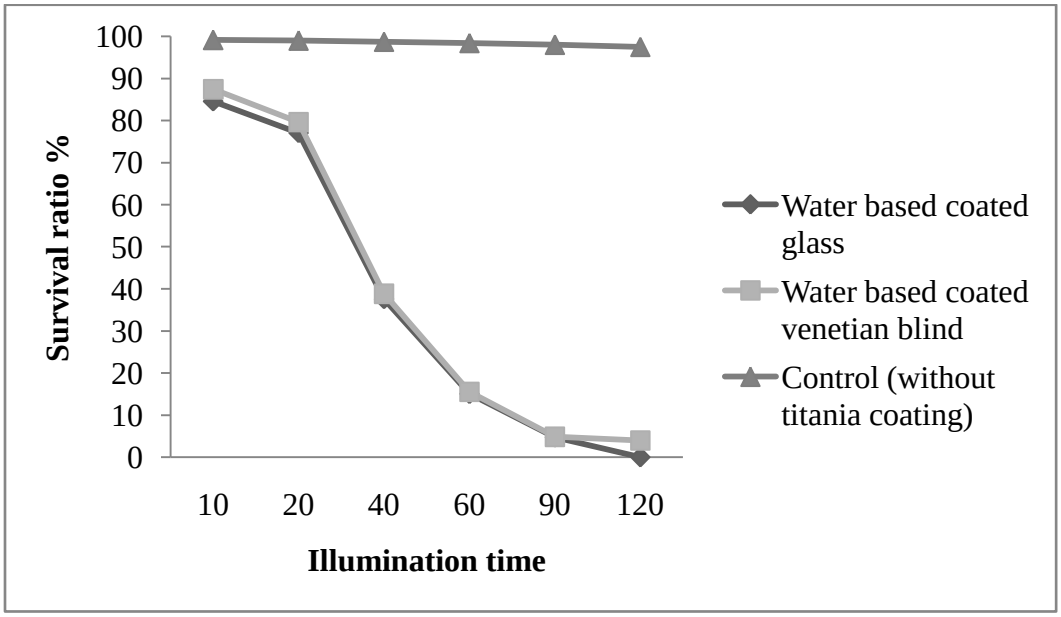


Figure 4.10 Survival rate of *B. Subtilis* on coated pyrex glass as a function of time

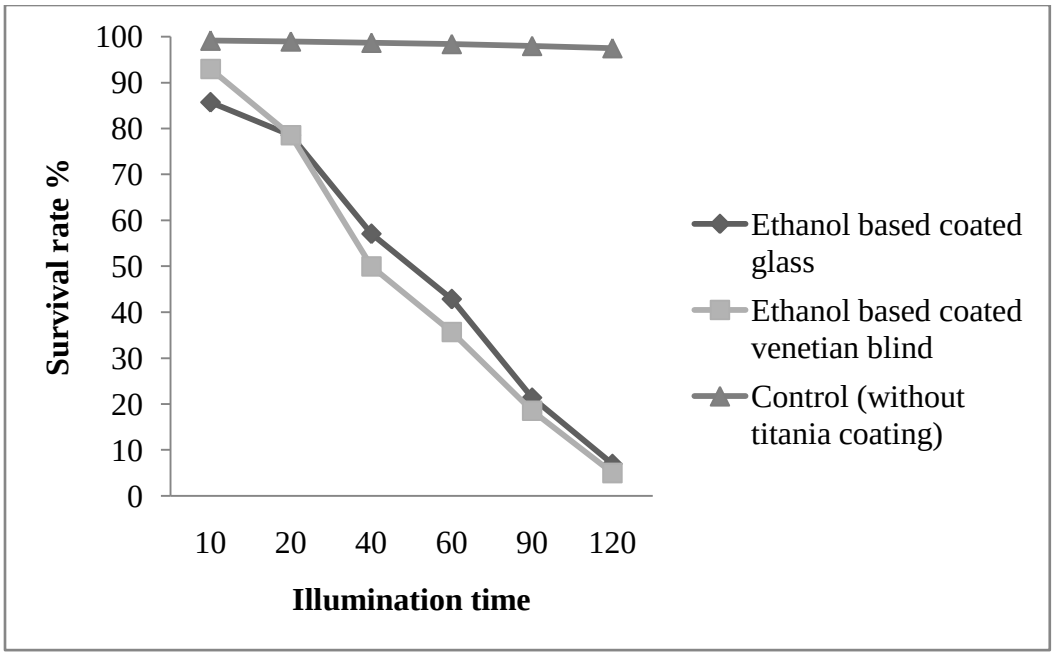


Figure 4.11 Survival rate of *B.subtilis* on coated venetian blinds as a function of time

The bacterial colonies of *P. aeruginosa* and *B.subtilis* above Ag-TiO_2 coated glass plates and venetian blinds were significantly dropped on various substrates as a function of time, while *P. aeruginosa* and *B.subtilis* proliferated well in the glass plate as well as venetian blinds which were not coated with Ag-TiO_2nanoparticles.It was observed that the bacterial count decreases as a function of time. When the initial cell count of *P.aeruginosa*was9 x 10^9cfu/ml, the bacterial load was reduced by almost 85% on Ag-TiO_2 nanoparticles coated glass petri dishes and 55 % on Ag-TiO_2 nanoparticles coated venetian blinds within ten minutes of exposure of light.

More than 90% decontamination was observed within 90 minutes exposure of light on Ag-TiO_2 nanoparticles coated layer over substrates. Almost complete

decontamination was achieved within 120 minutes of treatment. Control samples showed no reduction in bacterial load as a function of time.

The decay of bacteria survival by the photo-killing step was clearly demonstrated by bacteria viability assay. In control group of experiments, the number of bacterial count was above the countable range at the start of the experiment as well after 2 hrs. But a significant abatement in the bacterial count was observed within two hours; 0.4%, 9%, 4.7%, 6.36% survival rate of *P. aeruginosa* on water based coated glass, ethanol based coated glass, water based coated venetian blind, ethanol based coated venetian blind respectivelyand0%, 4%, 7.1%, 5.7% survival rate of *B. subtilis* on water based coated glass, ethanol based coated glass, water based coated venetian blind, ethanol based coated venetian blind respectively.

This illustrates that if 1 % Ag-TiO_2 would be coated on a surface like glass and venetian blinds, that surface may be considered as self sterilizing surface.

Chapter 5

5.0 Conclusion and recommendations

In this study, a simple and non expensive liquid impregnation method was used for the synthesis of 1% silver doped anatase TiO_2 nanoparticles. The antibacterial test of those nanoparticles gave promising results which showed significant inhibition on both bacteria, *P.aeruginosa* and *B.subtilis*, even under room light.

The results of characterization of the TiO_2 nanoparticles by EDS, XRD and SEM demonstrated that fairly uniform sized nanoparticles of 65-85 nm diameter with spherical-shaped anatase form were successfully obtained.

The method to demonstrate the antibacterial effect of those nanoparticles in two forms, an aqueous phase and thin coating substrates; glass and venetian blinds were successfully developed. Results demonstrated that Ag-TiO_2 nanoparticles in aqueous media as well as in coated substrates showed fast disinfection rate on two types of bacteria, *P.aeruginosa* and *B.subtilis*, even under room light. The results indicated the Ag-TiO_2 nanoparticles prepared in this study possess strong oxidizing ability and photocatalytic activity. The good antibacterial effect (100% killing efficiency) as observed in figure 4.6-4.11; may be due to those particles' small size, large surface area, large band gap energy, and more active sites for carrying out catalytic reactions.

Further testing on the survival ratio of *P.aeruginosa* and *B.subtilis* with silver-doped TiO_2 dispersed in coating formulations; coated glasses and coated venetian blind shows that silver doped titanium nanoparticles coating over different

substrates have 90-100 % killing efficiency towards bacteria by harnessing the power of light through photocatalytic oxidation without use of harmful and skin irritating materials. Ag-TiO_2 nanoparticle coating when applied to substrate like glass or plastic venetian blinds, they disinfect the air that comes in contact with that substrate. So ultimately these photocatalytic coatings containing silver doped titanium dioxide nanoparticles coating make surfaces self sanitizing that saves energy, and removes human error and harmful chemicals from the disinfecting process. It is strongly recommended that such Ag-TiO_2 coated surfaces should be employed in hospitals, public places and dwellings to reduce the spread of infectious diseases.

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